Effects of Bitter Substances on Energy Intake and Blood Glucose, and Associated Gastrointestinal Functions, in Healthy Humans

A thesis submitted by

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For the degree of

Doctor of Philosophy

Adelaide Medical School
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May 2020

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Abstract

The studies in this thesis investigated whether specific bitter compounds, administered intraduodenally or intragastrically, reduce postprandial blood glucose and/or energy intake in healthy humans, by modulating GI functions, e.g. gut hormones, gut motility and gastric emptying.

The key findings of the studies are:

1. Slow intraduodenal infusion of quinine, providing doses of 37.5, 75 and 225 mg, over 60 min, did not affect antropyloroduodenal motility, plasma CCK or energy intake, possibly because the delivery rate was insufficient to activate duodenal bitter taste receptors (**Chapter 2**).

2. Intragastric bolus administration of quinine, at doses of 275 and 600 mg, slightly stimulated insulin, and, in response to a mixed-nutrient drink, consumed 30 min later, lowered plasma glucose, associated with markedly increased insulin and modest increases in glucagon and GLP-1, but did not slow gastric emptying, suggesting that, in this study paradigm, postprandial blood glucose lowering was primarily due to insulin (**Chapter 3**).

3. Both intragastric and intraduodenal administration of quinine (600 mg), administered 60 min or 30 min, respectively, before consumption of a mixed-nutrient drink, markedly stimulated C-peptide and reduced plasma glucose, both alone and following the drink, and slowed gastric emptying, with no difference between the routes of administration. The data suggest that, intragastric quinine, when allowed sufficient time to interact with intestinal bitter-taste receptors, reduces blood glucose, by stimulating insulin and slowing gastric emptying, comparably with intraduodenal quinine (**Chapter 4**).

4. Intraduodenal administration of a bitter extract from hops flowers, *Humulus lupulus L.*, only had a modest, and transient, effect to stimulate pyloric pressure, and a delayed effect to stimulate PYY (~ 60 min post-administration), but did not affect antral or duodenal pressures, CCK or energy intake. While the intragastric study part needs to be completed, it appears that intragastric delivery of hops extract may have a more potent, and persistent, effect to stimulate pyloric pressure than intraduodenal delivery. While these findings may suggest that bitter hops extract, in contrast to quinine, may have a greater effect on gastric bitter receptors, the study will need to be completed to draw more definitive conclusions (**Chapter 5**).

In conclusion, the research presented in this thesis has established that bitter compounds, including quinine and hops extract, vary in their potency, and effects, on GI functions, i.e. secretion of gut hormones, modulation of gut motility and/or gastric emptying, that are associated with regulation of energy intake and/or blood glucose. Quinine had potent glucose-lowering effects, mediated by both gastric emptying and stimulation of glucoregulatory hormones, including insulin, while the role of GLP-1 is less clear. In contrast, the effects of hops extract remain less clear. Further research is warranted to investigate the suitability of these, and other, bitter compounds as novel and effective management or treatment strategies for type 2 diabetes and/or obesity.

Declaration of Originality

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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____

Vida Bitarafan,

M.Sc.

Dedication

To my parents

Who demonstrate daily that it is possible

Thank you for helping to give me the life I love today

"I never lose. I either win or learn"

Nelson Mandela

Acknowledgements

This thesis represents the work I have conducted at the Discipline of Medicine, University of Adelaide, in the last 3 ½ years – the time, despite its hardships, has certainly been full of overwhelmingly positive and valuable experiences. Its completion is due in large part to the special people who challenged and supported me along the way who I would like to thank below.

I would like to express my deep and sincere gratitude to my supervisors, Professor Christine Feinle-Bisset and Dr Tanya Little, without whose guidance I could not have completed this work. I would especially like to show my appreciation to Christine for giving me the chance to do this PhD so far from home. I am very grateful for your wisdom, dedication, friendship, and enthusiasm which all together have enabled me to accomplish the immense task that is a PhD. Your willingness to be available at a moment's notice for any small query, the vast amount of time you would spend reviewing my many drafts with such great detail, and your eagerness to help me improve my research abilities and professionalism, has been invaluable. I am extremely grateful to Tanya for her thoughtful and knowledgeable comments, support, and encouragement throughout this research. You were always so approachable and I always left our discussions feeling reassured. It was a great privilege and honour to have the opportunity to work and study under two such inspiring, dedicated and supportive supervisors.

I would like to thank our team research officer, Ms Penelope Fitzgerald, who has been a reliable source of support and also a wonderful friend to me throughout this entire PhD. I am very grateful for everything that I have learnt from you from laboratory techniques to organisational skills. I would not have been able to complete the practicalities of my PhD without your assistance in planning for, and performing, studies.

Besides my supervisors, I would like to thank those others who provided assistance to help me achieve my PhD. To Professor Michael Horowitz, Professor Karen Jones, Professor Wolfgang

Meyerhof, and Dr Tongzhi Wu; I appreciate your guidance in interpreting my data. Your thorough revisions of, and contribution to, my manuscripts, has undoubtedly improved their quality. To our international collaborators, Professor Sally Poppitt and Dr John Ingram, who have contributed to the studies presented in the last chapter of this thesis; your guidance in study designs and data interpretation was invaluable. To Professor Trygve Hausksen and Dr Hung Thai Pham; I am grateful for the hours you spent on teaching me the 2D-ultrasound gastric emptying technique.

My thanks also go to everyone from the CRE for a supportive and helpful work environment, especially to the reliable and experienced staff I was fortunate enough to work with. In particular, I would like to thank all past and current members of the "CFB-group". Having such an encouraging group of co-workers has made this time incredibly enjoyable and memorable. My thanks also go to Ms Kylie Lange for her valuable advice regarding statistical analysis and Mr Scott Standfield and Ms Judith Marie Wishart for their timely completion of hormone analysis. I would like to acknowledge the interns who assisted with study visits over the years. Finally, thank-you to the individuals who volunteered their time to participate in my projects, as without them this work would not have been possible.

I feel greatly indebted to my caring, loving, and supportive husband, Farshad: my deepest gratitude. You have always had my goals as your top priorities, and continued to support and encourage me to succeed even though you were many miles away. Thank you for tolerating all the hardships of a long distance marriage over the past years to help me complete my PhD.

Last, but certainly not least, I would like to thank my family: my brother Vahid, and, in particular, my parents Nahid and Naser, for being my constant source of love, support, inspiration and strength throughout my life, and imparting on me the value of education. Thanks for always being there for me no matter where I am.

Publications arising from this thesis

Original publications:

Hajishafiee M*, Bitarafan V*, Feinle-Bisset C. Gastrointestinal sensing of meal-related signals in humans, and dysregulations in eating-related disorders. *Nutrients. 2019; 11(6):1298.*
*Both authors contributed equally to this work

Bitarafan V, Fitzgerald PCE, Little TJ, Meyerhof W, Wu T, Horowitz M, Feinle-Bisset C. Effects of intraduodenal infusion of the bitter tastant, quinine, on antropyloroduodenal motility, plasma cholecystokinin, and energy intake in healthy men. *J Neurogastroenterol Motil. 2019; 25(3):413-422.*

Bitarafan V, Fitzgerald PCE, Little TJ, Meyerhof W, Jones KL, Wu T, Horowitz M, Feinle-Bisset C. Intragastric administration of the bitter tastant quinine lowers the glycemic response to a nutrient drink without slowing gastric emptying in healthy men. *Am J Physiol Regul Integr Comp Physiol. 2020; 318(2):R263-R273.*

Rose B*, Bitarafan V*, Rezaie P*, Fitzgerald PCE, Horowitz M, Feinle-Bisset C. Comparative effects of intragastric and intraduodenal administration of quinine on the glycaemic response to, and gastric emptying of, a mixed-nutrient drink, in healthy men. *Manuscript in preparation.*
*These authors contributed equally to this work

Bitarafan V, Ingram JR, Poppitt SD, Fitzgerald PCE, Feinle-Bisset C. Effects of intraduodenal and intragastric administration of a bitter extract of hops (*Humulus lupulus L.)* on plasma CCK and PYY concentrations, antropyloroduodenal motility and energy intake in healthy males. *Manuscript in preparation.*

Conference presentations:

Bitarafan V, Fitzgerald PCE, Little TJ, Wu T, Horowitz M, Feinle-Bisset C. Effects of intraduodenal infusion of the bitter tastant, quinine, on antropyloroduodenal motility, plasma cholecystokinin concentrations and energy intake in humans. Poster presentation at the 3rd meeting of the Federation of Neurogastroenterology and Motility (FNM), 29th August – 2nd September 2018, Amsterdam, Netherlands.

Bitarafan V, Fitzgerald PCE, Little TJ, Meyerhof W, Jones KL, Wu T, Horowitz M, Feinle-Bisset C. Effects of intragastric administration of the bitter tastant, quinine, attenuates the glycaemic response to a nutrient drink in healthy lean men. Oral presentation at the 27th annual meeting of the Society for the Study of Ingestive Behavior (SSIB), 9th – 13th July 2019, Utrecht, Netherlands.

Rose B, Rezaie P, Bitarafan V, Fitzgerald PCE, Horowitz M, Feinle-Bisset C. Effects of intragastric vs. intraduodenal administration of the bitter compound, quinine, on the glycaemic response to, and slowing of gastric emptying of, a mixed-nutrient drink, in healthy men. Accepted for poster presentation at the 4th meeting of the FNM, 23rd – 28th March 2020, Adelaide, Australia.

This meeting has been postponed to April 14th – 17th, 2021 due to COVID-19 situation.

Rezaie P, Rose B, Bitarafan V, Fitzgerald PCE, Horowitz M, Feinle-Bisset C. Effects of intragastric vs. intraduodenal administration of the bitter compound, quinine, on gut and gluco-regulatory hormones, antropyloroduodenal pressures and fasting glucose, in healthy men. Accepted for poster presentation at the 4th meeting of the FNM, 23rd – 28th March 2020, Adelaide, Australia.

This meeting has been postponed to April 14th – 17th, 2021 due to COVID-19 situation.

Abbreviations

WHO	World Health Organisation
OECD	Organisation for Economic Co-operation and Development
BMI	Body mass index
FTO	fat mass and obesity-associated gene
HbA1c	Glycated haemoglobin
OGTT	Oral glucose tolerance test
GI	Gastrointestinal
PYY	Peptide YY
NTS	nucleus tractus solitarius
CCK	Cholecystokinin
ENS	Enteric nervous system
CNS	Central nervous system
GPCR	G protein-coupled receptor
TAS2R	Taste 2 receptor
TAS1R	Taste 1 receptor
MMC	Migrating motor complex
GLP-1	Glucagon-like peptide-1
GIP	Glucose-dependent insulinotropic peptide
i.v.	Intravenous
GHS-R1a	Growth hormone secretagogue receptor 1a
NPY	Neuropeptide Y
DPP-IV	dipeptidyl-peptidase-IV
FD	Functional dyspepsia

FODMAPs	fermentable oligo-, di- and mono-saccharides and polyols
α-gust	α-gustducin
PDE	Phosphodiesterase
PLC	Phospholipaseβ2
IP3	1,4,5-trisphophate
DAG	Diacylglycerol
MHBA	Matured hop bitter acids
TRPM5	Transient Receptor Potential M5
Q37.5	Intraduodenal infusion of quinine hydrochloride at the load of 37.5 mg
Q75	Intraduodenal infusion of quinine hydrochloride at the load of 75 mg
Q225	Intraduodenal infusion of quinine hydrochloride at the load of 225 mg
QHCl	Quinine hydrochloride
VAS	Visual analogue scale
CVs	Coefficients of variation
ANOVA	Analysis of variance
MI	Motility indices
NS	Non-significant
SEM	Standard error of the mean
Q275	Intragastric administration of quinine hydrochloride at the dose of 275 mg
Q600	Intragastric administration of quinine hydrochloride at the dose of 600 mg
ELISA	Enzyme-linked immunosorbent assay
AUC	Areas under the curve
$AUC_{-31 - -1 min}$	Area under the curve between t = -31 – -1 min
$AUC_{15 - 120 min}$	Area under the curve between t = 15 – 120 min
$AUC_{0 - 120 min}$	Area under the curve between t = 0 – 120 min

$AUC_{0-30\ min}$	Area under the curve between t = 0 – 30 min
$AUC_{-1-30\ min}$	Area under the curve between t = -1 – 30 min
IG	Intragastric
ID	Intraduodenal
IG-QHCl	Intragastric administration of quinine hydrochloride at the dose of 600 mg
ID-QHCl	Intraduodenal administration of quinine hydrochloride at the dose of 600 mg
IG-control	Intragastric administration of control
ID-control	Intraduodenal administration of control
NHMRC	National Health and Medical Research Council
AUC/min	Area under the curve dividing by the number of min
$AUC_{10-120\ min}$	Area under the curve between t = 10 – 120 min
$AUC_{10-30\ min}$	Area under the curve between t = 10 – 30 min
$AUC_{0-30\ min}$	Area under the curve between t = 0 – 30 min
ID-HE100	Intraduodenal administration of hops extract at the dose of 100 mg
ID-HE250	Intraduodenal administration of hops extract at the dose of 250 mg
ID-control	Intraduodenal administration of control (canola oil)
IG-HE250	Intragastric administration of hops extract at the dose of 250 mg
IG-control	Intragastric administration of control (canola oil)
HE100	Administration of hops extract at the dose of 100 mg
HE250	Administration of hops extract at the dose of 250 mg

Chapter 1: Introduction

Statement of Authorship

Title of Paper	Gastrointestinal sensing of meal-related signals in humans, and dysregulations in eating-related disorders
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Hajishafiee M*, Bitarafan V*, Feinle-Bisset C., Nutrients. 2019; 11(6):1298. *Both authors contributed equally to this work.

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Name of Principal Author (Candidate)	Vida Bitarafan		
Contribution to the Paper	Wrote, reviewed, and edited the original draft.		
Overall percentage (%)	35%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. Maryam Hajishafiee and I are primary authors for this paper and have equally contributed to all aspects of this research.		
Signature		Date	07/05/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Wrote, reviewed, and edited the original draft.		
Signature		Date	30/04/2020

1.1 Obesity and related comorbidities

Obesity is a worldwide health problem, which, according to the World Health Organisation (WHO), is about to replace undernutrition and infectious diseases as the main health threat for humans (1). Globally, the prevalence of obesity among adults has increased by 28% between 1980 and 2013 (2). During this period, the number of overweight or obese individuals globally increased from ~ 921 million to ~ 2.1 billion (2). The most recent WHO statistics reported that in 2016, 40% of women and 39% of men aged 18 years and older were overweight (1). Obesity is now the leading cause of death globally (3), and was responsible for ~ 38 million (68%) of the 56 million deaths in the world in 2012 (4).

Obesity is associated with a number of comorbidities, including type 2 diabetes mellitus, hypertension and dyslipidaemia, gallbladder diseases, certain cancers, respiratory system problems, leading to asthma, breathing difficulties or sleep apnoea, and reproductive problems, causing menstrual irregularities or complications with pregnancies, as well as cardiovascular disease (5-7), which is the major cause of death among type 2 diabetes mellitus patients (8).

Obesity greatly increases the risk of developing type 2 diabetes (5, 9) because obesity is associated with an increased risk of insulin resistance, the predisposing factor for the development of type 2 diabetes (10). Therefore, similar to obesity, the incidence of type 2 diabetes is also rising worldwide. Estimates indicate that there were 415 million diabetic people, aged 20 – 79 years, in 2015, with the number predicted to rise to 642 million by 2040 (11). Type 2 diabetes is strongly associated with both microvascular (including retinopathy, nephropathy and neuropathy) and macrovascular (including peripheral vascular) disease, ischemic heart disease, and cerebrovascular disease, complications (12). Blood glucose control is tightly regulated by the rate of gastric emptying (13, 14). Patients with type 2 diabetes are

often diagnosed with diabetic gastroparesis (pathologically delayed gastric emptying), as a result of their neuropathy, leading to poor postprandial blood glucose control in these patients (15, 16).

The epidemiology and aetiology of both obesity and type 2 diabetes, as well as their diagnostic criteria, are summarised briefly in the following sections.

1.1.1 Obesity epidemiology and related metabolic conditions

Based on the Organisation for Economic Co-operation and Development (OECD) Obesity Update (17), in 2015, 19.5% of the adult population, across the member countries of the OECD, were obese, with the rate ranging from less than 6% in Korea and Japan to more than 30% in Hungary, New Zealand and Mexico, and 40% in the United States. OECD projections also predict a steady increase in obesity rates until at least 2030, for instance 47% of the population in the United States are projected to be obese in 2030. According to the OECD, in Australia (ranked number 9), ~ 28% of individuals aged 15 years or older are classified as obese (17). Furthermore, according to the 2017-18 Australian Bureau of Statistics Australian Health Survey (18), 31% of Australian adults are obese. Hence, it is not surprising that 8.4% of the economic burden of disease in Australia was attributed to obesity. The total annual direct cost of overweight and obesity in Australia in 2005 was estimated at AU$21 billion, substantially higher than previous estimates (19).

Based on the WHO definition, obesity is defined as "abnormal or excessive fat accumulation that presents a risk to health" (20). The aetiology of obesity is complex, due to its multifactorial nature, but is driven primarily by a sustained imbalance between energy intake and expenditure over a prolonged period of time (21). A crude population measure of obesity is the body mass

index (BMI), a person's weight (in kilograms) divided by the square of their height (in metres). A normal, or "healthy", weight is defined as a BMI in the range of 18.5 – 24.9 kg/m^2, overweight as 25 – 29.9 kg/m^2 and obesity as a BMI ≥ 30.0 kg/m^2. Obesity is frequently subdivided into categories: Class I: BMI of 30 – 34.9 kg/m^2. Class II: BMI of 35 – 39.9 kg/m^2, and Class III: BMI ≥ 40.0. The analysis of 57 large long-term prospective studies with 900,000 participants, mostly Western Europeans and North Americans, showed a U- or J-shaped curved relationship between mortality and BMI, so that BMIs of < 22.5 kg/m^2 and ≥ 25 kg/m^2 are associated with an increased mortality (22). However, the clinical limitations of BMI as an indicator of obesity should be considered. Factors such as age, sex, ethnicity and exercise can influence body fat, and consequently impact BMI. For example, a BMI that would indicate overweight in a population under the age of 55 years, is associated with a decreased mortality in an elderly population (23, 24); on average, women's total body fat mass is greater than in men with an equivalent BMI (25), and the above BMI categories may underestimate mortality risk in Asian populations (26). Furthermore, BMI is a measure of weight rather than body fat distribution or body composition, the main indicators of metabolic risks. For instance, individuals with a large muscle mass, or highly-trained athletes, may have a high BMI because of increased muscle mass, but not body fat. It is well-established that a relative excess of adipose tissue in the abdominal region is associated with an increased risk of co-morbidities, due to the release of non-esterified fatty acids and pro-inflammatory markers from abdominal visceral tissues (27-29). Therefore, waist circumference and waist-to-hip ratio better reflect body fat distribution (30). A waist circumference ≥ 80 cm for women, and ≥ 94 cm for men, has been found to increase the risk of developing co-morbidities (30). A waist-to-hip ratio of > 0.9, in men, and > 1, in women, indicates obesity (30). The advantage of both measurements is that they better reflect body fat distribution.

1.1.2 Obesity aetiology

Obesity is the result of a complex interaction of genetic, social and environmental factors, with the net effect being an overall energy imbalance, with energy intake higher than expenditure.

Overfeeding studies in twins have indicated a role for genetics in body weight regulation and the development of obesity (31, 32). For example, overfeeding of twins for 100 days by 100 kcal/day resulted in similar weight gain within, but substantial variations in weight gain between, twin pairs (33). In a study on adopted children, there was a strong relationship between the weight class of the adoptees and the BMI of their biological parents, but not their adoptive parents (34). Genome-wide association studies have reported variants in several genes that may contribute to weight gain (31, 32). Among these genes, the "fat mass and obesity-associated gene (FTO)" gene has been researched widely. FTO is associated with increased BMI, hip circumference and weight and yields a proportion of attributable risk of 22% toward obesity (35, 36). Moreover, in most cases, extreme obesity (defined as a BMI $\geq$ 40 kg/m^2) has been shown to be attributable to mutations of the *LEP* gene, the gene encoding leptin, and which results in a lack of leptin (37). Leptin is a hormone derived from adipose tissue and is involved in long-term regulation of body weight; therefore, absence of leptin signalling results in food overconsumption and subsequent obesity. In cases where leptin was genetically deficient, regular leptin injection has shown positive impact by preventing weight gain (38).

One of the driving forces behind the dramatic increase in obesity prevalence over the past few decades has been a sedentary lifestyle, i.e. a reduction in physical exertion (due to a reduction in manual work) and changes in leisure time activities, such as the increased use of electronic devices for entertainment. Therefore, energy expenditure during everyday life has decreased, relative to a sustained, or even increased, energy intake (39). Leading contributors to the latter

is the ready availability of relatively cheap, energy-dense "fast-food", high in sugar and fat, and sugar-sweetened soft drinks, as well as increased portion sizes (40-43). Therefore, weight and energy intake management have become increasingly complex and require effective solutions in order to prevent a further rise in obesity and related comorbidities, particularly type 2 diabetes.

1.1.3 Type 2 diabetes epidemiology, aetiology, and diagnosis

As mentioned earlier in this chapter, obese individuals have an increased risk for type 2 diabetes. For example, men and women with a BMI of ≥ 35 kg/m^2 have a relative risk of 42- and 49-fold, respectively, for diabetes (42, 43). Thus, it is not surprising that as the global incidence of obesity has rapidly increased, the incidence of diabetes has nearly quadrupled since 1980 (rising from 108 million in 1980 to 422 million in 2014) (44). The global prevalence of diabetes among adults, over 18 years of age, has risen from 4.7% in 1980 to 8.5% in 2014 (1), and is predicted to rise more in the future, particularly in developing countries (44). While these approximations include type 1, type 2 and gestational diabetes, type 2 diabetes (85%) represents the majority of the cases (45). In 2016, an estimated 1.6 million deaths were directly caused by diabetes and another 2.2 million deaths were attributable to glucose intolerance in 2012 (44).

In obese individuals, adipose tissue releases increased amounts of glycerol, non-esterified fatty acids, pro-inflammatory cytokines, hormones and other factors that are all involved in the development of insulin resistance. Insulin resistance refers to the impaired biological responses of target tissues, primarily the liver, muscle and adipose tissue, to insulin stimulation and, ultimately, compromised glucose disposal, resulting in a compensatory increase in β-cells insulin production and hyperinsulinaemia in order to maintain euglycaemia (46-48). Over time,

this leads to exhaustion, and a gradual decline in the function of β-cells. Once the β-cells can no longer compensate for the insulin resistance, hyperglycaemia develops and, ultimately, type 2 diabetes. Diets high in fat and/or high in sugar are known to induce obesity and promote insulin resistance (49, 50). Glucagon also plays an important role in contributing to hyperglycaemia in patients with diabetes, since postprandial glucagon suppression is diminished, probably due to hepatic insulin resistance, and glucagon secretion is enhanced as hepatic glucose output increases due to increased gluconeogenesis and glycogenolysis (51).

Both impaired glucose tolerance, where blood glucose levels are higher than normal but not high enough to be classified as diabetes, and impaired fasting glucose, where blood glucose levels are escalated in the fasting state, lead to diabetes, which each requires different treatment or management approaches; therefore, accurate diagnostic criteria are required. For diagnostic purposes, different acute measurements, including glycated haemoglobin (HbA1c), random blood glucose, fasting blood glucose, and oral glucose tolerance test (OGTT, 2-hour blood glucose responses to 75 g of glucose) are required (**Table 1.1**). HbA1c indicates the average blood glucose level for the past 3 months. It measures the percentage of blood glucose attached to haemoglobin in erythrocytes. This test is used to diagnose prediabetes or diabetes and for the long-term control of blood glucose levels, as well as to predict the risk of diabetes-related complications (52, 53).

Due to the pathophysiological changes associated with obesity and type 2 diabetes, it is critical to have effective strategies to manage, treat or, ideally, prevent both conditions.

Table 1.1: Diagnostic criteria for glycated haemoglobin (HbA1c), random blood glucose, fasting blood glucose, and 2-hour oral glucose tolerance (OGTT) tests (54)

Condition	Fasting blood glucose (mmol/L)	2-hour oral glucose tolerance OGTT (mmol/L)	HbA1c (%)	HbA1c mmol/mol	random blood glucose (mmol/L)
Health	< 5.5	< 7.8	< 6.6	< 42	–
Impaired fasting blood glucose	6.1 – 6.9	< 7.8	< 6.5	< 48	–
Impaired glucose tolerance	< 7.0	7.8 – 11.0	< 6.5	< 48	–
Type 2 diabetes	> 7.0	≥ 11.0	≥ 6.5	≥ 48	≥ 11.1

1.2. Therapeutic approaches for obesity and type 2 diabetes

To date most therapies for obesity have been relatively ineffective, associated with limited long-term maintenance of weight loss, or even weight regain. However, a weight loss of even ~ 5% is associated with a clinically meaningful reduction in cardiovascular risk factors, and a reduction in the risk for, or improvement of glycaemic control in, type 2 diabetes (55-57). Hence, reducing the risk of morbidity and mortality, rather than weight *per se*, is now considered the primary goal of obesity management (58). The main interventions include lifestyle interventions, including increased physical activity and dietary management, pharmaceutical treatment and surgical interventions. These are briefly reviewed in the following section.

1.2.1 Lifestyle interventions

Lifestyle modifications, including increased physical activity and dietary management, are used as the frontline approach for the treatment of both obesity and type 2 diabetes, given that they are safe and readily accessible (59). However, their efficacy is limited by the fact that achieving sustained changes in daily habits regarding food intake and physical activity is challenging and requires realistic goals.

1.2.1.1 Physical activity

Exercise has been shown to play an important role in reducing metabolic risk through preserving and/or enhancing lean muscle mass, which can enhance resting energy expenditure. Furthermore, exercise has beneficial effects on blood glucose control via directly mediating its anti-inflammatory effects or indirectly improving long-term metabolic markers, for example, physical fitness, body composition, lipid and glucose metabolism (60, 61). To achieve these benefits, 30 – 40 min/day of aerobic exercise, for at least 5 days/week has been recommended

by United Kingdom and United States guidelines (62), Based on the trials of exercise alone, exercise may be insufficient to induce significant weight loss, but in combination with dietary restriction, the probability of weight loss and its maintenance is increased (63).

1.2.1.2 Dietary management

The chief goal of dietary management is to reduce energy intake below the body's energy requirement in order to achieve weight loss. There are many different dietary strategies used that focus on manipulating the caloric or macronutrient composition of the diet. The following sections discuss weight loss diets, including very-low-calorie diets and low-calorie diets with varying macronutrient compositions.

1.2.1.2.1 Very-low-calorie diets

A very-low-calorie diet is a clinically supervised diet prescribed to achieve rapid initial weight loss by providing < 800 kcal/day or < 50% of resting energy expenditure. Very-low-calorie diets usually involve meal replacements, instead of common foods, to prevent nutrient deficiencies and ensure adequate intake of macronutrients, particularly protein, to prevent loss of lean mass. In the short-term, very-low-calorie diets are highly effective; however, there is disagreement in the literature concerning the relative efficacy of these diets vs. conventional low-calorie diets for long-term weight loss. For example, six randomised trials found that very-low-calorie diets, compared with low-calorie diets, induced significantly greater short-term weight loss (~ 7%), but similar long-term (over 4 years) loss (56).

Over the long-term, the probability of weight maintenance achieved by very-low-calorie diets is low (64, 65), therefore, in order to achieve maintenance of weight loss, very-low-calorie diets are usually followed with other interventions, such as a low-calorie diet; since low-calorie

diets are less extreme and easier to follow, less interruptive with normal daily activities, and less risky for health problems, e.g. loss of body muscle mass or bone mass (66).

1.2.1.2.2 Low-calorie diets with varying macronutrient compositions

Low-calorie diets aim to restrict energy intake by 500-1000 kcal/day, or achieve a total energy intake of ~ 1400-1800 kcal/day. This can be achieved by reducing overall food intake, or by decreasing intake of a specific macronutrient. Traditionally, low-fat diets were suggested due to the rationale that the high energy density of fat (~ 9 kcal/g) increases overall energy intake, compared with protein or carbohydrate, providing energy densities of ~ 4 kcal/g. Low-fat diets improve blood cholesterol and insulin sensitivity, but they often contain a high proportion of carbohydrate, which, in the long-term, limits weight loss to 2-4 kg (67, 68). Indeed, diets which restrict carbohydrate intake to < 20-30 g/day, and increase protein and monounsaturated fat intake, appear to be more successful for weight loss and achieve better health outcomes, including greater weight loss, improvements in cardiovascular risk factors and blood glucose control, than low-fat diets (67, 69, 70).

Low-carbohydrate diets (< 20% of energy from carbohydrate) have been shown to reduce body weight and improve glycaemic responses, including minimising fluctuations in blood glucose and the release of insulin. For example, in a study in 132 morbidly obese individuals, consumption of a low-carbohydrate diet (< 30 g carbohydrates/day), compared with a low-fat diet (energy deficit of 500 kcal/day and < 30% of energy from fat), resulted in greater weight loss over 6 months (~ 4%), as well as improvements in insulin sensitivity and triglyceride levels (~ 16%) (67).

High-protein diets (~ 20 – 30% of energy from protein) are probably the most effective diets in achieving weight loss, possibly because protein is highly satiating and decreases energy intake, while also increasing energy expenditure and maintaining muscle mass (71). For example, in healthy women in energy balance, a high-protein diet (% energy from protein/carbohydrates/fat: 30/40/30) for 4 days was associated with greater satiety over 24 hours, as well as increased thermogenesis and fat oxidation, than consumption of an adequate-protein diet (% energy from protein/carbohydrates/fat: 10/60/30) (65). Moreover, over a longer period of 21 days, a hypocaloric high-protein diet (% energy from protein/carbohydrates/fat: 45/35/20) resulted in comparable weight loss to a high-carbohydrate diet (% energy from protein/carbohydrates/fat: 20/60/20), however, the high-carbohydrate diet resulted in a greater loss of fat-free mass than the high-protein diet (~ 3 kg vs. ~ 1.4 kg) (72). In the long-term setting, preservation of fat-free mass is critical in facilitating the maintenance of weight. The potent energy intake-suppressant effects of a high-protein diet have been demonstrated in a study by Weigle *et al* (73). In this study, appetite, caloric intake, body weight and fat mass were measured in 19 healthy, normal-weight to obese, individuals, placed sequentially on the following diets: a weight-maintaining diet (% energy from protein/fat/carbohydrates: 15/35/50) for 2 weeks, an isocaloric, high-protein diet (% energy from protein/fat/carbohydrates: 30/20/50) for 2 weeks, and an *ad libitum* diet (% energy from protein/fat/carbohydrates: 30/20/50) for 12 weeks. During the isocaloric, high-protein diet, fullness increased, while hunger decreased, compared with the isocaloric adequate-protein diet. Interestingly, during the *ad libitum* phase, participants voluntarily decreased their daily energy intake by ~ 441 kcal, which resulted in a weight loss of ~ 4.9 kg over the 12-week period and fat mass decrease by ~ 3.7 kg. Hence, high-protein diets may result in weight loss, due to the potent effects of protein to suppress energy intake.

A large number of studies have evaluated the acute effects of protein ingestion and meals high in protein on food intake (74, 75). For example, cooked lunch meals with a high-protein (43% of energy, a meat casserole) content decreased energy intake at a subsequent *ad libitum* evening meal by 4 hours later 12%, compared with a high-carbohydrate (69% of energy, a vegetarian casserole) meal, in normal-weight individuals (75). Similarly, Brennan et al. (74) evaluated the acute effects of a pasta meal varying in its macronutrient content, including 1) high-fat (by adding full cream), 2) high-carbohydrate (by adding corn flour and raw sugar), 3) high-protein (by adding whey protein isolate) and 4) "adequate-protein", on appetite and energy intake at an *ad libitum* meal 180 min later in lean and obese participants. While fullness was greater after the high-fat, high-protein and "adequate-protein" lunches in the normal-weight group, only the high-protein and the high-fat lunches reduced energy intake at the following meal. In the obese group, hunger was reduced after the high-protein, and energy intake was less after the high- and adequate-protein meal, compared with the high-fat and high-carbohydrate meal. Therefore, it appears that, in the obese, the sensitivity to the satiating effects of protein, but not fat, is maintained, which supports the concept of a greater benefit of high-protein diets for weight loss.

It is important to appreciate the potential health risks of high-protein diets, given that there is some controversy about their long-term safety. A high protein consumption has been associated with increased risks of osteoporosis, particularly in diets containing > 2 g/kg/day of protein, and increased renal acid load, which may damage the kidneys over time (76, 77). Therefore, although protein appears to be effective in weight loss over the long-term, there is still controversy as to whether high-protein diets are safe for longer-term consumption, in particular in patients with type 2 diabetes.

1.2.2 Pharmaceutical treatment of obesity

Current obesity treatment guidelines recommend pharmaceutical strategies to be prescribed when weight loss efforts fail using lifestyle modifications (i.e. less than 5% total body weight loss), for overweight patients (BMI of 27-30 kg/m^2) with more than one comorbidity, or for patients with a BMI > 35 kg/m^2 (62). A number of drugs have been approved in the USA and Europe, including orlistat, liraglutide, phentermine, phentermine/topiramate extended release, bupropion sustained release and naltrexone sustained release (59). Among them, orlistat and phentermine are the most commonly used medications currently. Orlistat is a pancreatic lipase inhibitor, which decreases the absorption of ingested fat by up to 30%. It is considered to be the "safest" drug available and has also been approved for use in adolescents. However, as a result of its mechanism of action, it is associated with gastrointestinal (GI) side effects, including faecal incontinence and oily stools, apparent particularly after high fat intake, which limit its acceptance (78). Moreover, due to the prevention of the release of fatty acids in the GI lumen, it prevents activation of the GI mechanisms (discussed later) that are now recognised to play an important role in the regulation of energy intake and postprandial blood glucose (79, 80). Phentermine has a sympathicomimetic mechanism of action and includes primary pulmonary hypertension as a side effect (81). Naltrexone, an opioid antagonist, reduces food intake, and, in combination with bupropion SR, an antidepressant, reduces body weight. Because it increases blood pressure, the combination should only be prescribed to patients with controlled hypertension (59). Liraglutide, a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist with a 97% homology to GLP-1, is injected subcutaneously. Liraglutide stimulates insulin secretion and reduces food intake. It has a number of side effects, including nausea, diarrhoea, constipation and vomiting (82).

In conclusion, since these drugs often only achieve modest weight loss (< 5%), or are not effective in the longer-term (83-87), and are associated with a high cost and a number of adverse effects, as alluded to above, they have limited applicability and low patient compliance and, therefore, they can currently not be considered as an effective long-term solution for obesity. Thus, effective, long-term solutions are still required to achieve sufficient weight loss.

1.2.3 Surgical interventions

Bariatric surgery is the most effective intervention for obesity treatment (88), however, bariatric surgery is reserved for individuals with BMI ≥ 40 kg/m^2 or ≥ 35 kg/m^2 with at least one obesity-related comorbidity (88). The most common bariatric procedures are banded gastroplasty, gastric banding and Roux-en-Y gastric bypass. Not only are these surgical interventions associated with substantial weight loss (reductions in BMI by ~ 11 – 12 kg/m^2, after 3 years (59), particularly Roux-en-Y gastric bypass also results in dramatic improvements in glycaemic control in type 2 diabetes patients, even before major weight loss occurs (89, 90). Gastric bypass surgery divides the stomach to create a small gastric pouch (< 30 mL volume) (88), to which the distal small intestine is connected, bypassing the duodenum and upper jejunum. This procedure results in more rapid delivery of nutrients into the distal small intestine, leading to an enhanced postprandial increase in GI hormones, including peptide YY (PYY) and GLP-1, and more potent suppression of ghrelin, an orexigenic hormone (91). It is now increasingly recognised that the potent release of GI hormones after bariatric surgery is a key factor in bringing about improvements in postprandial blood glucose, as well as weight loss, providing evidence for a key role of the GI tract and GI hormones in the regulation of energy intake, body weight and glycaemic control.

Taken together, the major challenges in developing effective therapeutic approaches to obesity and related comorbidities, particularly type 2 diabetes, relate to the inability of current strategies to elicit increased fullness and reduced hunger, sustain energy expenditure, maintain weight loss and improve glycaemic control. In this regard, investigating the effects of specific nutrients on those upper GI functions, including gut hormone secretion and gastric emptying, that have been shown to be important for the reduction of energy intake and blood glucose control, might be critical to the development of novel nutrient-based approaches for obesity and type 2 diabetes treatment.

1.3 Anatomy and physiology of the GI tract

In order to appreciate the critical role of the upper GI tract in the regulation of postprandial blood glucose concentrations and energy intake, which will be discussed later, it is important to have an understanding of the anatomy and function of the upper GI tract, the motility patterns that contribute to the trituration and transport of ingested food, and the release of key GI hormones. These will be covered in the following sections.

The GI tract is a tubular organ, whose primary roles are to process and digest ingested food, extract and absorb nutrients, and excrete non-digestible material. The GI tract includes the oral cavity, the oesophagus, stomach and the small and large intestines, which each have distinct functions (92). The responses of the upper GI tract to food ingestion can, in a simplified way, be described in three phases: cephalic, gastric and intestinal (92).

The "cephalic phase" is activated by the smell, sight, taste and texture of food, before and during food ingestion. Gustatory and other visceral afferent inputs, resulting from the anticipation, sight, smell or taste of food, project to the nucleus tractus solitarius (NTS), the principal visceral sensory nucleus in the brainstem, from where the signals are transmitted, through the dorsal motor nucleus, via vagal efferents to the GI tract. These mechanisms initiate the secretion of saliva and digestive enzymes (93), gastric acid (94), modulations of the motor activity in the antropyloroduodenal region (95), and the secretion of hormones, including insulin and glucagon from the pancreas (96, 97) and cholecystokinin (CCK) and other hormones from the small intestine (98), preparing the GI tract for the arrival of food. The "gastric phase" is triggered by mechanical distension due to the arrival of ingested food in the stomach, activating vagovagal reflexes, and stimulating the secretion of hydrochloric acid in order to decrease the stomach pH. The "intestinal phase" is initiated once partially digested

food is transferred into the small intestine, and triggers the initiation of feedback signals to the stomach, either by local neural reflexes, secretion of hormones, such as gastrin or CCK, or via the vagus nerve, to first enhance and, as the duodenum fills with acidic content from the stomach, to inhibit, gastric secretion, and to stimulate pyloric pressures to slow gastric emptying. Thus, neuronal and humoral signals play key roles in mediating these three phases that regulate the responses of the upper GI tract to the ingested food.

1.3.1 Anatomy of the upper GI tract

The wall of the GI tract is made up of four layers of specialised tissue, which from the lumen outwards, include the mucosa, submucosa, muscular layer and serosa/adventitia. The structure of these layers varies in different regions of the digestive system, depending on their function. The submucosa consists of a dense network of neurones, named “the enteric nervous system” (ENS), which can function more or less independently of the central nervous system (CNS) and controls or modulates motility, exocrine and endocrine secretions, microcirculation and immune and inflammatory processes (99).

The GI tract itself is divided into upper and lower regions. The upper GI tract comprises of the oral cavity, pharynx, oesophagus, stomach, pylorus, duodenum, jejunum and ileum (**Figure 1.1**). Each of these regions play a distinct role in the ingestive, digestive and absorptive processes during food ingestion.

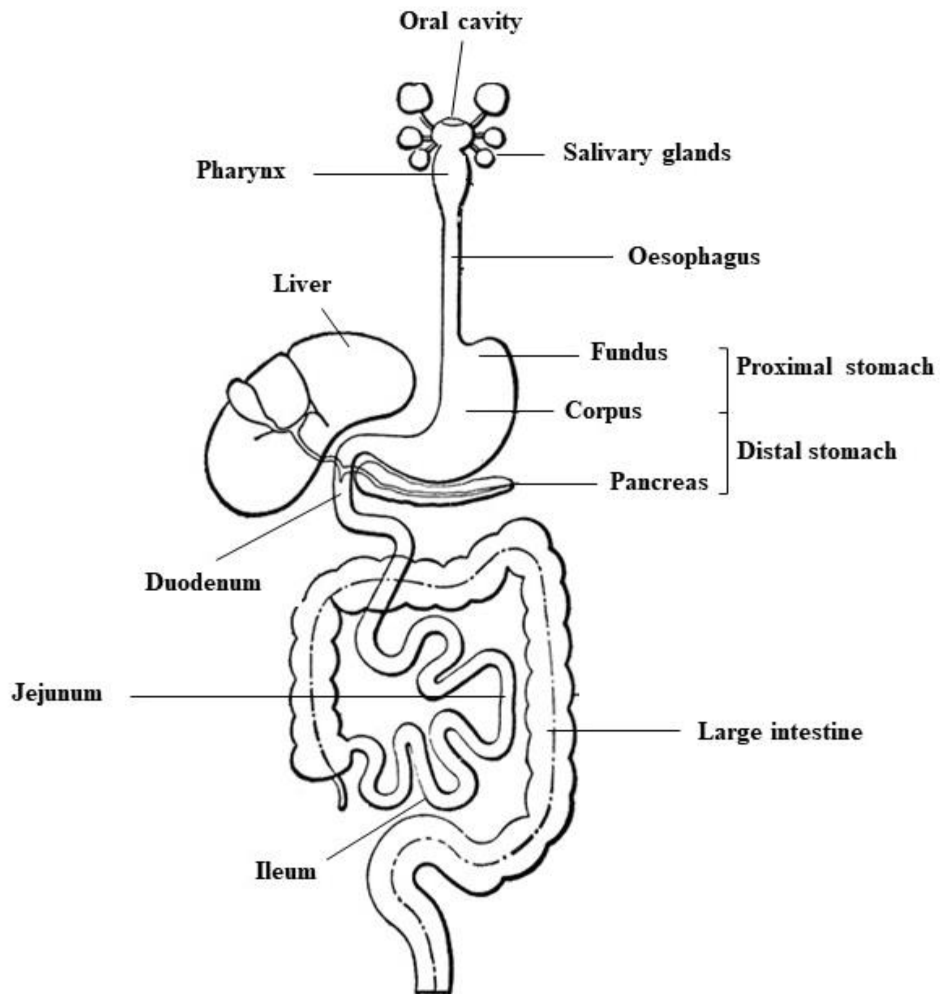

Figure 1.1: Basic anatomy of the human gastrointestinal tract.

1.3.1.1 Oral cavity

In the oral cavity, food is ground by mastication, and with the aid of enzymes secreted from the salivary glands, the digestion process commences. Food components interact with taste buds that detect sweet, salty, sour, bitter, and umami tastants, and fatty acids. These interactions generate neuronal signals that provide information about the composition of the food to the CNS. This aids the identification of nutrient components and the avoidance of toxin consumption (100).

Taste detection is initiated on the surface of the tongue and soft palate, where taste buds are distributed. Taste buds are onion-shaped structures, each consisting of 50 to 150 cells, including taste receptor, support and precursor cells (101). Taste cells have a bipolar structure, consisting of an apical tip, which has a projection called a taste pore, through which tastants make contact with the taste cell receptors, and the basolateral aspect, which synthesises neurotransmitters to trigger sensory nerve fibres to signal to higher brain centres where the taste is perceived (102). Among the five distinguishable tastes (mentioned above), sour and salty tastes are produced by ion movement through membrane channels and are due to the presence of hydrogen and sodium ions, respectively. Sweet, bitter and umami tastes are detected by members of the G protein-coupled receptors (GPCR family), including taste 2 (TAS2R) and taste 1 (TAS1R) receptor families (103). Heterodimers of TAS1R subtypes, including TAS1R2 – TAS1R3 and TAS1R1 – TAS1R3, detect sweet taste. Bitter tastants are currently known to be detected by 25 different TAS2Rs in humans, and more are known in animals, e.g. mice (100). In recent years, receptors for fatty acids have been identified as separate entities. Fatty acids are detected in the oral cavity with specific molecules and pathways which are less well defined yet, but include GPCRs, e.g. GPCR120 and GPCR40 (104), and the multi-functional protein, CD36 (105). Human taste perception is also influenced

by other factors, including smell (olfactory), texture (trigeminal), vision and memory (106). However, detailed discussion of these factors is beyond the scope of this thesis.

As food is swallowed, a number of reflexes, in the oral, pharyngeal and oesophageal passages, are activated, all of which facilitate the transport of masticated food into the stomach. In response to the bolus, the oesophagus, including the lower oesophageal sphincter, relaxes. Fluids move into the stomach by gravity, while coordinated contractions of the muscle layers in the oesophageal wall aid the transport of solids. Between swallows, the lower oesophageal sphincter closes, to protect the oesophagus from exposure to the acidic stomach content.

1.3.1.2 Stomach

The stomach, which is situated between the oesophagus and the small intestine, is a J-shaped compartment, divided anatomically into fundus, corpus and antrum. Functionally, it is considered to have two distinct regions: i) the proximal compartment, which includes the fundus and the proximal corpus, and predominantly acts as a temporary store for ingested food, and ii) the distal compartment, which comprises the distal corpus and antrum, with the key task of grinding food and mixing it with gastric secretions to produce small particles or a “paste”, the chyme (107).

The stomach wall consists of three muscle layers, including the inner oblique muscle, the circular and the outer longitudinal muscle layer, which contract in a highly coordinated, rhythmic fashion, independent of input from the CNS, but under the control of the ENS, i.e. autonomously (108). Contractions of the gastric wall are responsible for the physical break-down of food into smaller particles within the stomach, prior to evacuation through the pylorus into the small intestine.

1.3.1.3 Pylorus

The pylorus is an approximately 2-cm long sphincter located at the furthest part of the stomach connecting the antrum to the duodenum. The mechanical function of the pylorus, including tonic and phasic pressures, is determined by the underlying electrical activity of the smooth muscle myocytes (109) and regulated by the feedback arising from the GI lumen, including through gut hormones, such as CCK, in response to meal stimuli and mediated via the vagus nerve. The primary function of the pylorus is to regulate gastric emptying, by acting as a "sieve", regulating the outflow of gastric contents into the small intestine (110). The pylorus also protects the small intestine from exposure to the acidic stomach content (particularly in the fasting state), and the stomach from digestive enzymes and bile acids from the small intestine.

1.3.1.4 Small intestine

The small intestine is a muscular tube approximately 6 meters in length and divided into three regions: i) proximally, the duodenum (~ 25 cm long), ii) the jejunum (~ 2 m long), and iii) distally, the ileum (~ 3 m long). The small intestine has a distinct structure consisting of folds, villi and microvilli, which serve to enhance its surface area to more than 100 m^2. The large surface is important to optimise nutrient absorption through the mucosa, the main task of the small intestine. The small intestinal wall consists of an inner, circular, and an outer, longitudinal, muscle layer whose coordinated contractions, controlled by local neural reflexes, ensure mixing of the chyme with digestive secretions, including bile, pancreatic juice and intestinal digestive enzymes. A range of enzymes, including proteases, amylases and lipases, facilitate the digestion of macronutrients, breaking them into their individual building blocks, such as di- and tripeptides or amino acids, monosaccharides or fatty acids (111). This action facilitates the interaction of those nutrients with their specific transporters located in the

intestinal mucosa, for subsequent absorption from the intestinal lumen into the enterocytes. Moreover, nutrients can also activate the “taste” receptors, akin to those in the oral cavity, located on enteroendocrine cells along the length of the GI tract, but with varying densities (100, 112). Activation of GI taste receptors stimulates the release of a wide range of gut hormones, which will be discussed later in this chapter (section 1.3.3).

1.3.2 Motor function of the upper GI tract

The motor activity of the upper GI tract is characterised by distinct motor patterns, including an interdigestive motor pattern during fasting, and a postprandial motor pattern in response to food ingestion, which are described in the following sections.

1.3.2.1 Fasting motility pattern

In the fasted state, when the upper GI tract is essentially empty of food, the GI tract exhibits a distinct cyclic pattern of motility, termed “interdigestive” motility, or the migrating motor complex (MMC). Over a period of ~ 1.5 – 2 h (although the duration can vary considerably between cycles and also between individuals), the MMC migrates from the antrum to the terminal ileum ensuring that the upper GI tract is cleared of any undigested food, or particles ≥ 1 – 2 mm in size, digestive juices, indigestible "debris" or bacteria. The MMC comprises of four phases: i) phase I, a period of motor quiescence (approximately 40 – 60 min in duration); ii) phase II, a period of irregular, intermittent phasic contractions of low amplitude, whose frequency increases progressively (~ 20 – 40 min); iii) phase III, the most active phase characterised by regular, high-amplitude and rhythmic contractions, at the maximum frequency of the intrinsic pacemaker (in the antrum, 5 – 10 min, with ~ 3 contractions per minute; in the duodenum, either following the antral phase III or occurring simultaneously, with ~ 9 – 12 contractions per minute); and iv) phase IV, a period of declining activity that transitions into the next phase I (113).

1.3.2.2 Postprandial motility pattern and gastric emptying

Ingestion of a meal initiates the conversion of the fasting motor activity into a “fed”, or “postprandial”, motility pattern. Mechanical distension of the stomach, in the absence of nutrients, interrupts the MMC in the stomach and upper small intestine, while the presence of nutrients interrupts the MMC in the entire small intestine (114), indicating that nutrients, and small intestinal feedback, are required for full conversion from a fasted to a fed pattern.

The postprandial motility pattern is characterised by highly coordinated contractile events across the upper GI region. The proximal stomach initially displays a “receptive relaxation”, followed by a period of “adaptive relaxation”, to accommodate the meal without a substantial increase in intragastric pressure (115, 116). The volume of the food ingested is monitored by receptors for tension and stretch that transmit signals via vagal and splanchnic nerves to the brain to elicit a sensation of fullness (117).

The coordinated patterns of motor activity in the stomach, pylorus and duodenum carefully regulate the outflow of chyme from the stomach and the rate of nutrient delivery to the small intestine and, thus, underlie the process of gastric emptying (118). Before gastric contents can be transported into the small intestine, they are “churned” by contractions of the antral wall to facilitate the efficient digestion and absorption that occurs in the small intestine (119). For this purpose, peristaltic contractions of the antrum move towards the pylorus, and, when the pylorus is closed, reduce particle size and mix the food with gastric secretions to generate chyme. When the pylorus is open, chyme is transferred to the small intestine, and the arrival of nutrients generates feedback from the small intestine, which reinforces antral and pyloric motility, slowing gastric emptying to ensure that chyme arrives in the small intestine at a rate that allows for the optimal digestion and absorption of nutrients. Regular tonic and phasic pyloric

contractions act as a brake to regulate flow of chyme from the stomach to the duodenum. Thus, gastric emptying only occurs when the pylorus and duodenum are relaxed as a peristaltic antral wave approaches this region. Peristaltic waves in the duodenum transport the chyme, emptied from the stomach, distally (109).

1.3.2.3 Factors influencing gastric emptying

In health, rates of gastric emptying can vary widely between 1 – 4 kcal/min, influenced by a number of factors, including the physical characteristics (including solid, semi-solid, liquid, viscosity, volume) and chemical composition (e.g. nutrient and energy content, pH, osmolality) of a meal (92). Nutrient-containing liquids or liquefied solids empty from the stomach in an approximately linear fashion (120, 121), and non-nutritive liquid meals in a mono-exponential fashion driven partly by gravity (120, 122).

Emptying of solids differs from that of nutrient-containing liquids. Gastric emptying of solids commences after an initial lag phase, which is approximately 10 – 30 min in duration, reflecting the time taken for the initial grinding process to commence to triturate solids into particles of < 1 – 2 mm in size, which is a major rate-limiting step for gastric emptying (123, 124), as well as the gradual redistribution of food from the proximal to the distal stomach (125). Gastric emptying of solids then follows a linear pattern (122). When a mixed meal containing liquids and solids is ingested, the solids remain in the stomach until approximately 80% of the liquid has emptied (126). Non-digestible solids > 2 mm in size remain in the stomach and are emptied during the interdigestive phase (126).

As nutrient-containing liquids, or liquefied chyme, empty from the stomach into the small intestine, meal components interact with mucosal receptors that detect the pH and osmolarity

of the meal as well as nutrients, including glucose, amino acids and fatty acids. The subsequently released GI hormones activate their receptors on vagal afferents to provide feedback to the CNS and the stomach, reinforcing relaxation of the stomach and stimulating pyloric pressures, generating feedback slowing of gastric emptying.

1.3.3 GI hormones and their functions

As discussed, the interaction of nutrients with specific receptors on enteroendocrine cells, located in the GI mucosa, is associated with the release of a number of GI hormones, including CCK, PYY, the incretin hormones (including GLP-1 and glucose-dependent insulinotropic peptide (GIP)), which modulate the release of insulin and glucagon from the pancreas, and the suppression of ghrelin from the gastric mucosa. These hormones regulate a range of important functions, including GI motility, postprandial blood glucose and energy intake. The following section discusses these hormones, their release and physiological functions.

1.3.3.1 Ghrelin

Ghrelin is the only known orexigenic GI hormone. It is released predominantly by X/A-like cells located in the stomach mucosa and to a lesser extent in the duodenum and endocrine pancreas (127). Ghrelin consists of 28 amino acids with a characteristic octanoyl-group, which is necessary for ghrelin binding to the growth hormone secretagogue receptor 1a (GHS-R1a) (128), which is expressed in a number of tissues, including the brain and stomach (129, 130). When ghrelin is released, it is either transported via the blood stream to the hypothalamus, via the blood-brain barrier, where it binds to ghrelin receptors, or acts on GHS-R1s receptors on vagal afferents, to stimulate neuropeptide Y (NPY) (113). Plasma ghrelin concentrations are elevated during fasting (131), peak before meal consumption and decline in response to feeding, in a calorie-dependent manner, suggesting that ghrelin is important in meal initiation

and stimulation of food intake (132). For example, in humans intravenous (i.v.) infusion of 6 pmol/kg/min ghrelin has been shown to stimulate appetite and food intake from a buffet-meal by ~ 2-fold (133). Fasting concentrations of ghrelin are inversely correlated with nutritional status; they are higher in anorexia nervosa patients and lower in obese individuals, compared with normal-weight individuals. In obese individuals, ghrelin is not further suppressed by meal consumption (134), perhaps because fasting levels are already low. Obese people also appear to be more sensitive to the orexigenic effects of ghrelin, which may contribute to the aetiology of obesity. For example, i.v. infusion of a low dose (~ 1 pmol/kg/min) of ghrelin increased energy intake in obese, but was ineffective in normal-weight, volunteers, despite increasing plasma ghrelin concentrations comparably in both groups (135).

Ghrelin has also been reported to affect GI motility, leading to an acceleration of gastric emptying (136, 137). For example, i.v. infusion of ghrelin at the dose of 10 pmol/kg/min in healthy individuals, or 5 pmol/kg/min in diabetic patients with symptoms indicative of gastroparesis, accelerated gastric emptying of a solid meal (containing ~ 320 kcal) (136, 138). While the physiological relevance of this finding is unclear, given that meal ingestion suppresses ghrelin, there may be a therapeutic role for ghrelin to improve gastroparesis.

Ghrelin has been reported to have adverse effects on blood glucose control. For example, in ghrelin-receptor knock-out mice, ghrelin suppressed insulin release and improved glucose tolerance dose-dependently (139, 140). Moreover, in both men and women, i.v. administration of ghrelin, at the doses of 5, 15 or 25 pmol/kg/min, decreased insulin concentrations, associated with an increase in blood glucose, after an i.v. bolus of glucose (141). Therefore, it appears that ghrelin, at high plasma concentrations, as a result of the i.v. infusion, has adverse effects on

blood glucose control. However, the glycaemic effects of ghrelin, under physiological conditions, remain unclear.

1.3.3.2 CCK

CCK is synthesised by I-cells, located predominantly in the duodenum and jejunum (142). Many bioactive forms of CCK have been identified in human plasma. They are all generated by processing of pro-CCK, a 115-amino acid precursor, and are classified by their number of amino acids. Bioactive forms include CCK-5, -8, -22, -33, -39, -58 and -83, with the major bioactive forms in humans being CCK-8 in the brain, and CCK-8, -22, -33 and -58 in the intestine (143, 144). CCK-8 has also been identified to act as a neurotransmitter in the enteric nervous system. Two main receptors for CCK have been identified, including CCK-A (formerly CCK-1), expressed predominantly in the GI tract, hindbrain and hypothalamus, and CCK-B (or CCK-2), located in the CNS and stomach.

The first identified functions of CCK were stimulation of gallbladder contraction (98) and pancreatic secretion, hence its name cholecystokinin, or pancreozymin (104). However, other important functions have been discovered subsequently, including regulation of GI motility, associated with the slowing gastric emptying, and suppression of food intake (145, 146) 159. For example, in healthy volunteers, i.v. infusion of CCK at doses of 12 and 24 pmol/kg/h, which both reproduced average postprandial plasma levels, caused a significant slowing of the gastric emptying of water, and the effect of the higher dose was similar to that seen with ingestion of a mixed-liquid meal (consisting of 40% fat, 20% protein, and 40% carbohydrate) (147). Moreover, in healthy lean men, i.v. infusion of CCK-8, at a dose of 2.25mcg, slowed gastric emptying of a 500 g liquid meal (providing 0.35 kcal/g), when compared with saline (145). In healthy volunteers, i.v. infusion of exogenous CCK-8, at doses of 0.33, 0.66 or 2.0

ng/kg/min, suppressed antral and duodenal motility, and stimulated isolated pyloric pressures, in a dose-dependent manner (148). Also, in healthy females, i.v. infusion of CCK-33, at a dose of 0.72 nmol/kg/min, resulting in plasma levels comparable to those after a meal (~ 5 pmol/L), decreased basal gastric tone and increased gastric compliance (149).

A number of studies, in both humans and rodents, have shown that CCK suppresses energy intake by reducing meal size, without affecting the inter-meal interval (150-152). In healthy humans, this effect has been reported following i.v. doses of CCK that resulted in plasma concentrations comparable to physiological postprandial plasma CCK concentrations (~ 7 pmol/L) (153), and in supraphysiological concentrations (~ 12 pmol/L) (154). Although high doses of CCK may induce nausea, the effect on energy intake occurs apparently independently of nausea (148, 155).

The effects of CCK on energy intake and GI motility have been shown to be mediated via activation of CCK-A receptors, suggesting an important role for endogenous CCK in the regulation of gastric emptying. For example, i.v. infusion of the CCK-A receptor antagonist, loxiglumide, inhibited the slowing of gastric emptying of mixed-nutrient or glucose-containing drinks, and the reduction in intragastric pressure in response to a lipid-containing drink (156-158). However, it appears that endogenous CCK may only have a comparatively weak food intake-suppressant effect, since in healthy men blocking endogenous CCK, by using loxiglumide, only resulted in a modest increase in food intake from a meal (159).

In has also been reported that CCK can lower postprandial blood glucose (160, 161), possibly by slowing of gastric emptying. For example, in healthy volunteers, i.v. infusion of CCK-8 (24 pmol/kg), along with oral ingestions of glucose (60 gr), slowed gastric emptying of a standard

mixed liquid meal (containing 40% carbohydrate, 40% fat, and 20% protein), and increased insulin and decreased peak plasma glucose levels, while, when glucose (36 g) was infused directly into duodenum, there was no change in plasma glucose and insulin levels, suggesting that the glucose-lowering effect of CCK is mediated via slowing of gastric emptying (162). In support, i.v. infusion of loxiglumide, markedly accelerated the gastric emptying of both mixed and pure glucose meal and induced a rapid rise in insulin during the first hour after meal, followed by similar insulin levels in the second hour (157).

1.3.3.3 PYY

PYY is a 36-amino acids peptide, synthesised by enteroendocrine L-cells located predominantly in the distal small intestine, colon and rectum (142). PYY is secreted as PYY_{1-36} and then rapidly converted to PYY_{3-36} by enzymatic action of dipeptidyl-peptidase-IV (DPP-IV) (163). Both PYY peptides bind to NPY receptors, including Y_1, Y_2, Y_3, Y_4 and Y_5, with PYY_{3-36} preferentially binding to the Y_2 receptor, located in in the hypothalamic arcuate nucleus, to exert its anorectic effects (109). PYY has a short half-life of ~ 10 min.

After food ingestion, plasma concentrations of PYY are elevated within ~ 15 min, well before nutrients have reached distal small intestinal L-cells, plateau within ~ 90 min and remain elevated for up to 6 hrs (164). The magnitude of PYY release is related to the amount of calories ingested (165). Among macronutrients, both fat and protein appear to be potent triggers for PYY secretion in humans (166). The release of PYY in response to lipid or fatty acids depends, at least in part, on CCK (166, 167). This is supported by two studies, one in dogs in which plasma PYY increased after perfusion of oleate into the proximal half of the small intestine (166), and the other study in healthy males, in which intraduodenal infusion of orlistat, dissolved in olive oil, at the rate of 0.5 mL/min (load 41 g), reversed the effect of the oil on the

stimulation of PYY, due to the fact that lipid digestion was abolished, hence no free fatty acids were available to stimulate CCK secretion (167). In these studies (166, 167), i.v. administration of the CCK-A antagonists, devazepide or dexloxiglumide, blocked PYY secretion, supporting the role of CCK in PYY secretion. Both fasting and postprandial concentrations of PYY are lower in obese than in normal-weight individuals, however, i.v. infusion of PYY remains effective in obese people, e.g. it still reduces energy intake or suppresses ghrelin, suggesting that obese people are not resistant to the anorectic effects of PYY (168). Following weight loss induced by both gastric surgery (169, 170) or a 30% energy-restricted diet (171), PYY concentrations increased, and the number of PYY-secreting cells improved after surgery, suggesting that weight status influences the nutrient sensing capacity of the small intestine in obese.

PYY also affects GI motility, associated with slowing of gastric emptying (168, 169, 172-176). For example, in healthy volunteers, i.v. infusion of PYY at the doses of 0.18 or 0.51 pmol/kg/min, slowed both gastric emptying and the mouth-to-caecum transit of a lactulose-containing liquid meal (176). PYY was initially identified as a mediator of the so-called “ileal brake mechanism”, an inhibitory effect on upper GI motor activity triggered by intraluminal fat in the terminal ileum (177, 178). For example, in healthy volunteers, infusion of fat emulsions (containing either glycerol, oleic acid, triolein or medium chain triglycerides) into the ileum raised plasma concentrations of PYY, which correlated with the inhibition of jejunal pressure wave activity (179).

Some (180-182), but not all (183, 184), studies have reported an effect of PYY to suppress energy intake. It appears that this effect may only occur at higher doses associated with nausea, hence, it is not clear whether it is a true effect of PYY or secondary to nausea (185). For

example, in humans, i.v. infusion of PYY at the dose of 0.8 pmol/kg/min, increasing plasma concentrations from fasting levels of ~ 8 pmol/L to ~ 44 pmol/L, decreased energy intake by 33% over 24 hours, but had no effect on nausea (186). In contrast, another study in humans (185), evaluating a wide range of doses of i.v. PYY (0.2 – 0.8 pmol/kg/min), reported a reduction in food intake only at the highest doses of 0.4 and 0.8 pmol/kg/min, which also caused nausea. Therefore, it is possible that the observed reduction in energy intake by PYY is due to nausea. Unfortunately, there are currently no receptor antagonists available for use in humans to test the physiological role of endogenous PYY in food intake regulation.

Limited information is available about the effects of PYY on blood glucose control. For example, in mice, PYY_{1-36} did not affect fasting glucose or plasma glucose after the injection of glucose, but inhibited glucose-stimulated insulin (187), via a direct effect on islet cells (188), suggesting a paracrine role of PYY_{1-36} to inhibit insulin secretion. In another study in mice, intraperitoneal injection of either PYY_{3-36} or a Y_2-receptor agonist did not affect fasting blood glucose, but attenuated the blood glucose response to glucose (189). This effect was mediated via Y_2-receptors in peripheral, but not central, tissues and potentially by activation of GLP-1, (189). This indicates that locally, PYY_{1-36} may inhibit insulin secretion via a direct effect on the pancreas, while circulating PYY_{3-36} improves blood glucose by enhancing insulin secretion via increased GLP-1 release. In a study in humans, infusion of PYY at a low (1 pmol/kg/min), or a high (5 pmol/kg/min), dose did not affect fasting serum insulin or plasma glucose concentrations, or insulin secretion and glucose elimination rate in response to a bolus glucose injection (0.5 g/kg) (190). Thus, there may either be species differences between humans and rodents, or the human studies did not achieve the required concentrations at the β-cell to modulate insulin release.

1.3.3.4 Incretin hormones

The observation that oral ingestion of glucose stimulates insulin secretion more than i.v. infusion of the same amount of glucose is referred to as the "incretin effect" and is attributed to the action of incretin hormones (103, 104). Hence, after meal ingestion, the GI tract plays an important role in reducing postprandial blood glucose by increasing insulin, stimulated by incretin hormones (191). The only currently identified incretin hormones are GLP-1 and GIP. After food ingestion, both GLP-1 and GIP concentrations in plasma increase within 5 – 15 min and return to baseline after ~ 3 – 4 hours (192). DPP-IV rapidly inactivates GLP-1 and GIP within 1 – 4 min (193, 194), resulting in only 10 – 15% of active GLP-1 or GIP reaching the circulation (195).

1.3.3.4.1 GLP-1

GLP-1 is a 33-amino acids peptide released from L-cells, located in the mucosa of the small intestine and colon (142). GLP-1 is post-translationally cleaved from pro-glucagon to two bioactive forms, GLP-1_{7-36} and GLP-1_{7-37}, which are converted into their inactive forms, GLP-1_{9-36} and GLP-1_{9-37}, respectively, by DPP-IV. GLP-1 is released in response to the presence of nutrients, including carbohydrate and lipids, and to a lesser extent, protein, in the small intestine (196). GLP-1 receptors are expressed in different organs, including pancreatic islets, heart, subpopulations of immune cells, kidney and brain as well as on abdominal vagal afferents (195).

GLP-1 has well-established effects on GI motility (103, 197). For example, administration of exogenous GLP-1, at dose ranging from 0.3 – 1.2 pmol/kg/min, relaxes the fundus, which increases gastric compliance and enables the accommodation of larger volumes without extensive increase in pressure (198), suppresses antral and duodenal contraction, and stimulates

pyloric tone and isolated pyloric pressure waves (199). The involvement of endogenous GLP-1 is demonstrated in a study (200) in which i.v. infusion of the GLP-1 receptor antagonist, exendin(9-39)amide, prevented the suppression of antral and contraction and reduced, or completely inhibited, stimulation of pyloric pressures, during a 120-min intraduodenal glucose infusion at the rates of 1 or 2.5 kcal/min, suggesting that endogenous GLP-1 mediates the effect of glucose on antropyloroduodenal motility. The effect of GLP-1 to slow gastric emptying has been reported in different studies in healthy, normal-weight (201), obese (202) and type 2 diabetic (203, 204) people. For example, in normal-weight and obese volunteers, i.v. infusion of GLP-1, at doses ranging from 0.3 – 0.9 pmol/kg/min, slowed gastric emptying of solid/liquid meals (202, 205).

As discussed, GLP-1, as an incretin hormone, is critical to achieve postprandial blood glucose regulation within the physiological range, by stimulating insulin and inhibiting glucagon secretion, in a glucose-dependent manner (201). This was shown in a study, in healthy individuals, in which i.v. infusion of exendin(9-39)amide (500 pmol/kg/min) increased postprandial plasma glucose, reduced the increase in insulin, and blocked the suppression of glucagon, in response to a 150 g oral glucose tolerance test (206). Therefore, GLP-1 analogues have been developed to improve blood glucose in type 2 diabetes (207).

Of note, a number of studies have shown a reduction in postprandial insulin in response to GLP-1, probably because the potent effect of GLP-1 to slow gastric emptying, as explained above, may outweigh its insulinotropic effects (201, 208, 209). For example, in healthy volunteers, i.v. infusion of GLP-$1_{7\text{-}36}$, at doses ranging from 0.4 – 1.2 pmol/kg/min, slowed gastric emptying of, a liquid meal (327 kcal, 50 g sucrose) as well as the insulin response to that meal, yet reduced blood glucose substantially (209).

The available data about the effect of GLP-1 on energy intake are somewhat inconsistent, with some studies reporting a suppressant effect (210-212), while other studies found no effect (197, 202). For example, in healthy volunteers, i.v. infusion of ~ 0.8 pmol/kg/min GLP-1 increased fullness after a standardised breakfast and decreased energy intake at an *ad libitum* lunch 4.5 hours later (212). In contrast, in healthy males, i.v. infusion of 0.75 pmol/kg/min GLP-$1_{7\text{-}36}$, at the beginning of a test meal (cornflakes; 15 g sucrose and 150 mL milk), did not affect energy intake from the meal (202). Moreover, i.v. infusion of exendin(9-39)amide for 2 hr evoked no change in food intake from *ad libitum* meal 2 hr latter, in response to nutrient induced GLP-1 secretion (either oral glucose preload plus a 60-min intraduodenal infusion of glucose or oral mixed-liquid meal preload plus a 60-min intraduodenal infusion of oleic acid), suggesting that endogenous GLP-1 may be a relatively weak satiation signal (213). Therefore, the role of GLP-1 on energy intake is still uncertain and warrants further investigation.

1.3.3.4.2 GIP

GIP is a 42-amino acids peptide, secreted from K-cells, located predominantly in the duodenum and proximal jejunum (214). GIP was originally named "gastric inhibitory peptide" due to its role to inhibit gastric acid secretion (215). Later, its name was changed to "glucose-dependent insulinotropic polypeptide", to better reflect the important physiological role of stimulating insulin secretion in response to hyperglycaemia (216). Other roles of GIP include gluconeogenesis in the liver, increasing uptake of glucose into muscles and effects on cell proliferation, specifically differentiation of pancreatic β-cells (217). Limited information is available on the effect of GIP on GI motility. GIP does not appear to affect gastric emptying (218). GIP is released in response to carbohydrate and, to a lesser extent, fat (219). Furthermore, when fat is overconsumed, GIP activates lipoprotein lipase and induces body fat storage and, consequently, insulin resistance (220).

1.3.3.5 Insulin

Insulin is a 51-amino acids peptide released by the β-cells of the islets of Langerhans in the pancreas. Insulin is a derivative of proinsulin, which is cleaved into insulin and C-peptide, a 31-amino acid peptide. C peptide is released in equimolar amounts to insulin by pancreatic β-cells in the islets of Langerhans, but is not metabolised by the liver or other organs; therefore, it is often used as a marker for insulin secretion. Insulin secretion occurs, mainly, in response to carbohydrate ingestion, and to a lesser extent lipid (196). Protein and specific AAs, including arginine and leucine, can induce insulin secretion by stimulating the β-cell (221). While the response to carbohydrates is often impaired in type 2 diabetes, the response to protein and mixtures of amino acids is maintained (222).

The major function of insulin in the body is to ensure glucose uptake into almost all tissues, including skeletal muscle and adipose tissue, by binding to the insulin receptor, which belongs to a class of tyrosine kinase receptors. Beside the primary role of insulin on glucose homeostasis, it also has an effect on GI motility, including inhibition of phase III of fasting motility and fasting antral motor activity (223). In conscious dogs, i.v. infusion of insulin at physiological doses of 12 – 24 mU/kg/h failed to affect intestinal myoelectric activity, whereas i.v. infusion of higher doses of 48 – 96 mU/kg/h, increasing circulating insulin concentration above the levels occurring after a mixed meal, blocked the MMC mainly in the upper small intestine, and induced a postprandial motility pattern (224), suggesting that large concentrations are required to convert the fasting to a postprandial motility pattern. Based on these findings, it appears that insulin may not play a critical role in the regulation of GI motility. However, the effects of insulin on GI motility are difficult to assess, given their close relationship with blood glucose. In healthy individuals, under euglycaemic conditions,

hyperinsulinaemia (~ 46 mU/L) abolished phase III in the antrum, and slowed gastric emptying of a standardised omelette meal (225, 226).

The contribution of insulin to the regulation of energy intake also remains unclear, and the available data are inconsistent. I.v. infusion of low (0.8 mU/kg/min), and high (1.6 mU/kg/min), doses of insulin had no effect on energy intake, when euglycaemia was maintained over 120 min, but when the higher dose of insulin (1.6 mU/kg/min) was combined with i.v. glucose infusion, energy intake was reduced (227). However, in lean, but not obese, individuals, fasting plasma insulin, the insulin response over 3 hours after a fixed meal, and insulin concentrations immediately before an *ad libitum* meal were all inversely correlated with energy intake at that meal (228). Therefore, it appears that the presence of glucose is required for insulin to elicit a satiating effect in lean individuals, while these mechanisms may be interrupted in obese individuals.

1.3.3.6 Glucagon

Glucagon is a 29-amino acids peptide, produced by α-cells of the islets of Langerhans in the pancreas. Glucagon is a derivative of pro-glucagon, processed by tissue-specific cleaving. Glucagon receptors are located in the liver and extrahepatic tissues, including brain, heart, kidney, the GI tract and adipose tissue. During hypoglycaemia and long-term fasting, glucagon plays an important role in maintaining blood glucose levels by stimulating hepatic glycogenolysis and gluconeogenesis, synthesising glucose from other nutrients, including AAs and lactate (229). During the postprandial phase, glucagon secretion is suppressed by the action of insulin (229) and GLP-1 (230). Studies have reported inconsistent results for the effect of glucagon on GI motility. For example, i.v. administration of glucagon, at doses ranging from 0.25 – 2 mg, slowed gastric emptying and inhibited motility of the jejunum and colon in humans

(231, 232), while a much lower dose of 0.86 pmol/kg/min (~ 0.05 mg in a 65 kg person), infused continuously for 4 hours, did not affect gastric emptying of a liquid mixed-nutrient meal (233), suggesting that high glucagon concentrations may be required for an effect on GI motility and gastric emptying.

A number of studies have reported an effect of glucagon to reduce food intake (233-235); however, most of these studies evaluated the effects of exogenous glucagon, which resulted in plasma concentrations more than 3-fold higher than observed after a meal. Studies evaluating the effect of endogenous glucagon in mice have demonstrated that administration of two forms of glucagon receptor antagonists (desHis^{1}Pro^{4}Glu^{9}-glucagon or desHis^{1}Pro^{4}Glu^{9}Lys^{12}FA-glucagon (both at 25 nmol/kg) for 10 days had no effect on food intake, in both high-fat fed and obese diabetic mice (236). Since such data are not available in humans, the physiological effect of glucagon on energy intake is still unclear, and warrants further investigation.

Taken together, meal ingestion is associated with well-defined changes in GI functions, including highly coordinated alterations in GI motility, that underlie the slowing of gastric emptying, as well as potent effects on gut hormone secretion. Both are major determinants of energy intake and postprandial blood glucose. The relationship between GI functions with energy intake and glycaemic control is the focus of this thesis.

1.4 Nutrient-induced changes in GI functions in the regulation of food intake in health and the dysregulations in eating-related disorders

Has been published as "Hajishafiee M, Bitarafan V*, Feinle-Bisset C. Gastrointestinal sensing of meal-related signals in humans, and dysregulations in eating-related disorders. Nutrients, 2019; 11(6):1298".*

**Both authors contributed equally to this work.*

1.4.1 Abstract

The upper GI tract plays a critical role in sensing the arrival of a meal, including its volume as well as nutrient and non-nutrient contents. The presence of the meal in the stomach generates a mechanical distension signal, and, as gastric emptying progresses, nutrients increasingly interact with receptors on enteroendocrine cells, triggering the release of gut hormones, with lipid and protein being particularly potent. Collectively, these signals are transmitted to the brain to regulate appetite and energy intake, or in a feedback loop relayed back to the upper GI tract to further adjust GI functions, including gastric emptying. The research in this area to date has provided important insights into how sensing of intraluminal meal-related stimuli acutely regulates appetite and energy intake in humans. However, disturbances in the detection of these stimuli have been described in a number of eating-related disorders. This paper will review the GI sensing of meal-related stimuli and the relationship with appetite and energy intake, and examine changes in GI responses to luminal stimuli in obesity, functional dyspepsia (FD) and anorexia of ageing, as examples of eating-related disorders. A much better understanding of the mechanisms underlying these dysregulations is still required to assist in the development of effective management and treatment strategies in the future.

1.4.2 Introduction

Meal ingestion is associated with well-established changes in upper GI functions that serve to accommodate food in the stomach and break it down to particles of appropriate size for transfer into the small intestine for digestion and subsequent absorption. During these processes, the presence of food in the GI lumen generates a variety of signals arising from gastric distension, nutrient and non-nutrient compounds contained in the food, as well as gut hormones released from enteroendocrine cells in the gut wall (237-240). Distension of the stomach, induced by the volume of food ingested, activates mechano-sensitive vagal afferent fibres with nerve endings in the submucosa and smooth muscle layers of the gastric wall (241-243), and gives rise to a sensation of fullness (244). As gastric emptying progresses, the inputs from mechanical distension diminish gradually, while the small intestinal lumen is increasingly exposed to nutrients, including fats, proteins, carbohydrates and their digestion products. These are detected, or "sensed", by highly specialised receptors, primarily GPCRs, located on the luminal side of enteroendocrine cells, triggering a cascade of intracellular events to increase intracellular calcium, and culminating in the release of gut hormones from the basolateral side (238, 239, 245). Gut hormones, e.g. CCK and GLP-1, then activate receptors located on adjacent endings of submucosal vagal afferent, as well as enteric, neurons. This information, together with the signals from gastric distension, is transmitted to the brainstem, and from there to higher centres, including the hypothalamus, to modulate eating behaviour. Within the brainstem, signals are also relayed from the nucleus of the solitary tract to the dorsal motor nucleus of the vagus, from which vagal efferents trigger feedback regulation of GI motor functions, including stimulation of pyloric pressures, leading to the slowing of gastric emptying (142, 238, 240, 242). Following their release from enteroendocrine cells, gut hormones are also transported in the blood stream to peripheral organs, including the stomach, where they activate specific receptors expressed on smooth muscle cells and enteric neurons, e.g., in the pylorus,

to modulate gastropyloroduodenal motility associated with slowing of gastric emptying (**Figure 1.2**). Because the molecular and cellular processes involved in the sensing of these GI luminal signals cannot be investigated readily in humans, the changes in circulating concentrations of gut hormones, as well as effects on GI motor functions, including modulations in GI motility and slowing of gastric emptying, in response to these signals, are frequently evaluated as "markers" of GI luminal sensing in clinical studies.

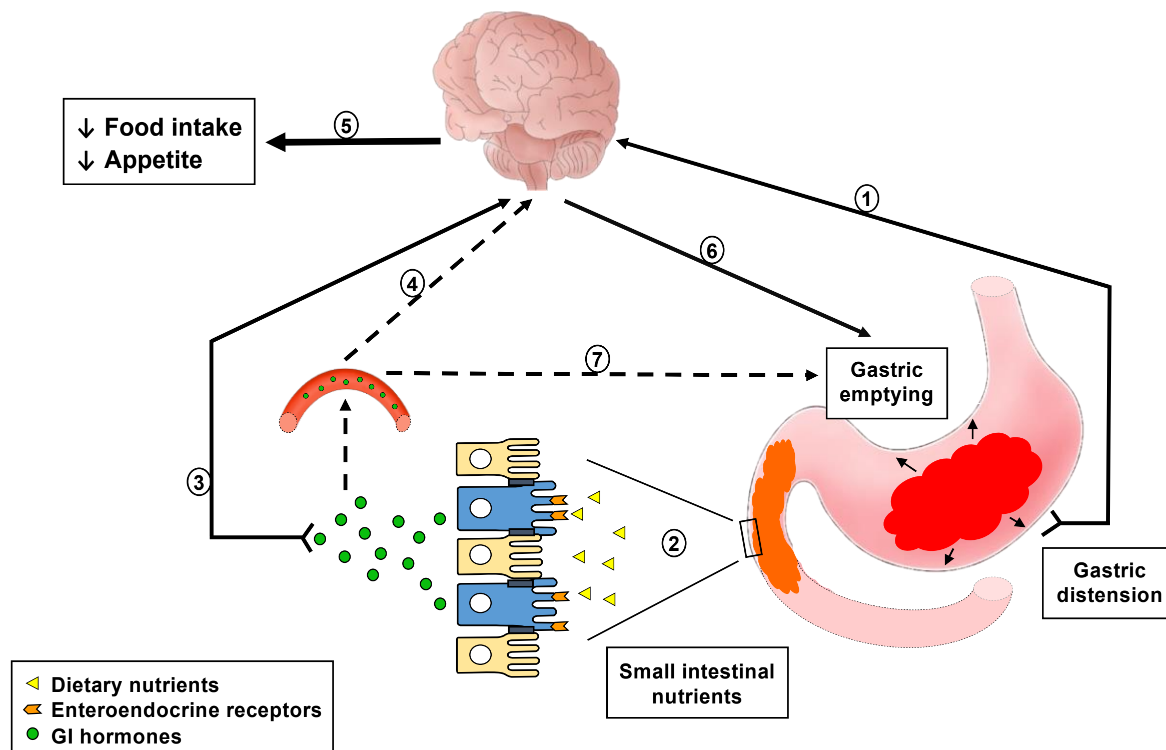

Figure 1.2 Schematic representation of the gastrointestinal (GI) sensing of meal-related stimuli, and effects on GI functions (specifically gut hormone release and slowing of gastric emptying), appetite and energy intake. Meal ingestion initially induces gastric distension, which activates mechanoreceptors on vagal afferents that terminate in the gastric wall and transmit this signal to the central nervous system (1). As chyme enters the small intestine in the process of gastric emptying, nutrients are sensed by receptors located on enteroendocrine cells, triggering GI hormone secretion (2). GI hormones convey meal-related information to the brain involving various pathways, including activation of hormone-specific receptors on vagal afferent endings (3) or following transport through the blood stream (4). Together, these inputs are conveyed to higher brain centres to modulate eating behaviour, appetite and energy intake (5), as well as feedback regulation of GI motor functions, particularly pyloric pressures, associated with the slowing of gastric emptying (6). The latter can also occur through endocrine pathways (7).

While the processes outlined above underlie the regulation of normal GI function, appetite and energy intake, dysregulations can occur in a range of disorders which adversely affect eating, or lead to GI symptoms, including exaggerated postprandial fullness, nausea and bloating (246-249). For example, in obesity, GI sensitivity to dietary fat appears to be decreased, possibly as a consequence of excess energy intake (250). Psychiatric eating disorders, including anorexia nervosa and bulimia nervosa, have been found to be associated with an enhanced sensitivity to gastric distension and alterations in GI hormone secretion in response to nutrients (251, 252). GI disorders (including gastro-oesophageal reflux disease, and the functional GI disorders, FD and irritable bowel syndrome), have in common a hypersensitivity to luminal stimuli, particularly fat, triggering postprandial GI symptoms (253). Critical illness (including sepsis, trauma, burns, head injuries or surgical emergencies in patients admitted to an intensive care unit) is also associated with hypersensitivity to small intestinal nutrients and GI motor dysfunctions, resulting in intolerance of gastric feeding (247, 254). Finally, ageing, while characterised by a reduced GI sensitivity to both fat and protein, has also been found to be associated with a range of changes in GI functions, including gastric emptying and hormone release (255), which may contribute to the characteristic loss of appetite, termed “anorexia of ageing”.

This review will provide a brief overview of the GI sensing of meal-related signals in humans, specifically, gastric distension and small intestinal nutrients, thus, focusing on preabsorptive signals, by describing their effects on GI functions, appetite and energy intake. We will also examine dysregulations in the GI responses to these signals. While, as described above, these can occur in a wide range of disorders, a comprehensive review is beyond the scope of this paper. Thus, we will illustrate key changes, and their functional implications, using obesity, FD and anorexia of ageing as examples. With regards to nutrients, we will focus on fat and

protein because their GI sensing is primarily altered in these disorders, as alluded to above. Thus, while carbohydrates are, of course, also sensed in the intestinal lumen (256), a discussion of their effects is beyond the scope of this paper.

1.4.3 GI sensing of intraluminal meal-related stimuli

The arrival of a meal in the upper GI tract in the process of food ingestion exerts powerful signals, including gastric distension as a result of the meal volume, as well as the chemical components of the meal, particularly macronutrients, that modulate postprandial GI functions, including gastric emptying, GI motility and the release of GI hormones, associated with changes in appetite perceptions and subsequent energy intake. As outlined above, while the investigation of GI sensing of intraluminal stimuli at the receptor level is currently not feasible in human studies in-vivo, the measurement of the downstream manifestations, including changes in upper GI motility, gut hormone release, as well as appetite perceptions and energy intake, provides a relatively non-invasive means to quantify the ability to "sense" these stimuli in the GI lumen in clinical research.

1.4.3.1 Sensing of gastric distension

Meal ingestion induces a gradual distension of the gastric wall, inducing fullness and acting as a first signal to control meal size (244, 257-259). For example, experimental distension of the proximal stomach using a bag attached to a gastric barostat gradually increases the perception of fullness, as the distension, induced either by increasing volume or pressure within the bag, increases (244, 258). Gastric distension before, or during, meal ingestion also reduces subsequent food intake (259). Filling of the antrum also plays a role in the perception of fullness and meal termination (260). For example, studies using scintigraphy or ultrasound to quantify intragastric volume and meal distribution showed that fullness after consumption of a 350-mL

glucose drink was directly related to the volume in the distal stomach (260). Moreover, suppression of energy intake after a mixed-nutrient drink was related inversely to antral area (a measure of antral filling) immediately before the meal (261). The relative importance of the proximal vs distal stomach cannot be determined from these studies. However, because a meal is initially stored primarily in the proximal stomach (as a result of proximal gastric relaxation) and, in the process of gastric emptying, gradually transferred into the antrum prior to evacuation into the small intestine, it is likely that the relative importance of the two regions changes as a result of changes in the intragastric distribution of the meal as gastric emptying progresses.

1.4.3.2 Effects of nutrients in the small intestinal lumen

As gastric emptying progresses and the signal from gastric distension diminishes, chyme enters the small intestinal lumen in a tightly regulated fashion. This is achieved by well-characterised effects of nutrients on pressures in the antropyloroduodenal region (262), mediated by gut hormones released in response to nutrients (142, 238, 239). Nutrients in the intestinal lumen, particularly lipid and protein, also modulate appetite and subsequent energy intake (263, 264), and changes in both motility and gut hormones play critical roles (265, 266).

1.4.3.2.1 Small intestinal sensing of lipid

The presence of lipid in the GI lumen provides a potent signal to stimulate the GI functions that are key to the regulation of appetite and energy intake (250). For example, infusion of lipid, at loads of 1 – 4 kcal/min, directly into the duodenum, to exclude any sensory inputs from the oral cavity or confounding effects of variations in the rate of gastric emptying, induces well-coordinated changes in upper GI motility, including the stimulation of pyloric pressures (263), which underlie the slowing of gastric emptying (262). Lipid also stimulates the release

of GI hormones, including CCK, GLP-1 and PYY, while the release of ghrelin from the stomach is suppressed (142). These effects occur in a load-dependent manner, and are associated with the suppression of energy intake (196, 263). In fact, the magnitude of the stimulation of pyloric pressures and plasma CCK, as indicators of the GI sensing of nutrients, have been identified as independent determinants of energy intake in response to intraduodenal administration of particularly fat and also protein (265, 266).

The above-mentioned effects of fat on GI functions, including gastric emptying, GI motility, gut hormone release and energy intake, are abolished by co-administration of the lipase inhibitor, orlistat, establishing that the GI effects of fat are dependent on fat digestion, and lipid digestion products, namely fatty acids, are essential for intestinal lipid sensing (267, 268). Thus, the digestibility of fat affects its sensing in the GI lumen. Once fatty acids are released in the process of lipid digestion, their effects on GI functions are chain-length dependent (269). Moreover, even within the group of fatty acids with $\geq$ 12 carbon atoms, fatty acids appear to have different potencies (270, 271). For example, only lauric acid (C_{12}), but not oleic acid (C18:1), reduced subsequent energy intake when infused at a load of 0.4 kcal/min (270), while C18:1 was effective at the higher load of ~ 0.75 kcal/min (271), suggesting that the threshold loads required for luminal detection differ between fatty acids. The sensing of fatty acids in the GI lumen (272, 273) is associated with the release of GI hormones, including CCK and GLP-1 (238, 239), which are involved in transmitting nutrient-related information to the brain, and, in case of CCK, at least in part, via CCK-A receptor-dependent mechanisms (274, 275).

1.4.3.2.2 Small intestinal sensing of protein

Dietary protein has been recognised to have potent effects to modulate GI functions and suppress appetite and food intake (276, 277). Amongst proteins, whey protein appears to be

particularly potent (278). For example, intraduodenal administration of whey protein, at loads of 0.5 – 3 kcal/min, stimulates pyloric pressures and modulates the release of gut hormones, including stimulation of CCK, GLP-1 and PYY, and suppression of ghrelin, and reduces subsequent energy intake, in a dose-dependent manner (264, 279). Moreover, the effects of whey protein, which is digested relatively rapidly, on GI hormone release, slowing of gastric emptying and suppression of energy intake are greater than those of casein, which coagulates in the stomach, suggesting a role for the digestibility of proteins in its sensing in the GI lumen (280). Thus, as with lipids and fatty acids, amino acids may mediate, at least in part, the effects of protein on GI functions and energy intake (281-283). There has, therefore, been an increased interest in evaluating the effects of specific amino acids on these outcomes (283, 284). However, the assessment of the effects of amino acids is complicated by the number of amino acids at play, their varying structures, their inter-dependence (e.g., for effective absorption) and the large range of their effects outside the GI tract. Nevertheless, a number of amino acids, when given in relatively small amounts, modulate gut functions and reduce energy intake (285-289). For example, L-tryptophan, given orally, intragastrically or intraduodenally, stimulates plasma CCK and pyloric pressures, slows gastric emptying and suppresses energy intake in healthy, lean individuals (286, 288, 289). In addition, the suppression of energy intake by amino acids, e.g., L-tryptophan and L-leucine, is also related to the circulating concentrations of these amino acids (285, 286), in line with the recognition that the effects of amino acids on energy intake are also regulated by extraintestinal factors, which may act in the periphery and/or the brain (290). This may explain, at least in part, why intraduodenal protein and lipid infusions have comparable effects to suppress subsequent energy intake, despite protein stimulating gut hormones and pyloric motility much less than lipid (196).

Taken together, the sensing of both lipid and protein, through their digestion products, has potent effects on GI functions, associated with a reduction in appetite and energy intake. While much work has been done in this area in humans, understanding the molecular processes involved in GI sensing still relies largely on preclinical studies, or ex-vivo investigations of clinical samples (e.g., human biopsies). Thus, technical advances are required that will enable in-vivo studies of these processes in healthy humans as well as relevant patient populations. A thorough understanding of the mechanisms underlying these processes is critical for a better understanding of the dysregulations in GI sensing underlying eating-related disorders, to develop effective management and treatment strategies.

1.4.4 Altered GI sensing of meal-related stimuli in eating-related disorders

While, as discussed, luminal meal-related stimuli contribute to the regulation of GI functions, appetite and energy intake, disturbances in the sensing of these stimuli have been found in a number of eating-related disorders, including a reduced intestinal sensitivity to the GI effects of fat in obesity, associated with dietary overconsumption (291), an exaggerated sensitivity to both gastric distension and intestinal lipid in patients with FD, associated with digestive symptoms (253), and reduced GI sensory perception associated with a loss of appetite with ageing (255) (**Table 1.2**).

Table 1.2. Changes in upper GI luminal sensing reported in eating-related disorders.

		GI Sensory Disturbances	References
Obesity	↓	Sensitivity to gastric distension	(276, 277, 291-300)
	↑	Gastric capacity	
	↓	Sensitivity to small intestinal lipid	
	? ↓	Sensitivity to CCK, PYY, ghrelin	
	↑ ↓ ↔	CCK secretion	
	↑ ↓ ↔	Gastric emptying	
Psychiatric eating disorders			
Anorexia nervosa	↑	Sensitivity to gastric distension	(248, 251, 252, 301)
	↑	Sensitivity to small intestinal nutrients	
	↑ ↔	CCK secretion	
	? ↑	Sensitivity to CCK	
	↑	Ghrelin, PYY secretion	
	↓	Gastric emptying	
	↓	Proximal gastric accommodation	
	↑	Antral filling	
Bulimia nervosa	↑	Sensitivity to gastric distension	(251, 252, 302)
	↑	Gastric capacity	
	↓	CCK secretion	
	↓ ↔	Gastric emptying	
	↓	Proximal gastric accommodation	
GI disorders			
Gastroesophageal reflux disease	↑	Sensitivity to gastric distension	(253, 303-305)
	↑	Sensitivity to small intestinal lipid	
	↓	Gastric emptying	
	↓	Gastric accommodation	
Functional dyspepsia	↑	Sensitivity to gastric distension	(246, 253, 306-317)
	↑	Sensitivity to small intestinal lipid	
	? ↑	Sensitivity to CCK	
	↓	Gastric emptying	
	↓	Gastric accommodation	
	↑	Antral distension	
Irritable bowel syndrome	↑	Sensitivity to gastric distension	(253, 318, 319)
	↑	Sensitivity to small intestinal lipid	
	↓	Gastric emptying	
	↑ ↓	Gut motility	
Critical illness	↓	Sensitivity to gastric distension	(247, 254, 320)
	↑	Sensitivity to small intestinal nutrients	
	↑	CCK, PYY secretion	
	↓	Ghrelin, motilin secretion	
	↓	Gastric emptying	
Anorexia of ageing	↓	Sensitivity to gastric distension	(261, 321-336)
	↓	Sensitivity to small intestinal nutrients	
	↑	CCK secretion	
	↑ ↓ ↔	PYY, GLP-1 and ghrelin secretion	
	? ↓	Sensitivity to CCK, PYY and GLP-1	
	↓ ↔	Gastric emptying	
	↑	Proximal gastric retention	

↑, increase; ↓, decrease; ↔, unchanged; ?, uncertain; GI, gastrointestinal; CCK, cholecystokinin; PYY, peptide YY; GLP-1, glucagon-like peptide-1.

1.4.4.1 Altered GI sensing in obesity

People with obesity, as a group, consume larger amounts of food, and have a preference for particularly high-fat and energy-dense foods; thus, it is conceivable that their ability to sense meal-related stimuli (e.g., distension of the stomach, dietary fat) in the GI lumen is compromised.

1.4.4.1.1 Sensitivity to gastric distension

Obese individuals have been found to have greater fasting gastric volumes (294), and in most, but not all, studies tolerate greater intragastric volumes, as measured by gradually filling a bag positioned in the stomach with air or water (292, 295, 296), or consume larger amounts of water or nutrient loads during drink challenges (294). Thus, obese individuals appear to be less sensitive to gastric distension and require larger intragastric volumes to experience fullness. While data relating to gastric meal emptying in obese have been inconsistent (with studies reporting slower or faster emptying, or no differences from lean individuals (293)), possibly in part due to differences in study design and methodological approaches (250), a comprehensive recent study of 328 participants found that gastric emptying of both solid and liquid components of a mixed meal was accelerated in obese individuals (294). Accelerated gastric emptying is associated with an enhanced exposure of the small intestine to nutrients, which has been shown to induce structural changes in the mucosa and facilitate nutrient absorption (337), therefore, differences in GI functions and energy intake in response to nutrients may be the result of reduced feedback from small intestinal nutrients, particularly fat.

1.4.4.1.2 Small intestinal sensing of fat

Experimental evidence that the overconsumption of energy-dense, high-fat foods is associated with a reduced GI sensitivity to fat has been derived mainly from short-term overfeeding

studies, often conducted in normal-weight people (338-341). For example, in normal-weight individuals, consumption of a high-fat diet for 2 weeks accelerated gastric emptying of a high-fat meal (338), and attenuated the pyloric motor response to an intraduodenal lipid infusion, when compared with the low-fat diet (297). The effect on gastric emptying was fat-specific, since gastric emptying of a high-carbohydrate meal was not accelerated after the high-fat diet (276). There is, indeed, also evidence that obese people are less sensitive to the appetite-suppressant effects of dietary fat (276, 277, 297, 298). For example, obese volunteers consumed a greater amount of food from a high-fat meal than healthy controls (297), and, unlike healthy controls, obese participants did not reduce subsequent energy intake after a high-fat meal (276). Only few studies have specifically evaluated the gut hormone responses to fat ingestion in obese people, and findings are somewhat conflicting. For example, male and female obese volunteers were reported to have a greater plasma CCK response to a soup containing 30 g of margarine than healthy controls, despite comparable gastric emptying in the two groups (299), although gastric emptying of fat was not specifically quantified, and thus, may have been faster, potentially resulting in greater CCK stimulation. In contrast, we found no differences in plasma CCK between obese and lean male adults during 3 h after ingestion of a solid high-fat meal (276). Solid meal emptying is slower than liquid emptying, possibly explaining the differences between the outcomes in the two studies; however, the latter study did not evaluate gastric emptying. Finally, we have reported reduced plasma CCK concentrations during 90-min intraduodenal administration of oleic acid in obese, compared with lean, men (300), indicating that the small intestinal response to a standardised fatty acid load is reduced in obese people, most likely due to compromised small intestinal lipid sensing. While PYY concentrations following consumption of a high-fat meal have been reported to be lower in obese than lean individuals (277), PYY and ghrelin responses to a high-fat meal have also been found to be comparable in the two groups (276). Taken together, the limited available

data suggest that obesity may be associated with a reduced ability to sense dietary fat, which may compromise the initiation of appropriate feedback mechanisms, including gut hormone responses. Fat-induced gut hormone secretion may be reduced, or, in the case of normal secretion, the sensitivity to hormones may be compromised, and these changes may contribute to altered energy intake regulation.

There is also limited evidence that dietary restriction can, at least in part, improve intestinal responses to intraluminal fat in obesity (342, 343), further supporting a contributory role of diet. For example, in obese volunteers, dietary restriction for 4 days (~ 1000 kcal/day) significantly enhanced plasma PYY, ghrelin suppression and pyloric contractions in response to intraduodenal lipid, associated with suppression of energy intake (342). Moreover, 30% dietary restriction for 12 weeks was associated with greater intraduodenal lipid-induced stimulation of PYY and basal pyloric pressures, and reduced energy intake (343).

1.4.4.1.3 Small intestinal sensing of protein

In contrast to lipids, obese people appear to remain sensitive to the GI and appetite-suppressant effects of protein, also evidenced by the potent effects of high-protein diets to achieve weight loss (344, 345). For example, energy intake 3 h after a high-protein meal was lower than after a high-carbohydrate control meal, while (as discussed above), a high-fat meal did not reduce subsequent intake (276). Similarly, a high-protein meal reduced hunger perceptions for 3 h post-meal substantially more than a high-fat meal, and the response to protein did not differ from those in lean participants (277). These effects of protein may be mediated, at least in part, by gut hormones; however, current evidence is limited and inconsistent. For example, the potent suppression of hunger by the high-protein meal was accompanied by marked stimulation of plasma PYY, although absolute concentrations were lower in the obese than in the lean

group, while no differences in plasma GLP-1 or ghrelin concentrations were observed between groups (277). In contrast, in the other study (276), the high-protein meal led to sustained CCK stimulation and ghrelin suppression in both lean and obese, while the PYY response did not differ between the high-protein and high-fat meal in lean or obese. Nevertheless, that these responses are, at least in part, mediated from the small intestine, is supported by a recent study (346) in which the antropyloroduodenal pressure, plasma CCK and GLP-1 responses to intraduodenal whey protein, at the load of 3-kcal/min, did not differ between lean and obese individuals, although energy intake was non-significantly higher in the obese.

The role of specific amino acids in the responses to protein is currently unclear, with limited information on the comparative effects of amino acids on GI functions and energy intake in health and obesity. Intraduodenal infusion of tryptophan had comparable effects on pyloroduodenal motility in lean and overweight participants (347), and intragastrically administered tryptophan slowed gastric emptying and reduced energy intake after a mixed-nutrient drink in ~ 50% of lean and obese individuals (348). In contrast, obese individuals have been reported to be less able to detect glutamate orally (349), suggesting that obese individuals may be less sensitive to palatable umami taste, which may contribute to higher food intakes.

Taken together, obese people appear to be less sensitive to gastric distension, and the GI and appetite-suppressant effects of fat, possibly as a result of overconsumption of high-fat, energy-dense diets, while the responses to protein remain relatively intact. Further research is needed to elucidate the mechanisms that underlie these changes, and the differential responses to protein and fat, as well as the responses to dietary restriction, at the level of the receptors, and along the pathways that transmit the information to the brain, to develop novel, and effective, strategies to better manage or treat, and ideally prevent, obesity.

1.4.4.2 Altered GI sensing in functional dyspepsia

FD is a multi-factorial disorder characterised by symptoms, including nausea, fullness, discomfort, bloating and vomiting, originating in the upper GI region, often triggered in close temporal association with meal ingestion, with patients unable to complete normal-sized meals (246, 350). This originally led to the assumption that FD was due to abnormalities in GI motor activity and gastric emptying; however, correlations between symptoms and changes in these functions are not strong. A number of contributing factors and mechanisms have been identified in FD, including gastroduodenal inflammation and changes in the epithelial barrier, GI infections, gut microbiota, genetic contributions, cognitive and psychological factors (307, 351), and a key feature is an increased GI sensitivity to meal-related stimuli, including gastric distension (potentially exacerbated by delayed gastric emptying, impaired proximal stomach accommodation, abnormal intragastric meal distribution and disordered antroduodenal motor function) and/or small intestinal nutrients (307).

1.4.4.2.1 Sensitivity to gastric distension

The frequent occurrence of FD symptoms in close temporal association with meal ingestion (308, 352) suggested an enhanced sensitivity to distension of the stomach by the meal volume. Indeed, studies evaluating the gastric sensory response to gastric distension have revealed that 30 – 48% of patients exhibit a hypersensitivity to mechanical distension of the stomach (309, 310). Thus, when either the proximal (309, 310) or distal (311) stomach was distended with an air-filled bag, FD patients reported both perception and discomfort at lower distension volumes or pressures than healthy controls. This hypersensitivity to gastric distension is also likely to underlie the inability of FD patients to complete normal-sized meals.

1.4.4.2.2 Alterations in the small intestinal sensing of nutrients

The frequent complaints by FD patients that certain foods or meals induce, or exacerbate, their symptoms suggest that FD might also be associated with a hypersensitivity to specific nutrients or other food components. Since rich and fatty foods appear to be particularly potent in triggering dyspeptic symptoms (246, 307), a body of research has investigated a specific hypersensitivity to fat. However, a range of other foods, or food groups, are also frequently reported by patients to lead to symptoms (307, 353-356), including milk and dairy products, meat, carbohydrate- or wheat-containing foods or drinks, certain vegetables (possible particularly those vegetables containing fermentable oligo-, di- and mono-saccharides and polyols, or "FODMAPs" (357)), sour, acid-secreting or irritant foods, including citrus fruit, spices, coffee and alcohol (354, 355, 358). Thus, in addition to an enhanced fat sensitivity, hypersensitivities to other nutrients or food components may also exist.

Hypersensitivity to lipid: Approximately 60 – 70% of FD patients display a hypersensitivity to fat (306, 312-314). For example, dyspeptic symptoms, including epigastric pain, bloating and nausea, were substantially greater in response to a high-fat soup than a bland soup (314). Similarly, a palatable high-fat yogurt was associated with significantly greater fullness, nausea and bloating than an equivolaemic fat-free yogurt (312, 313). The importance of a contribution from the small intestine is highlighted by the fact that intraduodenal infusion of a long-chain triglyceride emulsion induced typical symptoms, and exacerbated the sensitivity to gastric distension, in patients, but not healthy controls (306). This hypersensitivity appears to be fat-specific, since infusion of glucose did not induce symptoms (315). Moreover, administration of the CCK-A receptor antagonist, dexloxiglumide, reduced nausea, bloating and fullness, induced by duodenal lipid infusion, in patients (316), providing evidence that CCK mediates, at least in part, the effects of fat on symptoms in FD. Whether FD is associated with a

hypersensitivity to (316, 317), or altered secretion of (313), CCK, or both, and the involvement of other gut hormones, remains unclear and warrants investigation.

Responses to other nutrients: Since protein, similarly to fat, potently affects upper GI functions and energy intake in healthy people, it is conceivable that protein consumption could also generate FD symptoms; however, this has not been investigated. One study quantifying eating habits and the temporal relationship with dyspeptic symptoms in FD over one week indicated that although there was no difference in dietary protein consumption between FD and healthy individuals, postprandial fullness was related to protein in the patients (308). Moreover, some patients report dyspeptic symptoms after consumption of wheat-containing foods (353, 354, 359), which may be related to gluten (360, 361), and a gluten-free diet has been found to reduce dyspeptic symptoms (361). However, it is not clear whether such findings relate specifically to an intolerance of gluten or, more broadly, to other protein sources.

Findings relating to effects of different sources of carbohydrate on FD symptoms are limited. One study reported inverse relationships between overall symptoms and fullness with carbohydrate intake (308), suggesting that carbohydrates overall play a favourable role. The role of dietary fibre in FD is still uncertain (354, 362). No studies have evaluated the role of FODMAPs, or their elimination from the diet, in FD. Symptoms reported in response to milk ingestion may be due to lactose intolerance, or relate to the fat or protein content of milk, but this requires further study (354).

Taken together, FD is associated with hypersensitivities to both gastric distension and small intestinal nutrients, particularly fat; thus, these disturbances may, at least in part, address the patients' frequent complaints of an inability to complete normal-sized meals and intolerance of

fatty foods. Much more research is required to clarify the contributions of a large range of other foods, and food components, including protein, to FD symptoms, and mechanisms involved, ideally in large studies to allow sub-grouping of patients. Such approaches are vital, as they may eventually enable the development of specific dietary interventions for translation into effective therapeutic strategies.

1.4.4.3 Altered GI sensing in anorexia of ageing

Ageing, even in healthy people, is often associated with a loss of appetite, termed "anorexia of ageing". Older people consume smaller meals and fewer snacks, and eat more slowly, compared with young adults (363), resulting in a decline in energy intake and weight loss. Chronic weight-loss represents a major risk to the health and well-being of older people, hence, nutritional strategies, which include particularly the use of protein supplements, have been developed to address this problem (364). While the causes of appetite loss with ageing are not completely understood, ageing is associated with a gradual decline in metabolically active tissue, specifically muscle mass (364); thus, a reduction in basal metabolic rate, associated with reduced energy requirements, may lead to reduced appetite. However, there is evidence of altered GI sensory and motor functions, including slower gastric emptying (255), which would favour a reduction in energy intake (but also delays initiation of signals by nutrients in the small intestine), as well as, on the other hand, a reduced sensitivity to the energy intake-suppressant effects of nutrients (321, 322, 365), and changes in the secretion of, and/or sensitivity to, gut hormones (323). An improved understanding of these, apparently discrepant, changes in gastric function vs appetite signals arising from the small intestine in response to meal consumption is likely to assist in the development of improved management strategies to ensure that older people receive adequate nutrition.

1.4.4.3.1 Sensitivity to gastric distension

Older people frequently report reduced appetite before and during meal ingestion. For example, healthy older people were less hungry before and following the ingestion of a mixed-nutrient yogurt-based drink, and reported greater fullness after the drink than young controls (261). Early studies evaluated gastric emptying of meals and most (324-327), but not all (366), found that gastric emptying of both solid and liquid meal phases was slower in older than young people, although observed differences were often modest (325, 327). Increased gastric meal retention may enhance gastric distension in either proximal or distal stomach contributing to fullness, as described in healthy people (258, 260). While proximal and/or distal gastric retention has been found to be greater (261, 327), antral filling has also been reported to be less (324), in older people. In response to isovolumetric or isobaric proximal gastric distension, older people reported less fullness or bloating, and greater hunger, than young controls, at a given volume or pressure level, in the absence of any changes in gastric compliance (328), suggesting that healthy ageing is associated with a reduced perception of gastric distension. Reasons for the apparent discrepancies between responses to a meal, as opposed to experimental gastric distension, are currently unclear.

1.4.4.3.2 Alterations in the small intestinal sensing of nutrients

The effects of intestinal nutrient exposure, with a focus on protein, on GI functions and appetite in older people have been evaluated in a limited number of studies, and findings suggest that ageing is associated with a reduced responsiveness to the appetite-suppressant effects of nutrients (321, 329, 367, 368). This may be due, at least in part, to a reduced digestive capacity with ageing, since reductions in the secretions of gastric acid, pancreatic lipase and other enzymes, as well as bile salts have been reported (369); however, whether these changes have

any detrimental effects on the digestion of protein and fat, and whether, or how, that may alter the GI sensing of these nutrients, requires investigation.

Response to protein: In contrast to the use of high-protein diets to achieve weight loss in obesity (344, 345), in older people protein supplements are recommended to prevent weight loss and maintain functionality (364). Given the potent GI and appetite-suppressant effects of protein in young people (276, 277, 370), it is important to increase our knowledge of the alterations in the GI effects of protein in older people, and underlying mechanisms. The available literature indicates that ageing is associated with a reduced sensitivity to the satiating effect of protein (321, 322, 329). For example, despite a reduced desire to eat, as well as reduced fullness, in response to a protein drink containing either 30 g or 70 g whey protein, the suppression of energy intake relative to control from a meal consumed 180 min later was less in older people, associated with a greater cumulative intake (321). Interestingly, despite slower gastric emptying of the drinks in older people, energy intake from the meal was only related to gastric emptying in the younger people (321). Similarly, a 60-min intraduodenal infusion of whey protein suppressed appetite and energy intake less in healthy older than in young adults, associated with greater overall energy intake in older people (329). Moreover, ingestion of either a whey protein drink (70 g protein; 280 kcal), or a mixed-nutrient drink (70 g protein, 28 g carbohydrate, 12.4 g fat; 504 kcal) did not suppress energy intake differentially, so that total energy intake was increased, and most by the higher-energy mixed-nutrient drink (322).

Response to lipid: Only few studies have evaluated the effects of fat on appetite perception in ageing. For example, administration of a fat emulsion (30 mL, 120 kcal) 3 times/day for six weeks significantly increased daily energy intake by ~ 240 kcal (371), and either a high-fat or high-carbohydrate mixed-nutrient drink (250 mL, ~ 250 kcal) consumed after breakfast

increased intake over the following 24 h by ~ 200 kcal (365), with no differences between fat and carbohydrate-rich drinks. One earlier study evaluated the effects of ageing on the pyloric motor, appetite and energy intake responses to duodenal lipid and glucose infusion (330). Lipid stimulated pyloric pressures more in older people (which is likely to underlie the slower gastric emptying described above), and while baseline hunger was less in older people, and, unlike in young controls, not suppressed by either nutrient, subsequent energy intake did not differ between the two groups.

Collectively, these studies suggest that older people are less sensitive to the appetite-suppressant effects, but more sensitive to the inhibitory effects on the stomach, particularly gastric emptying, of small intestinal nutrients. It is possible that these changes are due to alterations in the release of, or sensitivity to, GI hormones.

Gut hormone responses: Available studies consistently report increased fasting plasma CCK concentrations (323, 324, 331, 332), as well as an exaggerated rise in response to oral or intraduodenal nutrients (322, 324, 331, 333), in older people. Furthermore, an inverse relationship between hunger and plasma CCK has been found in young, but not older people (331), and exogenous administration of CCK-8 suppressed food intake from a meal after the infusion twice as much in older, than young people (323), suggesting that older people remain responsive to CCK and their sensitivity to the appetite-suppressant effect of CCK may be enhanced. It is not known whether the stomach remains sensitive to CCK with ageing; a reduced gallbladder contraction and emptying has been reported previously (324). Studies evaluating the secretion of GLP-1 and PYY have yielded more inconsistent findings, with some studies reporting no differences in GLP-1 or PYY between older and young people (331-333), or lower (279) or greater (322, 324) levels in older people. It is conceivable, but has not been

investigated, that older people may be more sensitive to the effects of GLP-1 and/or PYY, resulting in an exaggerated ileal brake effect from the distal small intestine (372). The effects of ageing on ghrelin secretion are also unclear, with studies reporting greater (334) or lower (335) fasting acyl-ghrelin, greater fasting total ghrelin (332), no difference in fasting or postprandial ghrelin (336), or lower postprandial total ghrelin (322), in older and young people.

Taken together, current evidence suggests that even healthy ageing is associated with marked changes in upper GI functions, including delayed gastric emptying and a heightened sensitivity to particularly CCK, both of which would favour suppression of appetite and energy intake. However, and in apparent contrast, the appetite-suppressant effects of nutrients are reduced in older people. While the latter lends support to the utility of dietary supplements to improve energy intake in older people, the discrepancy between these findings and the consistently reported lack of appetite frequently leading to undernutrition in older people requires much further research to identify mechanisms, and other factors, that may help to explain the apparent divergence in current knowledge, with the aim to develop improved management strategies.

1.4.5 Summary and future directions

This article has reviewed the sensing of meal-related signals, including both mechanical and nutrient stimuli, in the upper GI tract, and their effects to modulate GI functions, appetite and energy intake, in humans. The appropriate sensing of these stimuli is altered in a number of eating-related disorders, including obesity, FD and anorexia of ageing, associated with compromised, or exaggerated, responses to meals. In obesity, there is evidence of an enhanced gastric capacity and reduced luminal sensing of gastric distension and duodenal lipid, associated with reduced inhibition of subsequent energy intake. FD, on the other hand, is associated with hypersensitivity to both gastric distension and small intestinal lipid, amongst

other food components, which, at least in part, underlies the induction of meal-related symptoms, particularly in response to fatty foods. Anorexia of ageing is characterised by reduced hunger perception and food intake, in part due to delayed gastric meal emptying and an enhanced secretion of, and/or sensitivity to, gut hormones, particularly CCK. In contrast, the satiating effects of nutrients are reduced, associated, in an apparent discrepancy to the free-living situation, with an increase in overall energy intake in the laboratory setting. These examples demonstrate the existence of a variety of sensory dysfunctions across eating-related disorders that may, at least in part, underlie the changes in food intake, or symptoms experienced, in these conditions. Much more research is required on the cause-effect relationships to better understand whether the sensory changes are causal, or occur as a result of particular dietary behaviours. For example, is over-eating in obesity the result of an inherently reduced GI sensitivity to meal-related stimuli, or does gradual over-eating lead to a desensitisation of the sensory systems with subsequent reductions in the ability to adequately sense these stimuli? The temporal relationship between the decline in GI sensitivity to meal-related stimuli and reduced basal metabolic rate with ageing also warrants investigation. In FD, studies in large cohorts are required to enable much more detailed investigations of the varied responses to different food groups, and how these may relate to specific changes in small intestinal nutrient sensing. Further technological advances will be required to investigate the alterations that occur in these disorders at the molecular and cellular levels in vivo, and to clarify the locations of the dysregulations along both directions of the gut-brain axis. While our knowledge in this field has advanced rapidly over the last decade, much more work is still required in order to develop novel and effective approaches for the management, treatment and/or prevention of these dysregulations in GI luminal sensing.

1.5 Effects of bitter compounds on GI functions, energy intake and glycaemic control

There has been an increasing interest in the effects of bitter compounds to provide a means to regulate energy intake and improve glycaemic control, due to the recognition that bitter substances, by activating bitter taste receptors, might trigger mechanisms in the gut to secrete GI hormones and alter gut motility, similar to nutrients. In addition, biter compounds are of particular interest, compared to other tastants, due to their calorie-free nature. Natural bitter-tasting compounds are predominantly found in plant-based foods, including phytochemicals (e.g. phenols, flavonoids and glucosinolates) and extracts from different plants (e.g. *Hoodia gordonii*, *Gentiana scabra*, *Humulus lupulus L.* flower, *cinchona* tree), and can also be found in animal-derived foods or generated during the process of food aging or spoilage (373-375). Maillard and fermentation reactions can also generate bitter compounds (376-378). In addition, there are many chemically synthesised compounds, including denatonium benzoate, phenylthiocarbamide or 6-N propylthiouracil, which have a strong bitter taste (379). To date, numerous bitter compounds have been found and studied (380), with each characterised by a unique structure, consisting of various molecules, i.e. fatty acids, hydroxyl fatty acids, amino acids, peptides, etc. (378). As some bitter compounds are the most poisonous compounds in nature, bitter taste usually causes withdrawal reflexes and negative affective responses, resulting in prevention from, or rejection of, consumption of these compounds (381-383). Although no clear association between bitter taste and toxicity has been found (384), it is believed that the ability to detect bitter substances is vital for humans and other mammals' survival, as it assists in the identification and avoidance of consumption of potentially harmful food constituents, or spoiled foods, to prevent poisoning (374, 385).

The purpose of this section is to review the mechanisms underlying the GI sensing of bitter-taste stimuli and the reported effects of bitter-taste stimuli on GI hormones, gut motility and gastric emptying, as well as energy intake and glycaemia. The rational of reviewing bitter tastants, compared with other tastants, is the due to the non-caloric nature of these compounds which enhances their effects on regulation of energy intake

1.5.1 Gastrointestinal sensing of bitter taste-related signals

Similar to nutrients, that are sensed by particular taste receptors in the oral cavity and along the GI lumen, bitter compounds are detected by TAS2Rs (386, 387). TAS2Rs were initially identified in the taste buds of the oral cavity (388), however, these receptors, as well as their downstream signalling molecules, have also been identified in extra-oral locations, including the kidney, airways, immune system, brain and the GI tract (100, 389). In mice and rats, evaluation of the putative TAS2Rs gene transcripts in the GI tract has demonstrated the expression of these receptors in both stomach and duodenum (390). Moreover, subsequent studies in rodents and humans have validated the expression of TAS2Rs in small and large intestinal tissue and enteroendocrine cells (391-393). Depending on the species, various TAS2R genes have been found in the genome. For instance, to date, 3 different subtypes of TAS2Rs have been detected in vertebrates, up to 50 in chickens, more than 30 in rodents, and 25 in the human genome (394, 395). Initially, it was thought that specific TAS2Rs only detect specific bitter compounds. For example, in mice, the TAS2R5 was reported to only be activated by cycloheximide, among 27 tested bitter compounds, and TAS2R8 and 4 by both propylthiouracil and denatonium benzoate (396). Subsequent studies in humans demonstrated that other TAS2Rs, e.g. TAS2R16 or 38, recognise a range of bitter compounds (397, 398). Thus, a particular TAS2R subtype can be activated by various bitter compounds. At the same time, it is now known that most known bitter compounds activate a range of TAS2R subtypes,

but the combination of subtypes varies (380). Comparison of the response profiles of 58 natural and 46 synthetic bitter compounds showed that diphenidol was the only compound that stimulated 15 different TAS2Rs subtypes (TAS2R1, 3, 7, 10, 13, 14, 16, 38-40, 43, 44, 46-49). Other promiscuous agonists included quinine, an extract from the bark of the *cinchona* tree which activates 9 subtypes (TAS2R4, 7, 10, 14, 39, 40, 43, 44, 46) and denatonium benzoate which activates 8 subtypes (TAS2R4, 8, 10, 13, 39, 43, 46, 47) (380). However, the specificity of many other bitter compounds for different TAS2Rs remains poorly defined, and the specific functions of individual receptor subtypes has not been thoroughly investigated in health or various disease states, e.g. obesity or diabetes.

Another important discovery was that in cell line models of human enteroendocrine L-cells and small and large intestinal tissues, gut hormones, including GLP-1 and PYY, are co-expressed with various TAS2Rs (399-402). Similarly, in rats, the co-expression of CCK with TAS2Rs has been reported in the taste buds of the posterior tongue (403). Moreover, activation of intestinal TAS2R by bitter agonists has been shown to modulate the secretion of gut hormones (399, 404). In enteroendocrine cells, binding of bitter agonists to TAS2Rs initiates an intracellular signalling cascade involving the dissociation of the G-protein, α-gustducin, into Gα and Gβγ subunits (399). The Gα subunit mediates taste by stimulating taste phosphodiesterase, resulting in decrease of the intracellular cAMP level, while Gβγ subunit stimulates phospholipaseβ2 for activation of the 1,4,5-trisphophate and diacylglycerol messengers (391, 399, 405), thereby leading to activation of the inositol trisphosphate receptor in the endoplasmic reticulum and release of intracellular Ca^{2+} into the cytosol (399, 405-408). The activation of the IP3 and DAG messengers is followed by Na^{+} influx through the membrane cation channel, transient receptor potential M5 (406, 409), which depolarizes the cells and causes the release of gut hormones and neurotransmitters, including ATP (399, 406)

(**Figure 1.3**). Once gut hormones are released, they regulate a range of important functions, including GI motility, energy intake and postprandial blood glucose, as explained in section 1.3.3.

One of the important factors that may influence the metabolic effects of bitter compounds is genetic variability in the genes involved in TAS2Rs function (410). For example, polymorphisms of TAS2Rs gene that impair the sensitivity to bitterness may be associated with changes in food intake and dysregulation of blood glucose (411-413). However, the details about this factor are outside the scope of this thesis.

A number of studies have evaluated the functional link between activation of TAS2Rs, by different bitter agonist, and GI hormone secretion, which will be summarised in the following section.

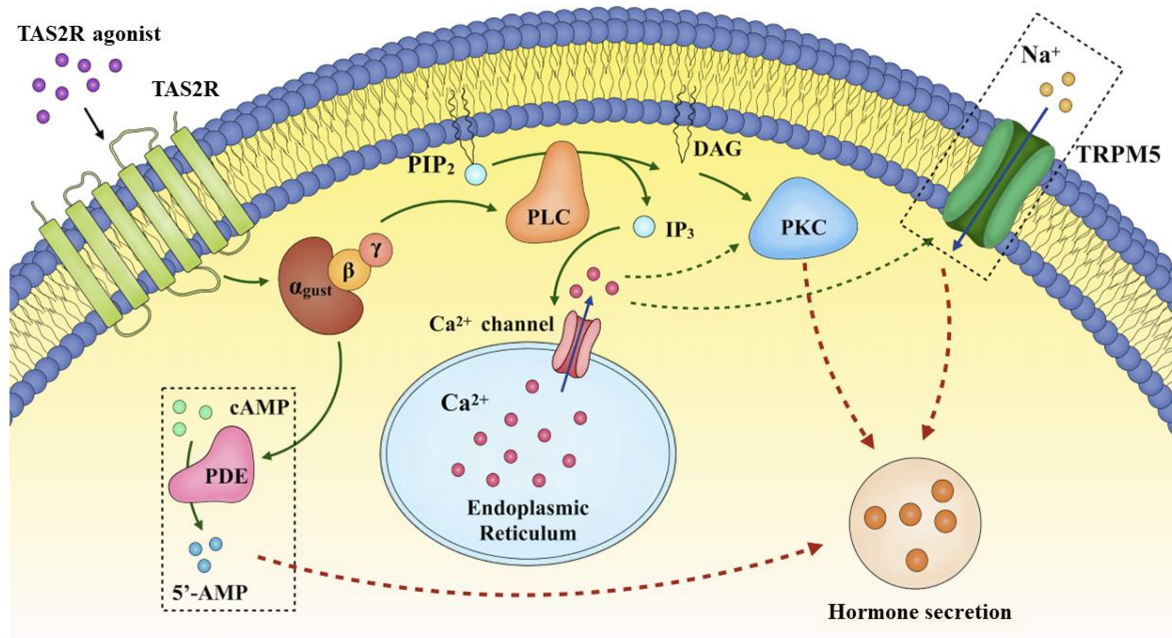

Figure 1.3: Proposed mechanisms underlying enteroendocrine secretion in response to bitter agonists. Binding of ligands (TAS2R agonists) to bitter taste receptors (TAS2R) triggers a signalling cascade involving the dissociation of the G-protein α-gustducin (α-gust) into Gα and Gβγ subunits. The Gα subunit stimulates phosphodiesterase (PDE) resulting in decrease of the intracellular cAMP level, while Gβγ subunit stimulates phospholipaseβ2 (PLC) for activation of the 1,4,5-trisphophate (IP3) and diacylglycerol (DAG) messengers, thereby leading to activation of the inositol trisphosphate receptor (Ca^{2+} channel) in the endoplasmic reticulum and release of intracellular Ca^{2+} into the cytosol and Na^{+} influx through the membrane cation channel Transient Receptor Potential M5 (TRPM5), which depolarizes the cells and causes the release of gut hormones. Adapted from Xie *et al*. (414).

1.5.2 Effects of bitter compounds on appetite- and glucoregulatory hormones

A number of studies, in both preclinical and clinical settings, have investigated the effects of bitter agonists on the secretion of appetite- and/or glucoregulatory hormones, including ghrelin, CCK, GLP-1, and PYY, via activation of various subtypes of TAS2Rs.

1.5.2.1 Preclinical studies on the effects of bitter compounds on appetite- and glucoregulatory hormones

Studies in cell cultures and tissue samples have indicated potent effects of bitter compounds on CCK, GLP-1 and PYY secretion. For example, in mice STC-1 cells, MHBA, matured hop bitter acids, (an oxidised product from bitter components in hops flower, *Humulus lupulus L.*), denatonium benzoate and phenylthiocarbamide stimulated CCK release dose-dependently, denatonium benzoate via activation of TAS2R108, 137, 138, 144, and 135, and phenylthiocarbamide via TAS2R35 (393, 415, 416). The CCK-stimulatory effect of MHBA was associated with increased intracellular Ca^{2+}, an effect eliminated by Ca^{2+} depletion or blockade of Ca^{2+} channels prior to hops treatment (416). In addition, hops bitter compounds have been shown to activate TAS2R1, 14, and 40 (417). In both excised rat intestinal tissue and the human enteroendocrine cell line, HuTu-80, steroid glycosides, extracted from the succulent plant, *Hoodia gordonii*, induced CCK secretion via activation of TAS2R14 (418), an effect abolished by administration of the TAS2R14 antagonist, compound 03A3 (418). A number of bitter compounds have been reported to stimulate GLP-1 secretion. For example, in human enteroendocrine NCI-H716 cells, a natural bitter plant, berberine, and in HuTu-80 cells, phenylthiourea, stimulated GLP-1, both by activating TAS2R38 (391, 401, 405), while, 1,10-phenanthroline stimulated GLP-1 via activation of TAS2R5, and denatonium benzoate via activating a range of TAS2R subtypes, including TAS2R4, 43, and 46 (399, 402). In addition, in mice SCT-1 cells, administration of hops bitter compounds, MHBA and KDT501 (pure

derivate of a hops component called "isohumulone"), stimulated GLP-1 release, with the effects of KDT501 reported to be mediated via activation of TAS2R108 (416, 419). In support of the GLP-1 secretory effects of bitter compounds, blockade of TAS2R signalling, by using small interfering RNA, suppressed the GLP-1-secretory effects of phenylthiourea (401). Also, the TAS2R inhibitor, probenecid, diminished the effects of denatonium benzoate, an extract from *Gentiana scabra* root, and wild bitter gourd to stimulate GLP-1 (399, 420, 421). Probenecid has been shown to inhibit TAS2R16, 38 and 43, but not TAS2R31, in mice (421, 422). Information regarding the effect of bitter compounds on PYY secretion is limited. In mice NCI-H716 cells, denatonium benzoate stimulated PYY release, mediated via activation of TAS2R3, 4 and 5 (399), whereas, in mice STC-1 cells, administration of MHBA had no effect on PYY (416).

Similar to cell line studies, there are promising results from animal studies on the effects of bitter compounds to stimulate gut hormone secretion, including ghrelin, CCK and GLP-1. For example, an intragastric infusion of a bitter mixture, containing denatonium benzoate, quinine hydrochloride, phenylthiocarbamide and salicin, stimulated plasma ghrelin in wild type mice, within 4 hours post-administration, an effect markedly attenuated in α-gustducin-/- mice (423). In contrast, in obese mice treated with a high-fat diet, daily gavage with quinine did not influence plasma ghrelin (424). A number of bitter compounds have been reported to stimulate GLP-1 in rodent models. For example, in mice, acute intragastric administration of denatonium benzoate and wild bitter gourd prior to glucose gavage, at doses of 2 – 5 g/kg, or phenylthiocarbamide alone, stimulated GLP-1 secretion, mediated via activation of TAS2R38 by phenylthiocarbamide, TAS2R4 by denatonium benzoate, and TAS2R1, 2, 3, 4, 6, 8, 9 and 12 by wild bitter gourd (399, 401, 421). In these studies (399, 401), administration of probenecid abolished the effect of denatonium benzoate, but not of phenylthiocarbamide, on

GLP-1, which is surprising because probenecid is known to block TAS2R38, but not TAS2R4 (421, 422). This discrepancy necessitates further investigation to confirm the TAS2R subtypes blocked by probenecid, as well as the possibility that the effects of phenylthiocarbamide might be mediated via pathways other than activation of TAS2Rs. In support of the latter, phenylthiocarbamide has also been shown to strongly induce responses in control cells devoid of bitter receptors (425). In diet-induced mice, acute oral gavage of KDT501 (150 mg/kg), prior to a glucose load (1 g/kg), stimulated GLP-1 levels 3-fold within 15 min of oral gavage, mediated via activation of TAS2R108 (419). Moreover, combined administration of KDT501 (150 mg/kg) with DPP-IV amplified the incretin-based benefits of this bitter compound, supporting the GLP-1-secretory effect of KDT501 (419). In a separate study, chronic (i.e. 17 weeks) treatment of mice with KDT501 (150 mg/kg daily) resulted in a more than 10-fold increase in GLP-1 levels; this effect only became apparent after 4 days of treatment and was then sustained over the treatment period (419). In another long-term study, in obese mice, 4 weeks intragastric administration of denatonium benzoate (60 μmol/kg) remained effective at stimulating GLP-1 secretion, whereas quinine hydrochloride (160 μmol/kg) only had a minimal effect on GLP-1 (424).

1.5.2.2 Clinical studies on the effects of bitter compounds on appetite- and glucoregulatory hormones

Only relatively few clinical studies have evaluated, in detail, the effects of bitter substances on GI hormone secretion in humans. These studies have yielded inconsistent results and modest effect sizes (426-429). For example, intragastric administration of 100 mg of bitter secoiridoids, extracted from the *Gentiana lutea* plant, had no effect on plasma ghrelin, PYY, GIP or insulin, and a trend towards increased GLP-1 secretion, in healthy male and female individuals (430). While, in healthy males, intragastric administration of quinine

hydrochloride, at a dose of 10 μmol/kg (~ 250 mg in a 65 kg person), moderately suppressed fasting plasma ghrelin and motilin concentrations (404), intragastric administration of denatonium benzoate, at a dose of 1 μmol/kg (~ 30 mg in a 65 kg person), suppressed motilin, but had no effect on ghrelin, in healthy females (431). Moreover, in healthy males, 18 mg of encapsulated quinine hydrochloride, ingested orally, increased secretion of CCK after consumption of an *ad libitum* meal, when differences were evaluated in respect to basal value, but not intra-individually between control and quinine (432), whereas, in a further study, 60 min-intraduodenal infusion of quinine hydrochloride (75 mg) had no effect on plasma levels of CCK, GLP-1 or PYY (433). Finally, in a recent study in healthy males, both intragastric and intraduodenal administration of encapsulated supercritical CO_2 extract from hops flowers, *Humulus lupulus L.*, at the dose of 500 mg, modestly, but significantly, enhanced the stimulation of CCK, GLP-1 and PYY in response to both breakfast and lunch 3 hours later, compared with placebo (434).

Taken together, the observations from preclinical studies suggest potent effects of bitter compounds on the stimulation of appetite- and glucoregulatory hormones, however, the data from clinical studies are still limited and less clear. Existing discrepancies may be due to species differences, differences in the characteristics of tested bitter compounds, differences in concentrations, or doses, of bitter compounds used in the studies, i.e. the concentration used in preclinical studies were often much higher than those in clinical studies, and differences in the specificities of bitter compounds for different TAS2R subtypes, amongst others. Therefore, much further research is required to clarify these questions.

1.5.3 Effects of bitter compounds on gut motor function and gastric emptying

Since, as discussed, bitter compounds potently stimulate the release of gut hormones, at least in preclinical models, and given the important role of these hormones in the regulation of GI motor functions and gastric emptying, effects of bitter compounds on aspects of GI motility and gastric emptying have also been evaluated in a limited number of studies.

1.5.3.1 Preclinical studies on the effects of bitter compounds on gut motor function and gastric emptying

The effects of bitter compounds on gastric emptying have been evaluated in a number of preclinical studies. For example, in normal-weight mice, gavage of phenylthiocarbamide (30 µmol/kg, equal to ~ 0.6 µmol in a 20 gr mice) or denatonium benzoate (60 µmol/kg, equal to ~ 1.2 µmol in a 20 gr mice) slowed, whereas, intragastric administration of a much higher dose of denatonium benzoate (10 mM) did not affect, gastric emptying (406, 423). While the reasons for the differences in observed effects of denatonium between the two studies are unclear, in the earlier study (406), the effect of denatonium benzoate, but not phenylthiocarbamide, was abolished by the TAS2R inhibitor, probenecid (50 mg/kg), raising the possibility that the effect of phenylthiocarbamide on gastric emptying might not be mediated via activation of TAS2Rs. In support, in wild type mice, intragastric gavage of phenylthiocarbamide, but not denatonium benzoate or quinine hydrochloride, slowed gastric emptying (11). In this study (11), the effect of phenylthiocarbamide on gastric emptying was unaffected by co-administration of the GLP-1 and CCK antagonists, exendin(9-39)amide and devazepide, respectively, supporting the idea that the effect of phenylthiocarbamide may not be mediated via activation of TAS2Rs on enteroendocrine cells.

A few studies have also evaluated the effect of bitter compounds on contractile activity in the upper GI tract. For example, in gastric tissue of mice, administration of both denatonium benzoate and chloroquine induced contractions in both fundus and antrum; in contrast, phenylthiocarbamide relaxed the fundic muscle and completely inhibited activity in the antrum, while salicin was ineffective (406). Moreover, swertiamarin, an extract from the *Swertia japonica* plant, was shown to stimulate small intestinal motility in mice (435). In the earlier study (406), the contractile pattern induced by denatonium benzoate was related to its effect to slow gastric emptying, whereas this was not seen for phenylthiocarbamide. Thus, it appears that bitter compounds may also vary in their effects on GI motility, possibly because they activate different receptor subtypes, which presumably not all contribute to the regulation of specific functions, including GI motility.

1.5.3.2 Clinical studies on the effects of bitter compounds on gut motor function and gastric emptying

Only a limited number of studies in humans have evaluated the effect of bitter compounds on gastric emptying. In healthy females, sham-feeding of quinine sulfate, at the dose of 10 mg, slowed gastric emptying of an electrolyte soup, compared with sham-feeding of a pleasant strawberry flavour or control (no sham-feeding) (436), although the unpleasant taste of quinine sulfate may have confounded the finding, since, in healthy males, consumption of encapsulated quinine hydrochloride (18 mg) and, in healthy females, intragastric administration of denatonium benzoate (1 µmol/kg equal to ~ 30 mg in a 65 kg person) failed to slow gastric emptying (431, 432). Similarly, in healthy male and female individuals, intragastric administrations of equi-bitter tasting solutions, containing either quinine (1 mM) or naringin (0.198 mM), had no effect on gastric emptying, compared with control (water) (437). In this study (437), both quinine and naringin, at the administered doses, yielded a medium intensity

of bitterness during an oral perception test, raising the possibility that the concentrations used were insufficient to exert any effect on gastric emptying.

There are few human studies evaluated the contractile potency of bitter agonists in different regions of the upper GI tract. For example, intragastric administration of denatonium benzoate (1 µmol/kg, equal to ~ 30 mg in a 65 kg person) impaired fundic relaxation after a mixed-nutrient drink (406), and decreased antral, but not duodenal, motility (431). In addition, intragastric administration of quinine hydrochloride (1 µmol/kg, equal to ~ 250 mg in a 65 kg person) reduced fluctuations in antral motility, and did not affect duodenal motility (438).

Despite of the limited and inconsistent data across preclinical and clinical studies, in general, preclinical studies report a slowing effect, while the few clinical studies that have been performed do not appear to find any substantial effect, of bitter compounds on gastric emptying. As with the differences in effects on gut hormones, further studies are required to establish the effects of bitter compounds.

1.5.4 Effects of bitter compounds on energy intake

A number of bitter compounds have been reported to have energy intake-suppressant effects, possibly, at least in part, related to their effects on GI functions, including secretion of appetite-regulatory hormones and slowing of gastric emptying, with potential implications as novel therapeutic strategies for body weight control. The absence of calories in bitter compounds represents a considerable asset for this approach, as their consumption does not provide additional calories for a substantial effect.

1.5.4.1 Preclinical studies on the effects of bitter compounds on energy intake

A number of preclinical studies have evaluated the effect of bitter compounds on energy intake and their capacity to reduce body weight. Acute intragastric administration of a mixture of bitter compounds, containing denatonium benzoate, phenylthiocarbamide, quinine hydrochloride and salicin, resulted in suppression of food intake, which was sustained for 4 hours, in male mice (423). Moreover, in rats, chronic oral gavage of an extract from *Hoodia gordonii* plant (6.25-50 mg/kg), quinine sulfate (diet containing 0.75%), or an extract from hops flower, *Humulus lupulus L.*, (diet containing 2%) resulted in a decrease in food intake from an *ad libitum* meal and reduction in weight gain over the study periods (439-442). Similarly, in rats, fed with a high-fat diet, 4 weeks i.v. injection of berberine (187.5 or 562.5 mg/kg) reduced both food intake and weight gain, compared with the high-fat diet (443). These outcomes suggest that bitter compounds, indeed, have the capacity to reduce food intake, as well as weight; however, the effect on body weight may not necessarily be as a consequence of a reduction in energy intake, but could be due to other effects, e.g. increased energy expenditure (444). In support, in a study in diet-induced obese mice, 4 weeks intragastric gavage with either quinine (160 μmol/kg) or denatonium benzoate (60 μmol/kg) resulted in less weight gain, although energy intake was only reduced by denatonium benzoate, but not quinine (424), suggesting that the effect of quinine on reducing weight gain may not be due to suppression of energy intake, but possibly mediated via other mechanisms.

1.5.4.2 Clinical studies on the effects of bitter compounds on energy intake

Few studies have evaluated the effects of bitter compounds on energy intake in humans. For example, after intragastric administration of denatonium benzoate (1 μmol/kg, equal to ~ 30 mg in a 65 kg person) participants felt satisfied ~ 64 min earlier and ingested ~ 129 mL less of a mixed-nutrient drink, compared with placebo treatment (406). While consumption of an

encapsulated extract of bitter secoiridoids, derived from the root of *Gentiana lutea* plant, with a standardised breakfast (biscuits, 20% of total daily energy intake), did not affect energy intake at the *ad libitum* lunch, offered 3 hr after the breakfast, it led to ~ 252 kcal reduction in energy intake over the rest of the day (post lunch) (430). In healthy males and females, intragastric administration of a low dose of quinine hydrochloride (18 mg) reduced energy intake by ~ 82 kcal at a subsequent *ad libitum* buffet meal (432), and at much higher dose (~ 250 mg), by ~ 67 kcal at a subsequent *ad libitum* liquid meal (chocolate milk shake), in healthy females (404). In apparent contrast, a 60-min intraduodenal infusion of 75 mg quinine hydrochloride, infused at the rate of 2 mL/min, did not affect energy intake at a subsequent *ad libitum* lunch (433). Finally, in healthy males, both intragastric and intraduodenal administration of a supercritical CO_2 extract from hops flower, *Humulus lupulus L.* (500 mg) prior *to ad libitum* lunch (consumed 1 hr later) and snack (consumed 3 hr latter), significantly reduced total (lunch and snack) energy intake by 217 kcal and 225 kcal, respectively (434).

While these studies provide evidence that some bitter compounds reduce energy intake, there are still important issues which have not been addressed or have yielded inconsistencies among these studies, including their effects across a range of doses, or their regional effects (that is administered into the stomach as opposed to the duodenum), as well as the relationship between the effects on energy intake and changes in GI functions.

1.5.5 Effects of bitter compounds on blood glucose

The effects of bitter compounds on blood glucose have received increasing interest, because preclinical studies have reported potent effects to stimulate gut hormones, particularly GLP-1, and, to a lesser extent, to slow gastric emptying, the major determinants of postprandial blood glucose. However, knowledge in this area is still limited.

1.5.5.1 Preclinical studies on the effects of bitter compounds on blood glucose

Few preclinical studies have indicated that bitter compounds and stimulation of intestinal TAS2Rs has the potential to regulate blood glucose. For example, in db/db mice, oral administration of denatonium benzoate (1 mg/kg), after glucose gavage (5 g/kg), lowered blood glucose levels by ~ 2 mg/dL, 20 – 40 min post-glucose gavage (399). Similarly, in db/db mice, oral administration of an extract from the root of the *Gentia scabra* plant (containing several bitter compounds such as gentiopicrin, loganic acid and rindoside), immediately before a glucose load (5 g/kg), at a dose of 100 mg/kg, reduced blood glucose by ~ 1 mg/dL, 90 min post-glucose gavage, and at a dose of 300 mg/kg, reduced blood glucose by ~ 2 mg/dL, 40 min after glucose gavage (420). Moreover, in high-fat diet fed mice, 5 weeks on a diet consisting of 5% extract of bitter gourd lowered the blood glucose response to an oral glucose tolerance test (2 g/kg) by ~ 2.5 mg/dL, 30 min after the glucose test (421). In the earlier studies (399, 420, 421), the glucose-lowering effects of denatonium benzoate, or the extracts of *Gentia scabra* root or bitter gourd were associated with stimulation of GLP-1 and insulin secretion. Moreover, the glucose-lowering effect of bitter gourd extract was substantially attenuated by co-administration of the GLP-1 receptor antagonist, exendin(9-39)amide (421), supporting the important role of GLP-1 for glucose lowering by this bitter compound. Finally, in a number of studies in rodents, isohumulones, one of the hops components, has been shown to lower blood glucose via improvement in glucose homeostasis, i.e. improved glucose tolerance and reduced insulin resistance (407, 445); however the role of GI functions, e.g. gut glucoregulatory hormone secretion or gastric emptying, in the glycaemic effect of this bitter compound has not been evaluated. In fact, despite of the well-established role of gastric emptying in the regulation of blood glucose (446), no animal study has evaluated this relationship.

1.5.5.2 Clinical studies on the effects of bitter compounds on blood glucose

Despite of the promising information from preclinical studies, the effects of bitter substances on postprandial blood glucose have not been evaluated in humans. While hypoglycaemia is a known side effect in patients with malaria treated with quinine, at the therapeutic dose of ~ 500 mg, administered intravenously, which also stimulates insulin (447-449), i.v. administration bypasses the GI tract, hence, does not involve GI bitter receptors. Thus, the mechanism(s) of action of quinine in stimulating insulin, as well as the effects of quinine administered into the GI lumen on blood glucose, remain unknown. Moreover, the contributions of GI functions, including gut hormones (particularly GLP-1), gut motility and gastric emptying, require investigation.

Taken together, despite the available evidence from preclinical and, to a lesser extent, clinical studies, the effects of bitter compounds, particularly quinine and hops extract, on appetite- and glucoregulatory-hormones secretion, GI motor activity and gastric emptying, as well as the association of these effects with the regulation of energy intake and glycaemia, are still poorly understood. Therefore, further studies, using a range of doses, evaluating administration into different GI regions (e.g. stomach vs. duodenum) and associated GI functions, are required to elucidate the GI-related effects of bitter compounds in humans.

1.6 Aims and hypotheses

To address the inconsistencies in the literature and gaps outlined above, the studies in this thesis aimed to:

1. Investigate the effects of intraduodenal infusion of a range of doses of quinine, given as quinine hydrochloride, on upper GI motor function, gut hormones, appetite perceptions and energy intake, in healthy, lean males. It was hypothesised that quinine would dose-dependently stimulate appetite-regulatory hormones, modulate antro-pyloroduodenal motility and suppress energy intake from a subsequent buffet meal (**Chapter 2**).

2. Investigate the effects of intragastric administration of quinine on the glycaemic response to, and gastric emptying of, a mixed-nutrient drink and the effects on subsequent energy intake, in healthy, lean males. It was hypothesised that quinine would dose-dependently lower the blood glucose response to a mixed-nutrient drink, associated with slowing of gastric emptying and stimulation of gut and pancreatic glucoregulatory hormones, and decrease subsequent energy intake from a buffet meal (**Chapter 3**).

3. Investigate the effects of intragastric and intraduodenal administration of quinine on the glycaemic response to, and gastric emptying of, a mixed-nutrient drink, in healthy, lean males. It was hypothesised that delivery of quinine to the duodenum might differentially affect the release of glucoregulatory hormones and gastric emptying, and consequently postprandial glycaemia, compare with delivery to the stomach (**Chapter 4**).

4. Investigate the effects of intragastric and intraduodenal administration of a supercritical CO_2 extract from hops flowers, *Humulus lupulus L.*, on upper GI motor function, gut hormones, appetite perceptions, and energy intake, in healthy, lean males. It was hypothesised that intraduodenal hops extract would stimulate appetite-regulatory hormones and antropyloroduodenal motility, and suppress energy intake from a subsequent buffet meal greater than intragastric hops extract (**Chapter 5**).

Addressing these hypotheses will substantially advance current knowledge on the effects of the selected bitter compounds on the modulation of GI functions, such as GI appetite- and glucoregulatory hormones release, gut motility and gastric emptying, as well as pancreatic glucoregulatory hormones, and the potential association between these effects and the regulation of energy intake and/or blood glucose. Moreover, the work will provide evidence for the potential utility of these bitter compounds as novel strategies for the treatment or management of obesity and type 2 diabetes mellitus.

Chapter 2: Effects of intraduodenal infusion of the bitter tastant, quinine, on antropyloroduodenal motility, plasma cholecystokinin, and energy intake in healthy men

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Published in:

Journal of Neurogastroenterology and Motility

2019; 25(3): 413-422

Statement of Authorship

Title of Paper	Effects of intraduodenal infusion of the bitter tastant, quinine, on antropyloroduodenal motility, plasma cholecystokinin, and energy intake in healthy men
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Bitarafan V, Fitzgerald PCE, Little TJ, Meyerhof W, Wu T, Horowitz M, Feinle-Bisset C., J Neurogastroenterol Motil. 2019; 25(3):413-422.

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Overall percentage (%)	50%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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2.1 Abstract

Background/Aims: Nutrient-induced gut hormone release (e.g. CCK) and the modulation of gut motility (particularly pyloric stimulation) contribute to the regulation of acute energy intake. Non-caloric bitter compounds, including quinine, have recently been shown in cell-line and animal studies to stimulate the release of GI hormones by activating bitter taste receptors expressed throughout the GI tract, and thus, may potentially suppress energy intake without providing additional calories. This study aims to evaluate the effects of intraduodenally administered quinine on antropyloroduodenal pressures, plasma CCK and energy intake.

Methods: Fourteen healthy, lean men (25 ± 5 years; BMI: 22.5 ± 2.0 kg/m2) received on 4 separate occasions, in randomized, double-blind fashion, 60-minute intraduodenal infusions of quinine hydrochloride at doses totaling 37.5 mg ("Q37.5"), 75 mg ("Q75") or 225 mg ("Q225"), or control (all 300 mOsmol). Antropyloroduodenal pressures (high-resolution manometry), plasma CCK (radioimmunoassay), and appetite perceptions/GI symptoms (visual analog questionnaires) were measured. *ad libitum* energy intake (buffet-meal) was quantified immediately post-infusion. Oral quinine taste-thresholds were assessed on a separate occasion using 3-alternative forced-choice procedure.

Results: All participants detected quinine orally (detection-threshold: 0.19 ± 0.07 mmol/L). Intraduodenal quinine did not affect antral, pyloric or duodenal pressures, plasma CCK (pmol/L [peak]; control: 3.6 ± 0.4, Q37.5: 3.6 ± 0.4, Q75: 3.7 ± 0.3, Q225: 3.9 ± 0.4), appetite perceptions, GI symptoms or energy intake (kcal; control: 1088 ± 90, Q37.5: 1057 ± 69, Q75: 1029 ±7 0, Q225: 1077 ± 88).

Conclusion: Quinine, administered intraduodenally over 60 minutes, even at moderately high doses, but low infusion rates, does not modulate appetite-related GI functions or energy intake.

2.2 Introduction

Dietary nutrients are known to potently modulate GI functions, including gut hormones and motor functions that underlie the slowing of gastric emptying, resulting in reductions in energy intake and blood glucose (142, 450, 451). There is increasing interest in the effects of bitter compounds to stimulate these GI functions by activating subtypes of the TAS2R family of G protein-coupled receptors expressed on enteroendocrine cells throughout the human GI tract (406, 414, 438, 452), particularly given the potential to suppress energy intake without providing additional calories.

The outcomes of studies in both cell lines and experimental animals suggest potent effects of bitter agonists to modulate gut hormone release (393, 399, 423). For example, denatonium benzoate and phenylthiocarbamide have been reported to stimulate CCK secretion from mouse STC-1 cells (393) and denatonium benzoate to stimulate GLP-1 and PYY secretion from both human NCI-H716 cells and isolated duodenal tissue from mice (399). Moreover, intragastric denatonium benzoate and phenylthiocarbamide, but not quinine hydrochloride (QHCl), stimulate the release of ghrelin in mice (423). Interestingly, in the latter study, phenylthiocarbamide, but not denatonium benzoate or QHCl, slowed gastric emptying, which was unaffected by administration of either the CCK-A receptor antagonist, devazepide, or the GLP-1 antagonist, exendin(9-39)amide (423). Finally, in mice a mixture of bitter tastants, including QHCl, denatonium benzoate, and phenylthiocarbamide, increased food intake in the first 30 minutes post-administration, while during the subsequent 4 hours there was a marked suppression of food intake (423). It is, however, important to note that some bitter agonists used in these studies (e.g. phenylthiocarbamide (393)), at the doses administered, have, in other studies, induced strong Ca^{2+} responses in control cells devoid of bitter receptors (425), thus, some of the reported effects may not be specific to bitter receptor activation.

In humans, studies relating to the effects of bitter agonists on gut hormone secretion, GI motility and/or energy intake have yielded inconsistent results and, if any, modest effects (404, 432, 438, 452). Intragastric administration of 18 mg QHCl in an acid-resistant capsule, had no effect on absolute plasma CCK concentrations, while the change in plasma CCK relative to baseline was slightly greater 30 minutes after consumption of a standardised meal following quinine compared with control (432), and intraduodenal infusion of a higher dose (75 mg) did not affect plasma CCK, GLP-1, or PYY (452). Intragastric administration of 10 µmol/kg QHCl (~ 250 mg in a 65 kg person) reduced plasma motilin and ghrelin moderately (438), while denatonium benzoate (431), or intraduodenal QHCl (432), had no effect on ghrelin. Intragastric administration of denatonium benzoate impaired fundic relaxation (406) and decreased antral, but not duodenal, motility (431), while QHCl reduced 'fluctuations in antral motility' (438). Finally, intragastric QHCl (18 mg) modestly reduced energy intake (432) and, at the much higher dose of ~ 250 mg, the amount consumed of a palatable chocolate milk shake in healthy women (404), while intraduodenal QHCl at an intermediate dose (75 mg) had no effect (452).

While these studies provide evidence that some bitter compounds modulate GI functions and energy intake, there are important issues which have not been addressed, including the effects of quinine across a wide range of doses, the relevance of the location of delivery (i.e. stomach vs small intestine), and also whether any effects on energy intake are related to those on GI hormone and/or motor functions. The aim of this study was, therefore, to investigate the effects of intraduodenal infusion of a wide range of doses of quinine, given as QHCl, on upper GI motor function, gut hormones, appetite perceptions, and energy intake. We chose intraduodenal infusion because it allows delivery in a standardised fashion, without potential confounding influences from the rate of gastric emptying.

2.3 Materials and methods

2.3.1 Participants

Fourteen healthy, lean, young men (mean age: 25 ± 5 years; BMI: 22.5 ± 2.0 kg/m2) participated in the study. Participants were recruited through flyers placed around the Royal Adelaide Hospital and local universities, and classified advertisements placed in local newspapers. Exclusion criteria were smoking, consumption of > 20 g alcohol/day, any medical condition, surgery, or the use of medications known to affect energy intake, appetite, or GI function. All participants were required to be weight-stable (< 5% change in body weight) for at least 3 months before participation, and unrestrained eaters with a score of 12 on the eating-restraint component of the Three-Factor Eating Questionnaire (453). The Central Adelaide Local Health Network Human Research Ethics Committee approved the study protocol (Protocol No. HREC/16/RAH/410), and the study was performed in accordance with the Declaration of Helsinki. Each participant provided informed, written consent before their inclusion. Once a participant was enrolled into the study, they were assigned to a randomized treatment order generated by a research officer who was not involved in the data analysis, using an online tool (454). The study was registered as a clinical trial with the Australian New Zealand Clinical Trial Registry (Trial No. 12617000719336) (455).

2.3.2 Study outline

The study evaluated the dose-related effects of 60-minute intraduodenal infusions of QHCl delivering either (1) 37.5 mg ("Q37.5", 0.625 mg/min), (2) 75 mg ("Q75", 1.25 mg/min) or (3) 225 mg ("Q225", 3.75 mg/min), or (4) 0.9% saline ("control"), on antropyloroduodenal pressures, plasma CCK, appetite perceptions, GI symptoms and energy intake in healthy men (**Figure 2.1**).

2.3.3 Test Solutions

QHCl solutions were prepared by dissolving 0.047 g, 0.094 g or 0.281 g QHCl (Sinkona Indonesia Lestari, West Java, Indonesia) and 1.44 g, 1.44 g, or 1.36 g NaCl, respectively, in 150 mL distilled water to achieve the required loads. The control solution contained 1.45 g NaCl in 150 mL distilled water. All solutions were isotonic (300 mOsmol) and administered intraduodenally at a rate of 2 mL/min for 60 minutes. The 2 lower doses of QHCl (37.5 mg and 75 mg) were selected based on studies in healthy subjects in which 18 – 75 mg QHCl reportedly stimulated CCK secretion (432), and reduced (432), or had no effect on energy intake (452). The dose of 225 mg was chosen based on recent reports that QHCl at ~ 250 mg reduced the release of both motilin and ghrelin as well as hedonic eating moderately (404, 438).

2.3.4 Study protocol

Each participant was studied on 4 occasions, separated by 3-10 days, in a randomized, double-blind fashion. Participants were instructed to refrain from vigorous exercise and alcohol consumption for 24 hours before each study and were provided with a standardised meal (Beef Lasagne; McCain Food, Wendouree, Victoria, Australia; energy content: 602 kcal) to be consumed by 7:00 PM on the night before each visit.

On each study day, the participant arrived at the Clinical Research Facility at the Adelaide Medical School, University of Adelaide, at 8:00 AM after an overnight fast (from food and fluids except water after 7:00 PM, and from water after 6:30 AM). On arrival, an i.v. cannula was placed in a right forearm vein for blood sampling, and the participant was intubated with a small diameter (external diameter: 3.5 mm), 17-channel manometric catheter (Dentsleeve International, Mui Scientific, Mississauga, Ontario, Canada), which was inserted into the stomach through an anaesthetized nostril and allowed to pass into the duodenum by peristalsis

(196). The manometric catheter consisted of 16 side-holes spaced at 1.5 cm intervals, measuring pressures in the antrum, pylorus, and duodenum (285, 456). An additional channel was positioned ~ 14.5 cm distal to the pylorus and used for intraduodenal infusion of QHCl or control solutions. The correct positioning of the catheter was maintained by continuous measurement of the transmucosal potential difference between the most distal antral, and the most proximal duodenal, channels (457). Once the catheter was positioned correctly (within 59 ± 4 minutes across study days and participants) and immediately after the occurrence of a phase III of the interdigestive MMC (time from catheter insertion to the occurrence of phase III (minutes); control: 67 ± 7, Q37.5: 72 ± 11, Q75: 84 ± 16, Q225: 76 ± 14, NS), during phase I (a period of motor quiescence), a baseline blood sample was collected, and the participant completed a visual analogue scale (VAS) questionnaire to assess appetite-related perceptions (hunger, fullness, desire to eat, prospective food consumption), and GI symptoms (nausea, bloating) (458), and fasting motility was monitored continuously for 10 minutes (t = -10 – 0 minutes). At t = 0 minutes, intraduodenal infusion of either QHCl or control commenced for 60 minutes. During the infusion, antropyloroduodenal pressures were recorded continuously, while blood samples for measurement of gut hormones and blood glucose were collected, and VAS questionnaires completed every 15 minutes. At t = 60 minutes, the intraduodenal infusion was terminated and the participant extubated. The participant was then presented with a standardised, cold, buffet-style meal and instructed to consume as much, or as little, food as they wished until they felt comfortably full, for up to 30 minutes (t = 60 – 90 minutes) (459). The meal comprised 4 slices (~ 120 g) of whole-meal bread, 4 slices (~ 120 g) of white bread, 100 g sliced ham, 100 g sliced chicken, 85 g sliced cheddar cheese, 100 g lettuce, 100 g sliced tomato, 100 g sliced cucumber, 22 g mayonnaise, 20 g margarine, 1 apple (~ 170 g), 1 banana (~ 190 g), 175 g strawberry yogurt, 100 g chocolate custard, 120 g fruit salad, 375 mL iced coffee, 300 mL orange juice, and 600 mL water. The buffet meal had a total energy content of

~ 2300 kcal (~ 27% fat, ~ 52% carbohydrate, and ~ 21% protein) and weight of ~ 2924 (459). At t = 90 minutes, after completion of the meal, a final blood sample was taken, and the VAS completed, and the participant was then allowed to leave the laboratory.

On a separate day, oral taste detection thresholds for QHCl were quantified (460).

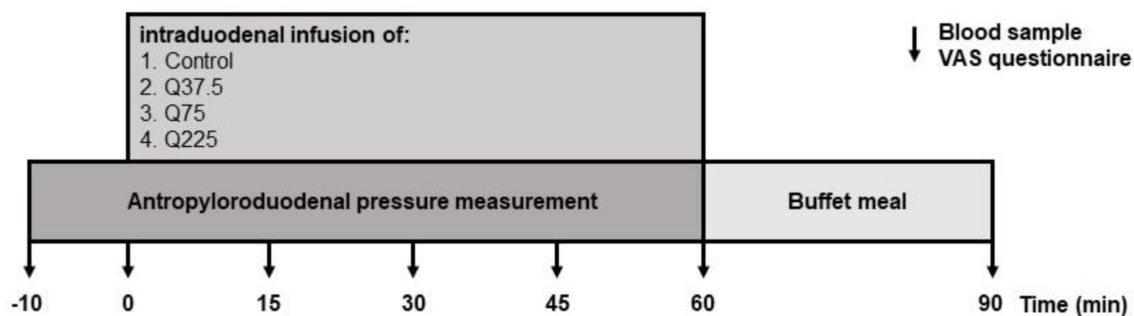

Figure 2.1. Schematic representation of the study design. At t = -10 minutes, immediately after the occurrence of a phase III, ie, during phase I, with the multi-lumen manometric catheter positioned across the pylorus, a baseline blood sample was collected, and the participant completed a visual analogue scale (VAS) questionnaire for the assessment of appetite-related perceptions. The recording of pressures in the antropyloroduodenal region was also commenced. At t = 0 minutes, intraduodenal infusion of quinine hydrochloride at 37.5 mg (Q37.5), 75 mg (Q75), 225 mg (Q225), or control commenced for 60 minutes. During the infusion, antropyloroduodenal pressures were recorded continuously, and blood samples and VAS ratings were collected at 15-minute intervals. At t = 60 minutes, the intraduodenal infusion was terminated, and the catheter removed. The participant was then presented with a buffet-style meal and instructed to eat until he was comfortably full. At t = 90 minutes, another blood sample was collected, and a VAS questionnaire administered, after which the participant was free to leave the laboratory.

2.3.5 Measurements

2.3.5.1 Oral quinine taste-thresholds

Detection thresholds for QHCl were determined using the ascending series 3-Alternate Forced Choice technique (460). Participants were asked to abstain from food, beverages and oral care products for at least 2 hours prior to the test, and to prevent confounding from non-taste sensory inputs, all tests were conducted while wearing nose clips. Taste samples were prepared by

adding QHCl at varying concentrations (0.00125, 0.0025, 0.0125, 0.025, 0.075, 0.25, 0.75, 1.5, 3, 7.5, 15, 30, and 75 mmol/L) to distilled water (461). Control samples consisted of distilled water. Samples were prepared fresh on the day of testing. Participants were presented with 3 samples per set, 2 controls and 1 containing QHCl, in ascending order from the lowest to the highest concentration. Participants rinsed their mouth with distilled water before beginning the task and between each sample set. In each set, participants were asked to identify the "odd" sample. If incorrect, they were presented with 3 samples at the next higher concentration and, if correct, with 3 more samples at the same concentration. This procedure continued until the participant identified the odd sample at a given concentration 3 consecutive times, and that concentration was defined as their detection threshold for QHCl.

2.3.5.2 Appetite perceptions, gastrointestinal symptoms, and energy intake

Appetite perceptions (including hunger, fullness, desire to eat, and prospective food consumption) were quantified with validated 100-mm VAS questionnaires (458). Nausea and bloating were also assessed. Energy intake (kcal) was calculated from the amount of food and liquids (g) consumed at the buffet meal, measured by weighing each food item before presentation and at the end of the meal, using commercial software (FoodWorks 8.0; Xyris Software, Highgate Hill, Queensland, Australia) (459).

2.3.5.3 Antropyloroduodenal motility

Antropyloroduodenal pressures were digitized and recorded using a computer-based system running commercially available software (Solar GI, MMS Data base software, version 8.17; Medical Measurement Systems BV, Enschede, The Netherlands), and stored for subsequent analysis. The number and amplitude of antral, isolated pyloric, and duodenal pressure waves, as well as basal pyloric pressure, using custom-written software modified to our requirements

(kindly provided by Professor Emeritus A Smout, University Medical Centre, Amsterdam, The Netherlands), as described previously (174).

2.3.5.4 Plasma cholecystokinin and blood glucose concentrations

Blood samples were collected into ice-chilled Ethylenediaminetetraacetic acid-coated tubes. Plasma was separated by centrifuga-tion at ~ 1830 g for 15 minutes at 4°C within 15 minutes of collection and stored at -70°C until assayed.

Plasma CCK-8 concentrations (pmol/L) were measured by radioimmunoassay after ethanol extraction using an adaptation of the method of Santangelo et al (462). Intra- and inter-assay coefficients of variation (CVs) were ~ 9.2% and 13.7%, respectively. The detection limit was 1 pmol/L.

Blood glucose was determined with a portable glucometer (Medisense Precision QLD; Abbott Laboratories, North Ryde, New South Wales, Australia), and was quantified because of the potential of quinine to cause hypoglycaemia.

2.3.5.5 Statistical methods

The number of participants was determined by power calculations based on our previous work (286). We calculated that with 14 participants, we would be able to detect a 15% decrease in energy intake at $\alpha = 0.05$, with a power of 80%. Baseline values for all data were calculated as means of values obtained between t = -10 and t = 0 minutes. For antral, isolated pyloric, and duodenal pressure waves, total numbers, and mean amplitudes were calculated over the 60-minute infusion period. Number and amplitude of antral and duodenal pressure waves were used to calculate motility indices (MI) using the following equation: MI (mmHg·number) =

natural logarithm {[sum of amplitudes × number of contractions (pressure waves)] + 1} (463). Basal pyloric pressures were averaged over the 60-minute infusion period. Plasma CCK and blood glucose concentrations were measured in duplicate and expressed as means at each time point. Statistical analyses were performed with SPSS software (version 24.0; IBM Corp, Somers, NY, USA). VAS scores, plasma CCK and blood glucose concentrations were analysed using repeated measures 2-way analysis of variance (ANOVA), with treatment (Q37.5, Q75, Q225, and control) and time (0 – 60 minutes) as factors. Sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P-value was reported. Motility data, energy intake and amount of food and liquids consumed from the test meal were analysed using one-way ANOVA. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects. Comparisons of post-meal (t = 90 minutes) vs pre-meal (t = 60 minutes) CCK and glucose concentrations were done using Student's paired t test. All data are reported as means ± SEMs. All tests were 2-tailed, and differences were considered statistically significant at $P \leq 0.05$.

2.4 Results

All subjects completed all 4 study visits and tolerated the experimental procedures well, and detected QHCl orally (detection threshold: 0.19 ± 0.07 mmol/L).

2.4.1 Energy intake

There was no effect of treatment on energy intake (kcal) or the amount consumed (g) from the buffet meal (**Table 2.1**).

2.4.2 Appetite perceptions and gastrointestinal symptoms

There were no differences in baseline ratings, or any effect of treatment or time, on ratings of hunger, desire to eat, prospective food consumption, fullness, nausea, or bloating (**Figure 2.2A-F**).

2.4.3 Antropyloroduodenal pressures

Baseline values for antral, pyloric or duodenal pressures did not differ between study days, and there was no effect of treatment on the total number, mean amplitude, or MI, of antral or duodenal pressures, basal pyloric pressure or the number or amplitude of isolated pyloric pressure waves (**Table 2.2**).

2.4.4 Plasma cholecystokinin concentrations

There were no differences in baseline plasma CCK concentrations between study days, and no effect of treatment on plasma CCK. Plasma CCK increased substantially in response to the buffet meal with all treatments (time effect: $P < 0.05$), with no difference between treatments (**Figure 2.3A**).

2.4.5 Blood glucose concentrations

There were no differences in baseline values for blood glucose between study days, and no effect of treatment on blood glucose. In response to the buffet meal, blood glucose increased with all treatments (time effect: $P < 0.05$), with no difference between treatments (**Figure 2.3B**).

Table 2.1. Energy Content and Amount of Food and Liquids Consumed at the Buffet Meal immediately After 60-minute Intraduodenal Infusions of Quinine Hydrochloride or Control

	C	Q37.5	Q75	Q225	P-value
Energy intake (kcal)	1088 ± 90	1057 ± 69	1029 ± 70	1077 ± 88	NS
Amount eaten (g)	1157 ± 90	1161 ± 84	1113 ± 76	1144 ± 98	NS

C, saline control; Q37.5, quinine hydrochloride load of 37.5 mg; Q75, quinine hydrochloride load of 75 mg; Q225, quinine hydrochloride load of 225 mg; NS, nonsignificant.
Data are means ± SEMs (n = 14).
P-values for main treatment effects were determined by one-way ANOVA.

Table 2.2. Number, Amplitude, and Motility Index of Antral and Duodenal Pressure Waves, Basal Pyloric Pressure, and Number and Amplitude of Isolated Pyloric Pressure Waves During 60-minute Intraduodenal Infusions of Quinine Hydrochloride or Control

	C	Q37.5	Q75	Q225	P-value
Antral pressure waves					
Number	39 ± 13	27 ± 11	37 ± 12	29 ± 13	NS
Amplitude (mmHg)	29 ± 6	21 ± 7	30 ± 5	43 ± 11	NS
Motility index (mmHg × min)	8 ± 1	6 ± 1	9 ± 1	7 ± 1	NS
Basal pyloric pressure (mmHg)	1 ± 1	−2 ± 1	0 ± 0	−1 ± 1	NS
Isolated pyloric pressure waves					
Number	21 ± 5	15 ± 3	22 ± 6	16 ± 4	NS
Amplitude (mmHg)	76 ± 9	73 ± 16	59 ± 8	51 ± 10	NS
Duodenal pressure waves					
Number	253 ± 31	266 ± 52	248 ± 32	235 ± 46	NS
Amplitude (mmHg)	26 ± 1	28 ± 2	24 ± 2	25 ± 1	NS
Motility index (mmHg × min)	14 ± 0	14 ± 0	14 ± 0	14 ± 0	NS

C, saline control; Q37.5, quinine hydrochloride load of 37.5 mg; Q75, quinine hydrochloride load of 75 mg; Q225, quinine hydrochloride load of 225 mg; NS, nonsignificant.
Data are means ± SEMs (n = 14).
P-values for main treatment effects were determined by one-way ANOVA.

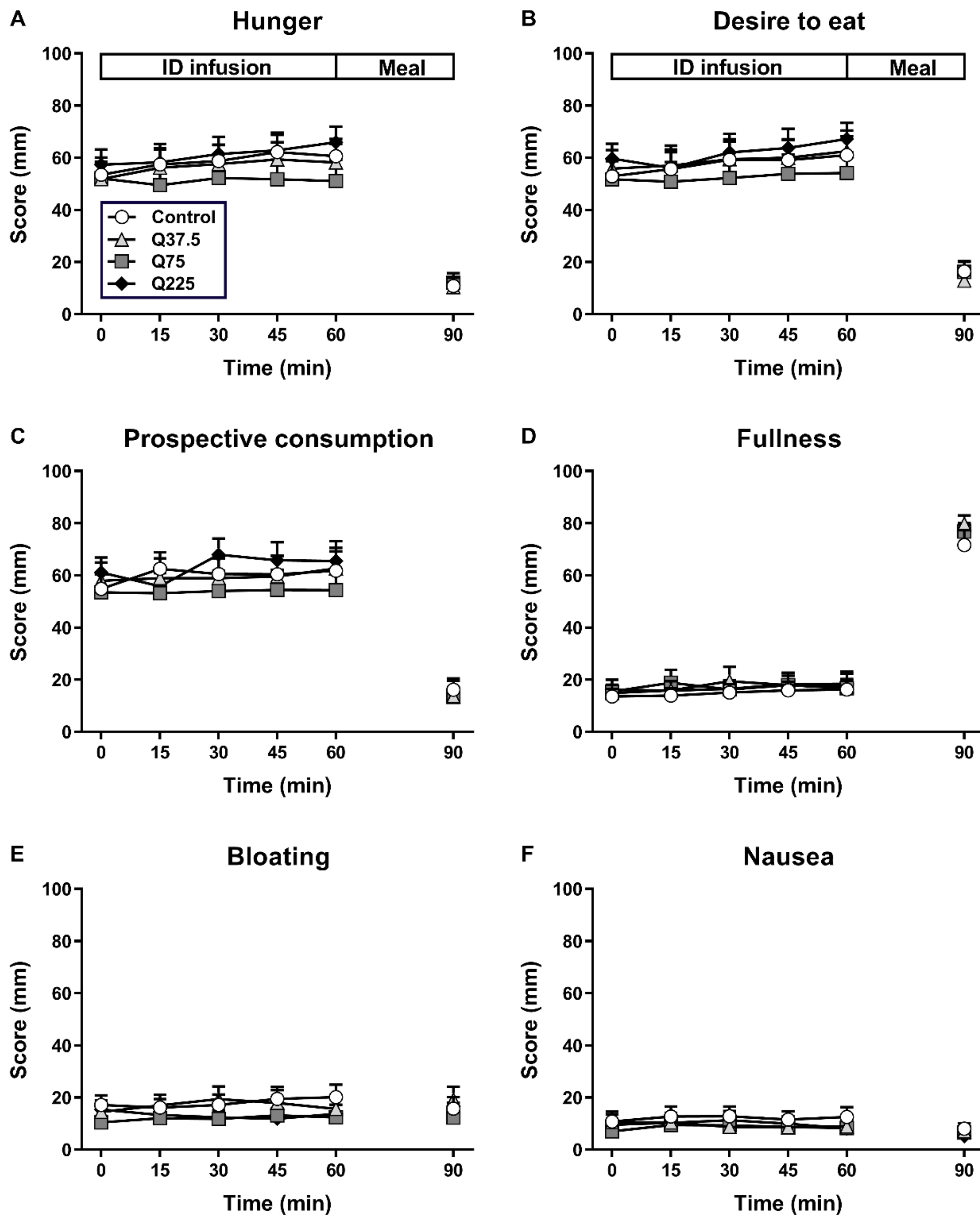

Figure 2.2. Scores for hunger (A), desire to eat (B), prospective food consumption (C), fullness (D), bloating (E), and nausea (F) during 60-minute intraduodenal infusions (t = 0 – 60 minutes) of quinine hydrochloride at 37.5 mg (Q37.5), 75 mg (Q75), 225 mg (Q225), or control, and after the buffet meal, at t = 90 minutes. Two-way ANOVAs, with treatment and time as factors, were used to assess differences between treatments. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were used to determine significant differences between either dose or control. Comparisons of post (t = 90 minutes) vs pre-meal (t = 60 minutes) data were done with Student's paired t test. Data are expressed as means ± SEMs; n = 14. ID, intraduodenal.

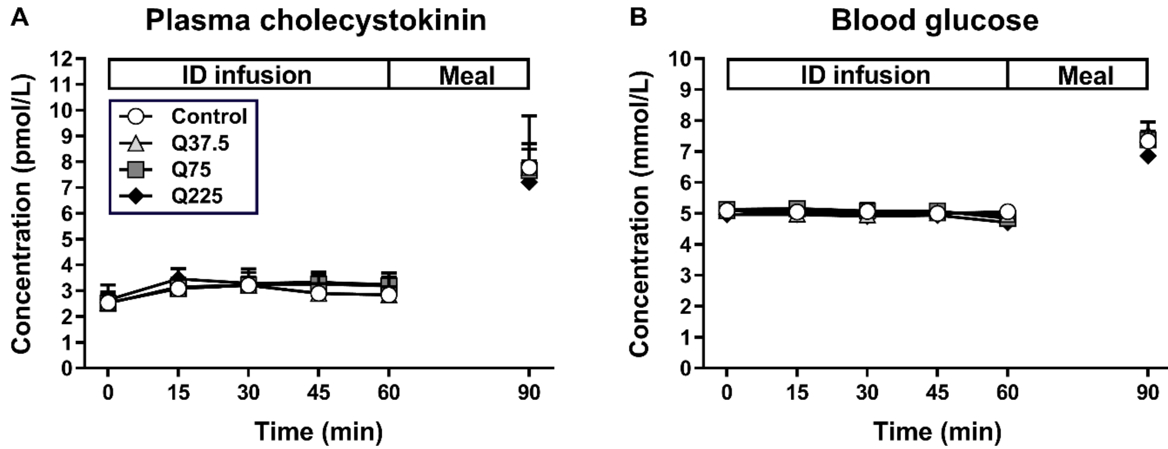

Figure 2.3. Plasma cholecystokinin (A) and blood glucose (B) concentrations during 60-minute intraduodenal infusions (t = 0 – 60 minutes) of quinine hydrochloride at 37.5 mg (Q37.5), 75 mg (Q75), 225 mg (Q225), or control, and after the buffet meal, at t = 90 minutes. Data were analysed using 2-way ANOVA, with treatment and time as factors. Comparison of post (t = 90 minutes) vs pre-meal (t = 60 minutes) concentrations were done with Student's paired t test. Data are expressed as means ± SEMs; n = 14. ID, intraduodenal.

2.5 Discussion

Our study indicates that slow intraduodenal administration of quinine over 60 minutes, at the loads used, does not affect the acute antropyloroduodenal motility, plasma CCK or energy intake responses to quinine, and, therefore, at least under these conditions, does not appear to be a potent stimulus for small-intestinal bitter taste receptors to modulate appetite-related GI functions and energy intake.

There has been much interest in characterising the effects of bitter compounds on gut hormone release (393, 399, 423, 432), since (1) bitter taste receptors are expressed on enteroendocrine cells in the GI lumen, (2) gut hormones, including CCK, GLP-1, and PYY, mediate the effects of nutrients on GI motor function and gastric emptying, and (3) gut hormones play a critical role in the regulation of appetite and energy intake (142, 464). Results from studies in humans have, however, been inconsistent, and effects, if any, appear to be modest (404, 431, 432, 438, 452). We were particularly interested in the effect of quinine on CCK, given the findings in preclinical studies of potent CCK release by other bitter substances (393) and our previous work in humans demonstrating potent release of CCK by dietary nutrients, associated with marked suppression of energy intake (286, 456). In line with the previous studies in humans on the effects of quinine on plasma CCK levels (432, 452), we found no effect of even the highest dose of intraduodenal quinine on plasma CCK concentrations. Thus, our data suggest that QHCl, administered at a slow rate, is not a potent stimulus for enteroendocrine cells that release CCK.

Previous studies on the effects of bitter compounds on GI motor functions have yielded diverse, and inconsistent, outcomes. For example, in mouse gastric tissue, denatonium benzoate and chloroquine stimulated, while phenylthiocarbamide inhibited, antral contractility (406).

Moreover, in mice, intragastric gavage of phenylthiocarbamide, but not denatonium benzoate or QHCl, slowed gastric emptying (423). In humans, intragastric administration of denatonium benzoate has been reported to impair nutrient-induced fundic relaxation (406) and decrease antral, but not duodenal, motility, but to have no effect on gastric emptying of a solid meal (431). Intragastric administration of ~ 250 mg reduced ‘fluctuations in antral motility’ (438). We found that intraduodenal infusion of QHCl, using a wide range of doses that spanned those used in previous studies of the effects of quinine on gut hormone release, motility, and energy intake (432, 438, 452), had no effect on antral, pyloric or duodenal pressures, suggesting that quinine, when administered intraduodenally at a slow rate, at the loads used, does not modulate antropyloroduodenal motility in humans. Since CCK potently modulates upper GI motor function (148, 465, 466), the absence of CCK release in the current study likely explains the lack of effect of QHCl on antropyloroduodenal pressures.

In line with the lack of effect of QHCl to modulate upper GI motility, particularly pyloric pressures, or stimulate CCK, both of which we have identified as key determinants of the subsequent suppression of energy intake in response to duodenal nutrients (450, 467), we found no effect of QHCl to reduce appetite or energy intake. In a recent study, denatonium benzoate reduced hunger ratings in the fasting state, and decreased hunger and increased satiety after a meal, but did not significantly reduce caloric intake from a standardised meal, with substantial variability between individuals (431). Moreover, while intragastric administration of 18 mg QHCl reduced energy intake in one study (432) and, at a much higher dose (~ 250 mg), modestly reduced the amount consumed of a palatable milk shake in healthy females (404), intraduodenal infusion of 75 mg QHCl was ineffective (452). Thus, overall findings are mixed, and more studies are warranted to determine the conditions under which bitter substances,

including QHCl, stimulate GI functions that underlie the regulation of energy intake, and suppress energy intake.

A key issue with the existing research on the effects of bitter compounds on GI function and energy intake is the substantial variation in outcomes between studies, which is potentially attributable to a number of factors. These include variations in the bitter compounds used and the doses applied, administration of the compounds to cell models vs into the stomach or small intestine of humans or animals, and using either bolus administration or slower infusions over time (393, 399, 404, 406, 431, 432, 438, 452). There are currently 25 known subtypes of TAS2Rs in humans (380), and different bitter agonists activate different combinations of receptor subtypes, for example, quinine activates 9 subtypes of TAS2Rs, including TAS2R4, 7, 10, 14, 39, 40, 43, 44, and 46, and denatonium benzoate activates 8 subtypes, including TAS2R4, 8, 10, 13, 39, 43, 46, and 47 (380). The difference in the subtypes of TAS2Rs that are activated by each bitter compound (380), as well as species differences in TAS2Rs (425), are likely to explain some of the differences in observed effects. Stimulation of the release of gut hormones by bitter compounds depends on the presence of specific bitter receptor subtypes on enteroendocrine cells; evidence of the identity, and functions, of some subtypes on enteroendocrine cells is emerging. For example, a recent study in humans reported that TAS2R38s are expressed on human colonic enteroendocrine cells and are colocalized with immunoreactivity for CCK, GLP-1 or PYY (468). Interestingly, neither quinine nor denatonium benzoate activate the TAS2R38 subtype (380). The regional distribution of bitter receptor subtypes across the stomach, small and large intestines may also vary (390). Thus, much more research is required to better understand the roles and functions of the various bitter receptor subtypes in humans, as well as their specific activation by particular bitter compounds. Interindividual variations in bitter taste perception that have been documented for oral taste

(379) may also exist in the gut and, thus, influence the responses to intraluminal bitter compounds. Finally, the GI responses to bitter substances may be influenced by the habitual diet, in analogy to fat (469), with evidence of an inverse relationship between intake of bitter vegetables and the ability to taste the bitter substance, 6-n-propylthiouracil in children (470); furthermore, the ability to taste bitter may also be modified by dietary energy and fat consumption (395, 471).

Some potential limitations of our study should be recognized. We only studied males, as they have been shown to be more sensitive to dietary manipulations (472), while, in contrast, it has been reported recently (431) that females appear to be more sensitive to bitter taste, however, all our volunteers readily detected QHCl. Our study evaluated the effects of intraduodenal, rather than intragastric or oral, QHCl administration, thus, studies are warranted to elucidate the relative contributions of gastric and oral perception of bitter taste to the regulation of GI functions and appetite. We only analysed the effects on plasma CCK concentrations, and not other gut hormones, including GLP-1, PYY, or ghrelin, but given that we found no effects on GI motility or energy intake, we believe that any effects on other hormones would be unlikely.

In conclusion, our study establishes that intraduodenal administration of QHCl, over a wide range of doses, when infused over a 60-minute period, does not affect antropyloroduodenal pressures, plasma CCK or energy intake. Further studies are warranted that evaluate conditions under which quinine may stimulate bitter receptors to modulate appetite-related GI functions and energy intake, including the effects of varying concentrations of quinine, and the relative effects of oral, intragastric and intraduodenal administration.

Chapter 3: Intragastric administration of the bitter tastant quinine lowers the glycemic response to a nutrient drink without slowing gastric emptying in healthy men

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Published in:

American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 2020; 318(2):R263-R273

Statement of Authorship

Title of Paper	Intragastric administration of the bitter tastant quinine lowers the glycemic response to a nutrient drink without slowing gastric emptying in healthy men.
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Bitarafan V, Fitzgerald PCE, Little TJ, Meyerhof W, Jones KL, Wu T, Horowitz M, Feinle-Bisset C. Am J Physiol Regul Integr Comp Physiol. 2020; 318(2):R263-R273.

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Contribution to the Paper	Performed experiments, analysed the data, interpreted results of experiments, prepared figures, drafted manuscript, edited and revised manuscript, and approved the final version of manuscript.		
Overall percentage (%)	50%		
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i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Signature		Date	05/05/2020

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3.1 Abstract

The rate of gastric emptying and the release of GI hormones are major determinants of postprandial blood-glucose concentrations and energy intake. Preclinical studies suggest that activation of GI bitter taste receptors potently stimulates GI hormones, including GLP-1, and thus may reduce postprandial glucose and energy intake. We evaluated the effects of intragastric quinine on the glycemic response to, and the gastric emptying of, a mixed-nutrient drink and the effects on subsequent energy intake in healthy men. The study consisted of 2 parts: part A included 15 lean men, and part B included 12 lean men (aged 26 ± 2 yr). In each part, participants received, on 3 separate occasions, in double-blind, randomized fashion, intragastric quinine (275 or 600 mg) or control, 30 min before a mixed-nutrient drink (part A) or before a buffet meal (part B). In part A, plasma glucose, insulin, glucagon, and GLP-1 concentrations were measured at baseline, after quinine alone, and for 2 h following the drink. Gastric emptying of the drink was also measured. In part B, energy intake at the buffet meal was quantified. Quinine in 600 mg (Q600) and 275 mg (Q275) doses alone stimulated insulin modestly ($P < 0.05$). After the drink, Q600 and Q275 reduced plasma glucose and stimulated insulin ($P < 0.05$), Q275 stimulated GLP-1 ($P < 0.05$), and Q600 tended to stimulate GLP-1 ($P = 0.066$) and glucagon ($P = 0.073$) compared with control. Quinine did not affect gastric emptying of the drink or energy intake. In conclusion, in healthy men, intragastric quinine reduces postprandial blood glucose and stimulates insulin and GLP-1 but does not slow gastric emptying or reduce energy intake under our experimental conditions.

3.2 Introduction

The release of gut hormones and the slowing of gastric emptying, which is induced by ingestion of nutrients, are important in the regulation of postprandial blood glucose and energy intake (142, 473). GLP-1 is of particular interest, as it slows gastric emptying, stimulates insulin in the presence of glucose, and reduces energy intake (205, 212, 474). Gastric emptying is a major determinant of the initial (i.e., within ~ 30 min) glycemic response to carbohydrate-containing meals (446). Moreover, agonists of GLP-1 are now used widely in the management of type 2 diabetes and obesity (475, 476).

There has been substantial interest in the role of bitter-taste receptors (TAS2Rs) to activate these GI functions. TAS2Rs are present in the oral cavity (380, 477), where they are activated by bitter compounds. In humans, 25 subtypes of TAS2Rs have been identified, with different bitter compounds activating varying combinations of receptor subtypes. For example, quinine activates 9 subtypes: TAS2R4, 7, 10, 14, 39, 40, 43, 44, and 46, whereas denatonium benzoate activates 8 subtypes: TAS2R4, 8, 10, 13, 39, 43, 46, and 47 (380). TAS2Rs are also expressed throughout the GI tract, where they are located on enteroendocrine cells (390, 478) and thought to mediate the GI responses to bitter compounds present in the GI lumen. In preclinical studies, a number of bitter compounds potently stimulate the release of GLP-1 by activating TAS2Rs (399, 401, 406). For example, in mice, intragastric administration of denatonium benzoate before an intragastric glucose load augmented circulating GLP-1 concentrations and lowered the glycemic response to the glucose load (399). Moreover, oral administration of an extract of bitter gourd, a tropical vegetable, stimulated GLP-1 and insulin and reduced the glycemic response to intraperitoneal glucose (421). The latter effect was markedly attenuated by concomitant administration of the GLP-1 receptor antagonist exendin(9-39)amide (421), supporting the concept that GLP-1 is integral to glucose lowering by bitter compounds. The

effects of bitter compounds on gastric emptying are inconsistent. For example, although intragastric phenylthiocarbamide appears to slow gastric emptying in mice (406, 423), denatonium benzoate has been reported to either slow gastric emptying (406) or have no effect (423), whereas propylthiouracil and quinine have been found to be ineffective (423). Interestingly, the slowing effect of denatonium benzoate, but not of phenylthiocarbamide, was reversed by coadministeration of the TAS2R antagonist probenecid (406), whereas phenylthiocarbamide-induced slowing of gastric emptying was unaffected by the GLP-1 antagonist exendin(9-39)amide (423), raising the possibility that the effects of some bitter compounds that slow gastric emptying may not be mediated via activation of specific receptors on enteroendocrine cells.

The observations in animals have stimulated interest in the effects of bitter agonists in humans, particularly whether any effects on GI functions may lead to reductions in postprandial blood glucose or energy intake, which would have major implications for novel treatment and management strategies for people with type 2 diabetes and/or obesity (451). Moreover, bitter compounds, unlike dietary nutrients with appetite-regulatory and glucoregulatory effects (e.g., protein), do not provide additional calories and thus do not increase overall energy intake. However, comprehensive studies in humans are limited, and the outcomes of existing studies are inconsistent. For example, in healthy subjects, although intraduodenal infusion of quinine [as QHCl] administered at doses of 37.5 – 225 mg over 60 min did not stimulate GLP-1 or CCK (452, 479), an intragastric bolus of 18 mg QHCl slightly increased plasma CCK after a standardised meal (432). Sham-feeding quinine sulfate (10 mg) was reported to slow gastric emptying of a bland soup (436), although this may reflect its aversive taste, as 18 mg QHCl consumed in an acid-resistant capsule or intragastric denatonium benzoate (1 μmol/kg) both failed to slow gastric emptying of solid meals (431, 432). The effects of bitter substances on

postprandial blood glucose, insulin, or glucagon have hitherto not been evaluated in humans. This is surprising, because quinine, when given intravenously at much higher doses to treat malaria (~ 500 mg, equivalent to ~ 600 mg QHCl), stimulates insulin and frequently induces hypoglycaemia (480, 481); it is not known whether this effect is related to effects on GI functions and/or glucoregulatory hormones. Finally, denatonium benzoate has been reported to reduce hunger both in the fasting and postprandial states but did not affect energy intake from a standardised meal (431). In addition, although intragastric administration of ~ 10 μmol QHCl/kg body weight (~ 275 mg in a 70-kg person) reduced consumption of a palatable milk shake (404), intraduodenal infusion of QHCl delivering doses of 37.5 – 225 mg over 60 min was ineffective (452, 479).

The aim of this study was to investigate the effects of intragastric administration of QHCl, at doses of 275 and 600 mg, on 1) gastric emptying of a mixed-nutrient drink, 2) plasma glucose, insulin, glucagon, and GLP-1 concentrations in response to the drink, and 3) energy intake from a buffet meal. We hypothesized that QHCl would stimulate GLP-1 and insulin release and slow gastric emptying, leading to reduced fasting and postprandial glucose concentrations.

3.3 Materials and methods

3.3.1 Study participants

Healthy, lean, young men participated in this study, 15 (mean age: 26 ± 2 years, BMI: 23.2 ± 0.4 kg/m²) in part A, and 12 (mean age: 26 ± 2 years, BMI: 23.1 ± 0.4 kg/m²) in part B. The number of participants was determined by power calculations based on our previous studies (285, 286). We calculated that n = 15 participants would allow detection of a 1.0 mmol/L reduction in plasma glucose, and n = 12 participants a 170 kcal difference in energy intake, both at $\alpha = 0.05$, with a power of 80%. The parts were performed concurrently, and 11 of the volunteers participated in both parts. Participants were screened before inclusion in the study to exclude GI symptoms or a history of GI disease or surgery, low plasma ferritin or iron levels (as stipulated by the local Human Research Ethics Committee), vegetarians, smokers, alcohol consumption of > 2 drinks (20 g ethanol) on > 5 days/week, use of medications known to affect energy intake, appetite or GI function, high-performance athletes, unstable body weight (≥ 5% change over the last 3 months before participation) and restrained eaters (score >12 on the restrained eating component of the 3-factor eating questionnaire (453)). The study protocol was approved by the Human Research Ethics Committee of the Central Adelaide Local Health Network and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent prior to their inclusion. Once participants were enrolled, they were assigned to a treatment order of balanced randomization that was generated with an online tool (www.randomization.com) by a research officer who was not involved in data analysis. Both study parts were registered as clinical trials with the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au; part A: ACTRN12618000813280, part B: ACTRN12618000814279).

3.3.2 Study design

The study evaluated the dose-related effects of QHCl at doses of 275 mg (Q275) or 600 mg (Q600) or control on 1) gastric emptying of a mixed-nutrient drink, 2) plasma glucose, insulin, glucagon, and GLP-1 concentrations in responses to the drink (**part A; Figure 3.1A**), and 3) appetite perceptions and energy intake from a buffet meal (**part B; Figure 3.1B**). We performed the study in healthy, lean men to establish physiological responses.

3.3.3 Preparation of QHCl and control solutions

The lower dose QHCl solution was prepared by dissolving 275 mg of QHCl (Sinkona Indonesia Lestari, Subang, West Java, Indonesia) and 0.23 g NaCl in 20 mL distilled water, and the high-dose solution was prepared by incorporating 600 mg QHCl powder in 20 mL saline (0.9%). The control consisted of 0.23 g NaCl and 20 mL distilled water. The NaCl content was varied to match the osmolarity (~ 373 mosmol) of the 3 solutions. All solutions had a pH of ~ 7 and were administered at a temperature of ~ 30°C. Solutions were made fresh in the morning of each study day by a research officer who had no involvement in data analysis who filled syringes and administered the solutions via a nasogastric catheter directly into the stomach. The syringes were covered to blind both the study participant and the investigator performing the study as to the nature of the infusate. The 275-mg dose (Q275) was selected based on a recent study that reported an effect on ghrelin suppression and hedonic eating (404), and the dose of 600 mg (Q600; equivalent to ~ 500 mg quinine) was used as a “proof of principle”: it represents the recommended therapeutic dose of quinine in the treatment of malaria (480).

3.3.4 Study protocol

In both parts A and B, each participant was studied on three occasions separated by 3 – 7 days in a randomized, double-blind fashion. Participants were instructed to refrain from strenuous exercise and alcohol consumption for 24 h before each study and were provided with a

standardised meal (beef lasagne; total energy content: 602 kcal; McCain Food, Wendouree, Victoria, Australia) to be consumed by 7:00 PM the night before each study visit. The following morning, participants attended the Clinical Research Facility at the Adelaide Medical School, University of Adelaide, at 8:15 AM after an overnight fast (from food and all fluids except water after 7 PM and from water after 6:30 AM).

On study days in part A, upon arrival, an i.v. cannula was placed into a forearm vein, and the arm was kept warm with a heat pad for regular sampling of "arterialized" blood. participants were then seated in an upright position, and a custom-built nasogastric, soft silicon feeding tube (outer diameter: 4 mm; Dentsleeve International, Mississauga, Ontario, Canada) was inserted through an anesthetized nostril, with the tip placed in the stomach. At t = -31 min (~ 8:30 AM), participants received the 20-mL intragastric bolus of one of the QHCl doses or control, and the nasogastric tube was removed. Thirty minutes later, i.e., at t = -1 min, participants consumed 350 mL of a mixed-nutrient drink within 1 min [Resource plus; Nestle, Tongala, Victoria, Australia (325 mL); 500 kcal, 74 g carbohydrates, including maltodextrin and sucrose, 18 g protein, and 15 g fat, plus 25 mL water to make up the final volume] labeled with 100 mg of [^{13}C]acetate for measurement of gastric emptying by breath test (482). The macronutrient composition of the drink reflects the composition of the "average" Australian diet, thus mimicking the effect of a normal meal. Arterialized venous blood samples for measurement of plasma glucose, insulin, glucagon, and GLP-1 concentrations; VAS questionnaires to assess appetite-related perceptions and GI symptoms; and breath samples for subsequent analysis of $^{13}CO_2$ were collected at baseline (t = -31 min) and at regular time points throughout the study (**Figure 3.1A**). At t = 120 min, the i.v. cannula was removed and the participant was served a light lunch, after which they were free to leave the laboratory.

On study days in part B, upon arrival, participants were provided with a light breakfast (1 slice of wholemeal toast, 11 g peanut butter, and a cup of black tea; ~ 140 kcal), and an i.v. cannula was positioned. At t = -31 min (~ 11:30 AM), participants received a 20-mL intragastric bolus of one of the QHCl doses or control. At t = 0 min, i.e., 30 min later, participants were presented with a standardised, cold, buffet-style meal for the assessment of energy intake and were instructed to eat until they were comfortably full (459). The participants were unaware of the purpose of the meal. VAS ratings were collected at baseline (t = -31 min) and at regular time points throughout the study (**Figure 3.1B**). At t = 90 min, the i.v. cannula was removed, and participants were allowed to leave the laboratory.

On a separate study day, oral taste detection thresholds for QHCl were determined.

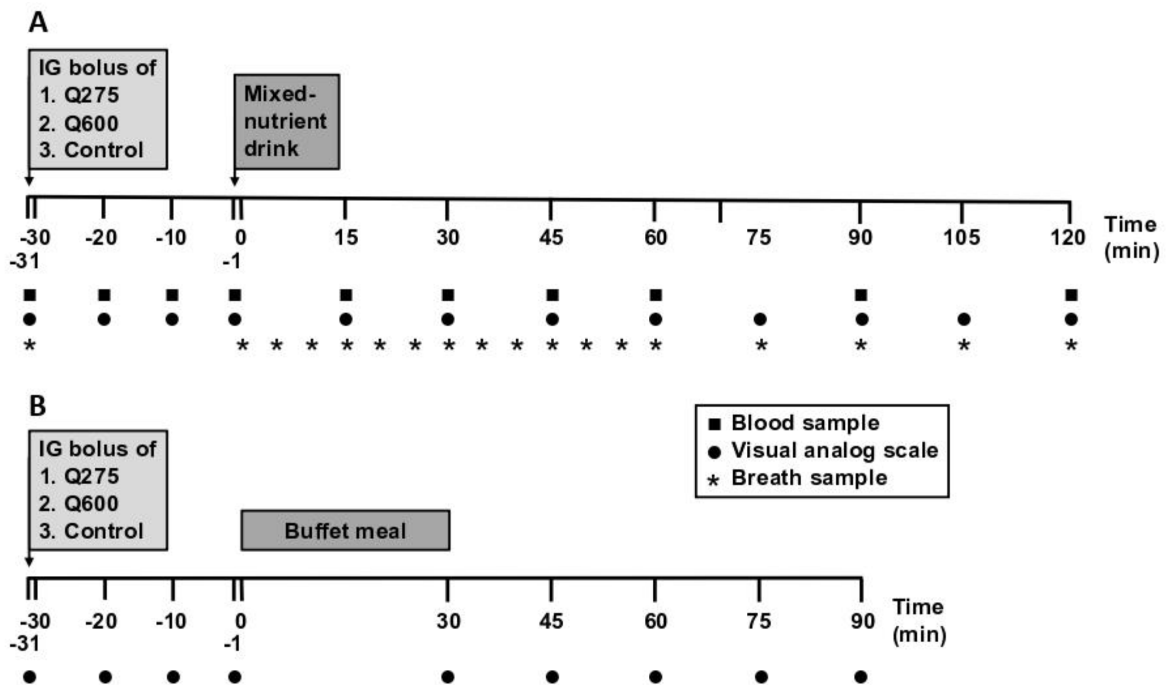

Figure 3.1: Schematic representation of the study design. A: study protocol of part A. B: study protocol of part B. On study days in part A, at t = -31 min, an intragastric (IG) bolus (20 mL) of quinine-HCl at doses of either 275 mg (Q275) or 600 mg (Q600) or control was administered into the stomach via a nasogastric tube within 1 min, and the tube was then removed. Thirty minutes later, at t = -1 min, participants consumed 350 mL of a mixed-nutrient drink containing 500 kcal and 74 g of carbohydrates within 1 min. The drink was labeled with 100 mg of [^{13}C]acetate for measurement of gastric emptying by $^{13}CO_2$ breath test. Arterialized venous blood samples were collected for measurement of plasma glucose, insulin, glucagon, and GLP-1 concentrations, and breath samples were collected at baseline and at regular time points throughout the study as indicated for subsequent analysis of $^{13}CO_2$. At t = 120 min, the intravenous cannula was removed and the participant was served a light lunch, after which they were free to leave the laboratory. On study days in part B, to standardise study conditions, participants were provided with a light breakfast upon arrival in the morning. Three hours later, at t = -31 min, as in part A, participants received the bolus of quinine-HCl or control. At t = 0 min, they were presented with a standardised, buffet-style meal for the assessment of energy intake and instructed to eat until they were comfortably full. Visual analog scale ratings were collected at baseline (t = -31 min) and at regular time points throughout the study, as indicated. At t = 90 min, the intravenous cannula was removed, and participants were free to leave the laboratory.

3.3.5 Measurements

3.3.5.1 Oral quinine taste-thresholds

The ascending-series 3-alternative forced-choice technique was used to quantify detection thresholds for QHCl (460). Participants were asked to abstain from food, beverages and oral care products for at least 2 h before the test, and, to prevent potential confounding from nontaste sensory inputs, all tests were conducted while wearing nose clips. Taste samples were prepared by adding QHCl at varying concentrations (0.00125, 0.0025, 0.0125, 0.025, 0.075, 0.25, 0.75, 1.5, 3, 7.5, 15, 30, and 75 mmol/L) to distilled water (461). Control samples consisted of distilled water. Samples were prepared fresh on the day of testing. Participants were presented with three samples per set, two controls and one containing QHCl, in ascending order from the lowest to the highest concentration. Participants rinsed their mouths with distilled water before beginning the task and between each sample set. In each set, participants were asked to identify the “odd” sample. If incorrect, they were presented with three samples at the next highest concentration and, if correct, with three more samples at the same concentration. This procedure continued until the participant identified the odd sample at a given concentration three consecutive times, and that concentration was defined as their QHCl detection threshold.

3.3.5.2 Plasma glucose and hormone analyses

Blood samples were collected into ice-chilled tubes containing ethylenediaminetetraacetic acid. Plasma was obtained by centrifuging samples at ~ 1832 g-force for 15 min at 4 ºC within 15 min of collection and stored at -80 ºC until subsequent analysis.

Plasma glucose concentrations (mmol/L) were measured by the glucose oxidase method using a glucose analyser (YSI 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, Ohio, USA).

Plasma insulin concentrations (mU/L) were measured using an enzyme-linked immunosorbent assay (ELISA) (10-1113, Mercodia, Uppsala, Sweden). The minimum detectable concentration was 1 mU/L, and intra- and inter-assay CVs were 2.1% and 9.3%, respectively.

Plasma glucagon concentrations (pg/mL) were measured by radioimmunoassay (GL-32K, Millipore, Billerica, Massachusetts, USA). The minimum detectable concentration was 15 pg/mL, and intra- and inter-assay CVs were 3.7% and 2.9%, respectively.

Plasma total GLP-1 concentrations (pmol/L) were measured using a radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA). The minimum detectable concentration was 3 pmol/L, and intra- and inter-assay CVs were 6.8% and 9.7%, respectively.

3.3.5.3 Measurement of gastric emptying

$^{13}CO_2$ concentrations in end-expiratory breath samples were measured using an isotope ratio mass spectrometer (FANCi; Fisher Analysen Instrumente GmbH, Germany) with an online gas chromatographic purification system. The results were expressed as percentage of $^{13}CO_2$ recovery/hour and profiles plotted as cumulative values over the sampling period (482).

3.3.5.4 Appetite perceptions and GI symptoms

Appetite-related perceptions (fullness, hunger, desire to eat and prospective food consumption) were assessed using a validated 100-mm VAS questionnaire (458). Nausea and bloating were also assessed. Each VAS evaluated a sensation on a 100-mm horizontal line, where 0 mm represented “sensation not felt at all” and 100 mm represented “sensation felt the greatest”. Participants rated how they were feeling at a given time-point by placing a vertical stroke at the appropriate point on the line.

3.3.5.5 Energy intake

Energy intake at the buffet meal was calculated from the amount of food and drinks (g) consumed at the meal, obtained by weighing all food items before and after being offered to the participant. The meal comprised 4 slices (~ 120 g) of whole-meal bread, 4 slices (~ 120 g) of white bread, 100 g sliced ham, 100 g sliced chicken, 85 g sliced cheddar cheese, 100 g lettuce, 100 g sliced tomato, 100 g sliced cucumber, 22 g mayonnaise, 20 g margarine, 1 apple (~ 170 g), 1 banana (~ 190 g), 175 g strawberry yogurt, 100 g chocolate custard, 120 g fruit salad, 375 mL iced coffee, 300 mL orange juice and 600 mL water, and had a total energy content of ~ 2300 kcal (~ 27% fat, ~ 52% carbohydrate and ~ 21% protein) and weight of ~ 2924 g (459). Energy intake (kcal) was then calculated using commercial software (FoodWorks 8.0; Xyris Software, Highgate Hill, Queensland, Australia) (459).

3.3.6 Data and statistical analysis

Plasma glucose, insulin, glucagon and GLP-1 concentrations and gastric emptying data were expressed as absolute values, while VAS data were expressed as changes from baseline (i.e. t = -31 min) to account for variations in baseline values. Effects of quinine alone on plasma glucose, insulin, glucagon and GLP-1 data were summarized by calculating areas under the curve (AUC), from t = -31 – -1 min ($AUC_{-31 - -1\ min}$), using the trapezoidal rule. For the response to the mixed-nutrient drink, AUCs from t = 15 – 120 min ($AUC_{15 - 120\ min}$) were calculated for plasma glucose and hormone data, and AUCs from t = 0 – 120 min ($AUC_{0 - 120\ min}$) for gastric emptying. To assess relationships between plasma glucose at t = 30 min with early changes in plasma insulin, glucagon and GLP-1, and gastric emptying, we also calculated AUCs from t = -1 – 30 min ($AUC_{-1 - 30\ min}$) for hormones, and from t = 0 – 30 min ($AUC_{0 - 30\ min}$) for gastric emptying.

Statistical analysis was performed using SPSS software (version 24.0; SPSS Inc). $AUC_{-31 - -1 \, min}$ for plasma glucose and hormones, and $AUC_{15 - 120 \, min}$ for plasma glucose and hormones, $AUC_{0 - 120 \, min}$ for gastric emptying, energy intake and the amount consumed were analysed using repeated-measures one-way ANOVA with treatment as a factor. Plasma glucose, hormone and VAS data were also analysed using two-way repeated-measures ANOVA, with treatment (control, Q275, Q600) and time (time points relevant for individual variables) as factors. Sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P-value was reported. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects. Linear within-subject correlation analysis of pooled quinine and control data was used to calculate correlations between plasma glucose at t = 30 min with $AUCs_{-1 - 30 \, min}$ for hormones or $AUC_{0 - 30 \, min}$ for gastric emptying. All data are reported as means ± SEM. All tests were two-tailed, and differences were considered statistically significant at $P \leq 0.05$.

3.4 Results

All participants completed all study visits of the respective part(s) and tolerated the study conditions well. All participants were able to detect QHCl orally (detection threshold: 0.10 ± 0.08 mmol/L), and, thus, no one was excluded from participation because of an inability to detect QHCl orally. Because of technical problems, plasma glucose data in one participant, plasma GLP-1 data in one participant, and breath test data in two participants were unavailable.

3.4.1 Part A

3.4.1.1 Plasma glucose

There were no differences in fasting plasma glucose between study days (**Figure 3.2A**).

Response to quinine: There was no effect of treatment on plasma glucose concentrations or $AUC_{-31 - -1min}$ (**Figure 3.2A**, **Table 3.1**).

Response to mixed-nutrient drink: Plasma glucose increased on all study days. There was a treatment × time interaction for plasma glucose (P = 0.001) and an effect of treatment on $AUC_{15 - 120min}$ (P = 0.024) (**Figure 3.2A, Table 3.1**). At t = 30 min, both Q600 (P = 0.005) and Q275 (P = 0.001) lowered plasma glucose compared with control, and at t = 45 min, Q275 (P = 0.021) lowered plasma glucose compared with control. Q600, but not Q275, also decreased plasma glucose $AUC_{15 - 120\ min}$ compared with control (P = 0.04).

3.4.1.2 Plasma insulin

There were no differences in fasting plasma insulin concentrations between study days (**Figure 3.2B**).

Response to quinine: There was a treatment × time interaction for plasma insulin (P = 0.019), but no effect on $AUC_{-31 - -1\ min}$ (**Figure 3.2B**, **Table 3.1**). Both Q600 (P = 0.03) and Q275 (P =

0.001) increased plasma insulin at t = -1 min, compared with control, although the magnitude of the effect was small.

Response to mixed-nutrient drink: Plasma insulin increased on all study days. There was a trend for a treatment × time interaction for plasma insulin concentrations (P = 0.082) and an effect of treatment on insulin $AUC_{15-120\ min}$ (P = 0.014) (**Figure 3.2B**, **Table 3.1**). Both Q600 (P = 0.005) and Q275 (P = 0.039) substantially increased plasma insulin at t = 15 min compared with control, and the response to Q600 was sustained until the end of the study. In contrast, post-hoc tests only revealed a trend for Q600 to increase $AUC_{15-120\ min}$ compared with Q275 (P = 0.058).

3.4.1.3 Plasma glucagon

There were no differences in fasting plasma glucagon concentrations between study days (**Figure 3.2C**).

Response to quinine: There was no effect of treatment on plasma glucagon or $AUC_{-31--1\ min}$ (**Figure 3.2C**, **Table 3.1**).

Response to mixed-nutrient drink: Plasma glucagon increased modestly on all study days. There were trends for effects of treatment on plasma-glucagon concentrations (P = 0.065) and glucagon $AUC_{15-120\ min}$ (P = 0.071); Q600 tended to increase plasma glucagon (P = 0.073) and $AUC_{15-120\ min}$ (P = 0.074) compared with control (**Figure 3.2C**, **Table 3.1**).

3.4.1.4 Plasma GLP-1

There were no differences in fasting plasma GLP-1 concentrations between study days (**Figure 3.2D**).

Response to quinine: There was no effect of treatment on plasma GLP-1 concentrations or $AUC_{-31--1\ min}$ (**Figure 3.2D**, **Table 3.1**).

Response to mixed-nutrient drink: GLP-1 increased on all study days, and there were effects of treatment on plasma GLP-1 (P = 0.04) and GLP-1 $AUC_{15-120\ min}$ (P = 0.036); Q600 tended to increase (P = 0.066) and Q275 increased (P = 0.04) plasma GLP-1 and $AUC_{15-120\ min}$ compared with control, although effects were modest overall (**Figure 3.2D**, **Table 3.1**).

3.4.1.5 Gastric emptying

There was no effect of treatment on gastric emptying of the mixed-nutrient drink (**Figure 3.3**, **Table 3.1**).

3.4.1.6 Relationships between plasma glucose with gastric emptying, plasma insulin, glucagon or GLP-1

There were no significant correlations between plasma glucose at t = 30 min with the $AUC_{0-30\ min}$ for gastric emptying (r = 0.2, P = 0.97) or $AUC_{-1-30\ min}$ for insulin (r = 0.08, P = 0.58) or glucagon (r = 0.3, P = 0.08), but there was an inverse correlation with $AUC_{-1-30\ min}$ for GLP-1 (r = 0.4, P = 0.008).

3.4.1.7 Appetite perceptions and GI symptoms

Response to quinine: There were no effects of treatment on hunger, desire to eat, prospective food consumption, fullness, nausea or bloating (**Figure 3.4**).

Response to mixed-nutrient drink: There were no effects of treatment on hunger, desire to eat, prospective food consumption, fullness or bloating (**Figure 3.4**). There was an effect of treatment on nausea (P = 0.036), however, post-hoc tests revealed no differences between treatments.

3.4.2 Part B

3.4.2.1 Energy intake

There was no effect of treatment on energy intake (kcal) or the amount consumed (g) from the buffet meal (**Table 3.1**).

3.4.2.2 Appetite perceptions and GI symptoms

Response to quinine: There was a treatment × time interaction for fullness (P = 0.079) and a trend for an effect of treatment on nausea (P = 0.096), however, post-hoc tests revealed no differences between treatments. There were no effects of treatment on hunger, desire to eat, prospective food consumption or bloating (**Figure 3.5**).

After buffet-meal: There was an effect of treatment on fullness (P = 0.025); Q600 tended to increase fullness compared with control (P = 0.082). There were no effects of treatment on hunger, desire to eat, prospective food consumption, nausea or bloating (**Figure 3.5**).

Table 3.1: Glycemic and GLP-1 responses to, and gastric emptying of, a mixed-nutrient drink after intragastric administration of quinine or control solutions, and effects on energy intake [1]

Parameter	n	Control	Q275	Q600
Plasma glucose				
$AUC_{-31-−1\ min}$, mmol/L × min	14[2]	126 ± 6	126 ± 4	120 ± 7
$AUC_{15-120\ min}$, mmol/L × min	14[2]	567 ± 29	516 ± 26	486 ± 13*
Plasma insulin				
$AUC_{-31-−1\ min}$, pmol/L × min	15	108 ± 16	118 ± 15	127 ± 22
$AUC_{15-120\ min}$, pmol/L × min	15	4002 ± 464	3913 ± 514	4854 ± 672$^{\gamma}$
Plasma glucagon				
$AUC_{-31-−1\ min}$, pg/mL × min	15	1165 ± 73	1100 ± 75	1173 ± 76
$AUC_{15-120\ min}$, pg/mL × min	15	4496 ± 232	5057 ± 296	5247 ± 344$^{\beta}$
Plasma GLP-1				
$AUC_{-31-−1\ min}$, mU/L × min	14[2]	462 ± 46	465 ± 39	494 ± 49
$AUC_{15-120\ min}$, mU/L × min	14[2]	2261 ± 125	2584 ± 143*	2566 ± 137$^{\beta}$
Gastric emptying				
$AUC_{0-120\ min}$, %recovery of $^{13}CO_2$	13[2]	2099 ± 73	1987 ± 98	2116 ± 111
Food intake at buffet meal				
Energy intake, kcal	12	1067 ± 82	1093 ± 93	1014 ± 66
Amount consumed, g	12	1051 ± 69	1018 ± 66	995 ± 60

[1] Data are means ± SEMs. n, number of participants; Q275, quinine hydrochloride load of 275 mg; Q600, quinine hydrochloride load of 600 mg; AUC, area under the curve, GLP-1, glucagon-like peptide-1. All parameters are expressed as absolute data and were analysed using repeated-measures one-way ANOVA with treatment as a factor. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted where ANOVAs revealed significant effects. * Significantly different from control ($P < 0.05$). γ Trend for difference from Q275 ($P = 0.058$). β Trend for difference from control ($0.05 < P < 0.1$).
[2] n < 15, due to technical difficulties with the measurements.

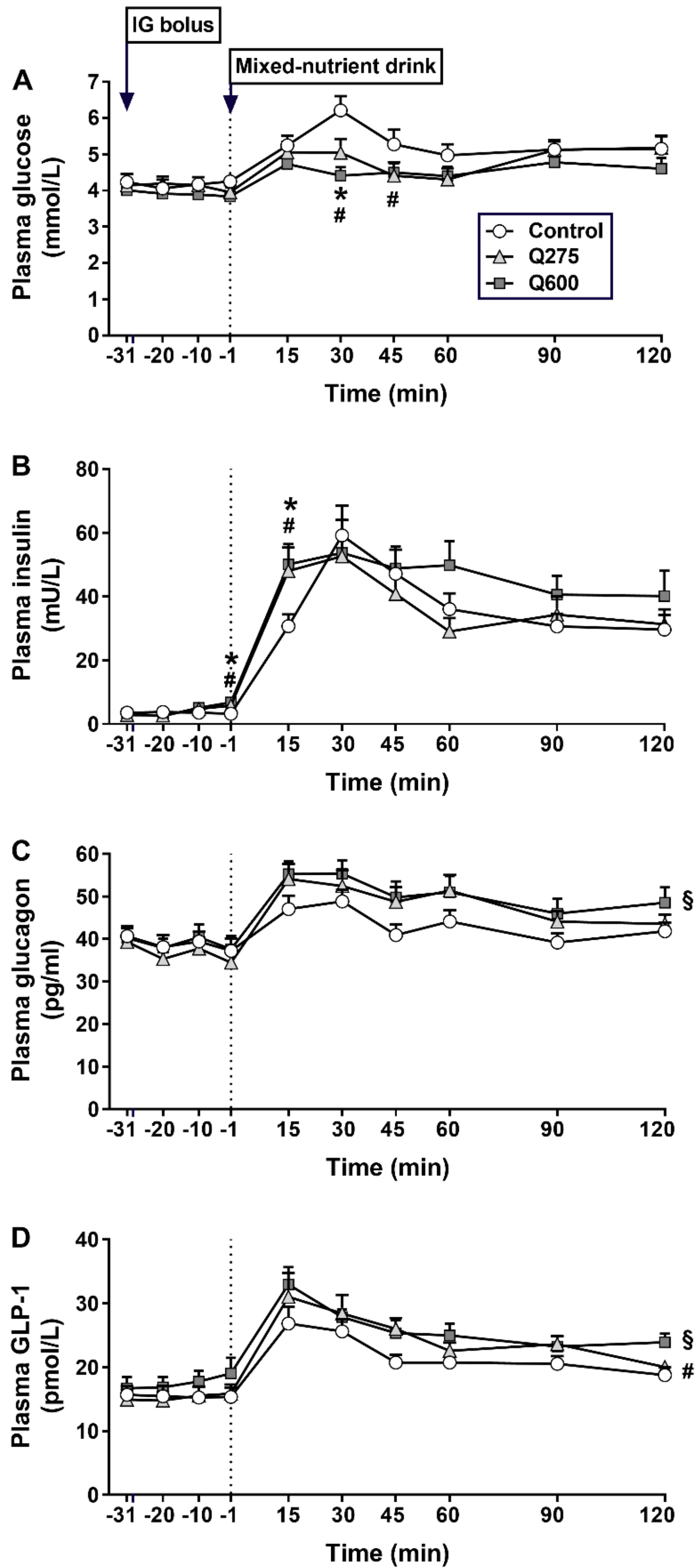
IG bolus
Mixed-nutrient drink
A
Plasma glucose (mmol/L)
Control
Q275
Q600
Time (min)
B
Plasma insulin (mU/L)
Time (min)
C
Plasma glucagon (pg/ml)
Time (min)
D
Plasma GLP-1 (pmol/L)
Time (min)

Figure 3.2: Plasma glucose (A), insulin (B), glucagon (C) and glucagon-like peptide-1 (GLP-1) (D) concentrations at baseline (t = -31 min), in response to an intragastric (IG) bolus of quinine-HCl at doses of 275 mg ('Q275') or 600 mg ('Q600'), or control (t = -20, -10, -1 min) and after a mixed-nutrient drink (t = 15 – 120 min). Data were analysed using repeated-measures two-way ANOVA with treatment and time as factors. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted where ANOVAs revealed significant effects. (A) There was a treatment × time interaction for plasma glucose after the drink (P = 0.001). Q600 reduced plasma glucose at t = 30 min (* P = 0.005), and Q275 at t = 30 (# P = 0.001) and 45 min (#P = 0.021), compared with control. (B) There was a treatment × time interaction for plasma insulin concentrations after quinine alone (P = 0.019). Both Q600 (* P = 0.03) and Q275 (# P = 0.001) increased plasma insulin at t = -1 min, compared with control. There was also a trend for a treatment × time interaction for plasma insulin after the drink (P = 0.082). Both Q600 (* P = 0.005) and Q275 (# P = 0.039) increased insulin at t = 15 min, compared with control, and the response to Q600 was sustained until the end of the study. (C) There was a trend for an effect of treatment on plasma glucagon after the drink (P = 0.065). Q600 tended to increase glucagon compared with control (§ P = 0.073). (D) There was an effect of treatment on plasma GLP-1 after the drink (P = 0.04). Q600 tended to increase (§ P = 0.066) and Q275 increased (# P = 0.04), plasma GLP-1, compared with control. Data are means ± SEM, n = 15 for plasma insulin and glucagon and n = 14 for plasma glucose and GLP-1.

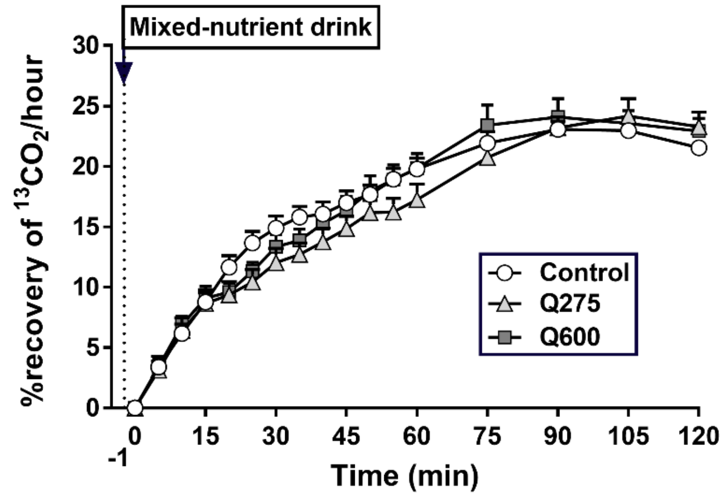

Figure 3.3: Recovery of $^{13}CO_2$ in breath samples to measure gastric emptying of a mixed-nutrient drink, containing 100 mg ^{13}C-acetate, consumed at t = -1 min, 30 min after intragastric administration of quinine-HCl, at doses of 275 mg ('Q275') or 600 mg ('Q600'), or control. Data were analysed using repeated-measures one-way ANOVA with treatment as a factor. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted where ANOVAs revealed significant effects. There was no effect of treatment on gastric emptying of the drink. Data are means ± SEM, n = 13.

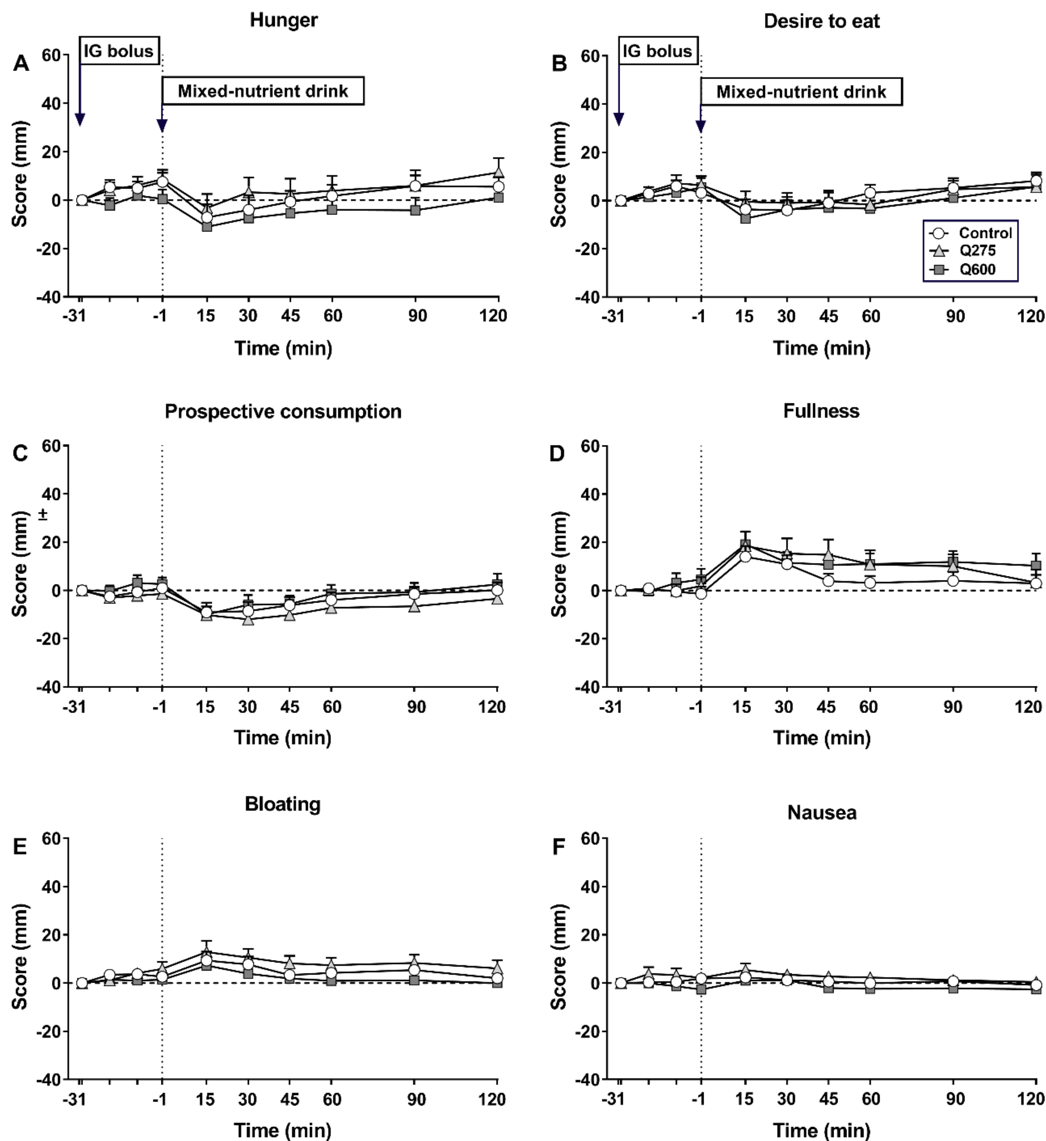

Figure 3.4: Scores for hunger (A), desire to eat (B), prospective food consumption (C), fullness (D), bloating (E) and nausea (F), at baseline (t = -31 min), in response to an intragastric (IG) bolus of quinine-HCl at doses of 275 mg ('Q275') or 600 mg ('Q600'), or control (t = -20, -10, -1 min) and after a mixed-nutrient drink (t = 15 – 120 min). Data were expressed as changes from baseline (i.e. t = -31 min) to account for variations in baseline values. To evaluate the effects of quinine alone, data from t = -31 – -1 min were analysed, and to assess the response to the mixed-nutrient drink, data from t = 15 – 120 min were analysed, using repeated-measures ANOVA with treatment and time as factors. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects. There was an effect of treatment on nausea (P = 0.036) after the mixed-nutrient drink, however, post-hoc tests revealed no differences between treatments. There were no effects on appetite perceptions or bloating. Data are means ± SEM, n = 15.

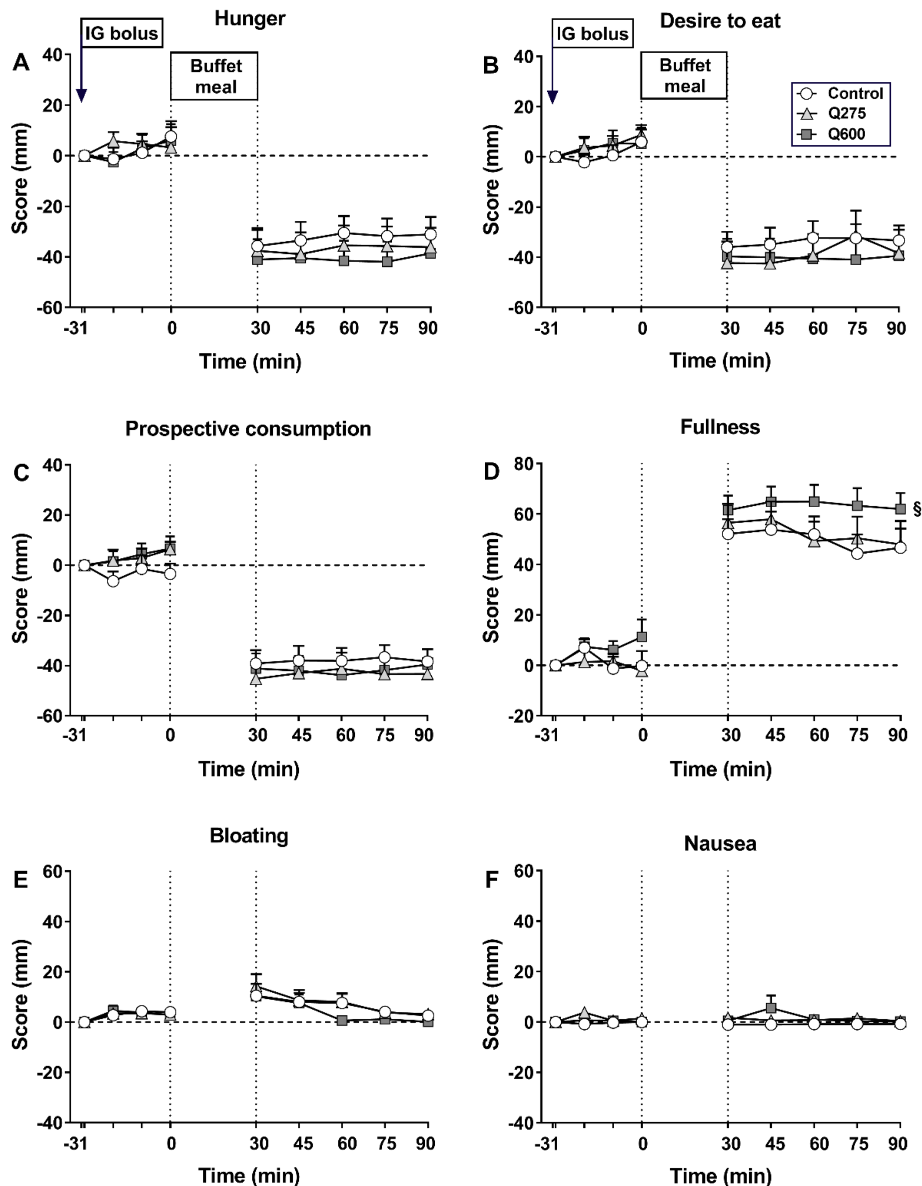

Figure 3.5: Scores for hunger (A), desire to eat (B), prospective food consumption (C), fullness (D), bloating (E), and nausea (F), at baseline (t = -31 min), in response to an intragastric (IG) bolus of quinine-HCl at doses of 275 mg ('Q275') or 600 mg ('Q600'), or control (t = -20, -10, 0 min) and after consumption of a standardised buffet-meal (t = 30 – 90 min). Data were expressed as changes from baseline (i.e. t = -31 min) to account for variations in baseline values. To assess the effects of quinine alone, data from t = -31 – 0 min were analysed, and to evaluate responses after the buffet-meal, data from t = 30 – 90 min were analysed, using repeated-measures ANOVA with treatment and time as factors. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects. There was a treatment × time interaction for fullness (P = 0.079) and a trend for an effect of treatment on nausea (P = 0.096) in response to quinine alone, however, post-hoc tests revealed no differences between treatments. There was also an effect of treatment on fullness after the buffet-meal (P = 0.025); Q600 tended to increase fullness compared with control (§ P = 0.082). There were no effects on other appetite perceptions or bloating. Data are means ± SEM, n = 12.

3.5 Discussion

Our study evaluated the effects of intragastrically administered QHCl at doses of 275 mg and 600 mg on 1) gastric emptying of a mixed-nutrient drink, 2) plasma glucose, insulin, glucagon, and GLP-1 concentrations, as well as appetite perceptions in response to the drink, and 3) appetite perceptions and energy intake from a buffet meal in healthy, lean men. Key findings were that 1) QHCl alone stimulated insulin modestly but did not affect plasma GLP-1 or glucose, 2) QHCl significantly lowered the plasma glucose response to the drink, which was associated with increased insulin and modest increases in glucagon and GLP-1 compared with control, and 3) QHCl did not affect gastric emptying or energy intake but increased fullness after the buffet meal. The data suggest that the reduction in plasma glucose by intragastrically administered quinine reflects the stimulation of insulin, possibly independent of GLP-1, and that quinine, even at doses used clinically, does not appear to have major effects to decrease appetite or energy intake.

Bitter compounds have received increasing interest because preclinical studies have reported potent effects to stimulate GI hormones, particularly GLP-1 and CCK, from enteroendocrine cell lines (399, 401), suggesting that they may have effects to lower elevated blood-glucose levels and reduce energy intake, which are regulated by these hormones (142, 483), with major implications for novel treatment approaches to type 2 diabetes and obesity. In the current study, intragastric QHCl, given 30 min before a carbohydrate-containing drink, substantially reduced the blood-glucose response to the drink, with a reduction in glucose at 30 min of ~ 1.2 (Q275) to 1.9 (Q600) mmol/L. This effect was observed in healthy men with normal blood glucose homeostasis, and, intuitively, when blood-glucose levels are higher, as in people with type 2 diabetes, the fall is likely to be even greater.

In both cell lines and experimental animals, numerous bitter compounds have been reported to stimulate GLP-1 secretion (399, 401, 406, 419, 420, 424), potentially mediated by TAS2R pathways. For example, berberine, a natural bitter plant alkaloid, stimulated GLP-1 in both NCI-h716 and STC-1 cells (391, 405), via TAS2R38 and denatonium benzoate stimulated GLP-1 in NCI-h716 cells via a range of TAS2R, including TAS2R4, TAS2R43, and TAS2R46 (399), whereas KDT501, a pure derivative of a hops isohumulone, stimulated GLP-1 in STC-1 cells via TAS2R108 (419). In the current study, quinine alone, administered as a bolus into the stomach, did not stimulate GLP-1; however, in response to the mixed-nutrient drink, GLP-1 was modestly greater after quinine compared with control. The reasons underlying the lack of a potent effect of quinine to stimulate GLP-1 are unclear. Although quinine does not stimulate the TAS2R38 subtype, like denatonium benzoate, it stimulates TAS2R4, TAS2R43, and TAS2R46. Although the doses given in our study were relatively high, the concentrations that reached small intestinal bitter receptors may not have achieved a critical threshold required for potent release of GLP-1. The role of GLP-1 in the observed glucose lowering is uncertain, since the insulinotropic effect of GLP-1 appears to only be evident when blood-glucose levels exceed ~ 7 – 8 mmol/L (484), although a direct role of GLP-1, e.g., on portal vagal afferents, to enhance hepatic and peripheral glucose uptake (485), may also be a possibility, given that peak GLP-1 concentrations preceded maximum glucose lowering by ~ 15 min.

To our knowledge, this is the first study in humans to evaluate the effects of a bitter compound administered into the lumen of the GI tract on insulin secretion. In rodents, both intragastric denatonium benzoate (1 mg/kg) before an enteral glucose administration (399), and orally administered bitter gourd extract before an intraperitoneal glucose load (421), stimulated insulin secretion and lowered blood glucose. Moreover, in rats with impaired glucose tolerance, oral administration of berberine lowered the blood-glucose response to an oral glucose-

tolerance test compared with control, although insulin levels were not evaluated (443). In diet-induced obese mice, oral administration of KDT501 was found to substantially improve insulin sensitivity, associated with marked glucose lowering (419). In the current study, intragastric quinine stimulated insulin within 30 min of administration. Insulin substantially increased further after the drink, with a markedly greater rise following quinine compared with control, within 15 min of consumption of the drink, preceding the substantial lowering of plasma glucose at 30 min, indicating that insulin stimulation by quinine was most likely responsible for the observed blood glucose lowering. Although the mechanisms underlying the effect of quinine on blood glucose are as yet unknown, our data suggest that circulating quinine may have acted directly on pancreatic beta cells to stimulate insulin. Although the presence of bitter-taste receptors on pancreatic beta cells is unclear, a direct effect of quinine is supported by studies in patients with malaria, in whom i.v. quinine potently stimulates insulin, associated with hypoglycemia (447, 481), and by in vitro studies in which berberine dose-dependently stimulated insulin release from murine isolated pancreatic islet cells in the presence of glucose (443).

Although quinine alone did not affect glucagon release, it slightly enhanced the plasma-glucagon response to the mixed-nutrient drink compared with control, with maximum levels occurring within 15 min of drink ingestion. Thus, although glucagon may have somewhat counteracted the glucose-lowering effect of insulin, any effect was minor, and glucagon was not able to prevent the potent action of insulin to reduce postprandial blood glucose.

Gastric emptying is a key determinant of postprandial blood glucose regulation (142, 483). In contrast to studies in experimental animals (406, 423), the effects of bitter compounds on gastric emptying and motility in humans are less clear (431, 436, 437). Findings that

intragastric administration of denatonium benzoate impaired nutrient-induced fundic relaxation (406), while reducing antral motility (431), would suggest that overall gastric emptying remains unaffected. Indeed, we found no effect on gastric emptying and no correlation between early gastric emptying with plasma glucose concentrations. These observations indicate that slowing of gastric emptying does not contribute to the potent effect of quinine to reduce postprandial blood glucose, at least in the current setting.

We found no effect of quinine on energy intake, in line with our recent study, in which slow intraduodenal infusion of quinine over 60 min, delivering doses of 37.5 – 225 mg, had no effect on energy intake (479). In contrast, studies administering quinine at doses of 18 to ~ 250 mg intragastrically as boluses have reported a modest intake suppression (404, 432). The latter may have resulted in larger concentrations reaching the small intestine, which has been reported to be the major site of bitter receptors in animals (390), because quinine was either given in an acid-resistant capsule (432), or in a smaller volume of water (404) than in our current study. However, recent studies using human tissue receptors may vary according to the TAS2R subtype, and for some TAS2Rs, the expression was higher in the fundus than in the small intestine (486). Interestingly, the higher dose of quinine (Q600) modestly increased fullness before the buffet meal, although this was apparently insufficient to reduce intake from the meal. Whether the prolonged experience of fullness after the meal may delay subsequent food intake or reduce intake from a subsequent meal warrants evaluation. Nevertheless, the conditions under which quinine modulates appetite or energy intake remain to be determined, including whether blood levels of quinine play a role or whether quinine was associated with nausea in previous studies, which was not the case in the current study.

Some limitations of our study should be noted. We included only male volunteers to exclude the potential confounding effects of the menstrual cycle on energy intake (487). Although women have been reported to have lower oral detection thresholds for denatonium benzoate than men (431), and differences in the origin of phase III of fasting motility and hunger perceptions in response to denatonium benzoate have also been found between men and women (431), it remains unclear whether sex differences may also affect other GI or glucoregulatory functions. All our male volunteers detected quinine orally, and the observed effects on glucose lowering were marked. Although the duration of 2 h for breath sampling in our study is sufficient to measure overall gastric emptying (482), the calculation of half-emptying and lag times is not feasible with these data (488). Since we found no effect on energy intake or gastric emptying, analysis of plasma samples for CCK and other gut hormones was not performed. We did not measure plasma quinine concentrations, which may provide insights into the role of circulating quinine. Since our participants detected quinine orally within a narrow range of concentrations, we did not investigate relationships between oral bitter-taste thresholds and downstream (i.e., small intestinal, glucoregulatory) effects. Such correlations have been found for other nutrients, e.g., oleic acid (489), and, therefore, warrant evaluation in future studies in people characterized by a wide range of oral bitter-sensing abilities. Finally, we cannot exclude that the effects of quinine observed in the current study and previous studies using high doses of quinine are “nonspecific,” i.e., may not come about by specific activation of bitter-receptor subtypes. Unfortunately, there are currently no receptor antagonists available for use in humans to clarify the involvement of specific bitter-receptor subtypes in the described effects.

In conclusion, our study established that intragastric QHCl potently reduced postprandial blood glucose, at least in part by stimulating insulin but not via slowing of gastric emptying. Although

there was modest stimulation of GLP-1, in the current setting, the effect of quinine to lower blood glucose was probably due primarily to stimulation of pancreatic insulin secretion.

3.6 Perspectives and significance

In this study, we report that intragastric QHCl, at the administered doses, potently reduces the postprandial blood glucose response to a carbohydrate-containing drink. Our data suggest that this effect is most likely mediated by insulin, whereas the role of GLP-1 is uncertain, and slowing of gastric emptying does not appear to play a role in the current experimental setting. Therefore, the observed effects of quinine on blood glucose may not be mediated primarily via changes in GI functions through activation of TAS2Rs located in the GI tract but possibly via a direct insulinotropic effect of circulating quinine. However, the mechanisms underlying the observed effects of QHCl require further investigation. Nevertheless, the effects of QHCl to lower postprandial blood glucose were marked and observed in healthy volunteers with good blood glucose control; thus, studies are warranted to evaluate the utility of quinine to improve postprandial blood glucose in people with type 2 diabetes, or people with obesity and impaired glucose tolerance.

Chapter 4: Comparative effects of intragastric and intraduodenal administration of quinine on the glycaemic response to, and gastric emptying of, a mixed-nutrient drink, in healthy men

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Manuscript in preparation

Statement of Authorship

Title of Paper	Comparative effects of intragastric and intraduodenal administration of quinine on the glycaemic response to, and gastric emptying of, a mixed-nutrient drink, in healthy men.
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Rose B*, Bitarafan V*, Rezaie P*, Fitzgerald PCE, Horowitz M, Feinle-Bisset C Manuscript in preparation. *These authors contributed equally to this work.

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Name of Principal Author (Candidate)	Vida Bitarafan		
Contribution to the Paper	Designed the research, conducted the experiments, analysed the data and conducted the statistical analysis, interpreted the data, prepared the manuscript, and contributed to the revision of the manuscript.		
Overall percentage (%)	30%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. Braden Rose, Peyman Rezaie, and I are primary authors for this paper and have equally contributed to all aspects of this research.		
Signature		Date	05/05/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Designed the research, conducted the experiments, analysed the data and conducted the statistical analysis, interpreted the data, prepared the manuscript, and contributed to the revision of the manuscript.		
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Contribution to the Paper	Interpreted the data and contributed to the revision of the manuscript.		
Signature		Date	05/05/2020

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Contribution to the Paper	Designed the research, interpreted the data, prepared the manuscript, contributed to the revision of the manuscript, obtained funding and had overall responsibility for the project.		
Signature		Date	30/04/2020

4.1 Abstract

Background: In preclinical studies, bitter compounds, including QHCl, have been shown to interact with TAS2Rs on enteroendocrine cells to stimulate the secretion of glucoregulatory hormones (e.g. GLP-1) and slow gastric emptying, both of which are key determinants of postprandial glycaemia. There appear to be regional variations in the distribution of TAS2R subtypes, specifically a greater density in the duodenum than the stomach. Accordingly, delivery of bitter compounds directly into the duodenum may be more effective in stimulating GI functions to lower postprandial glucose.

Objective: To compare the effects of intragastric (IG) and intraduodenal (ID) administration of QHCl on gastric emptying of, C-peptide (as a measure of insulin secretion) and plasma glucose responses to, a mixed-nutrient drink, in healthy men.

Methods: 14 men (25 ± 3 yr) participated on 4 study days on which they received 600 mg QHCl or control, IG or ID, 60 min or 30 min, respectively, before a mixed-nutrient drink. Plasma glucose and C-peptide were measured before, and for 2h following, the drink. Gastric emptying of the drink was measured using ^{13}C-acetate breath test.

Results: Before the nutrient drink, QHCl stimulated plasma C-peptide and lowered plasma glucose (both $P < 0.05$), with no difference between IG- and ID-QHCl. After the drink, QHCl stimulated C-peptide, slowed gastric emptying and reduced plasma glucose (all $P < 0.05$), with no differences between IG- and ID-QHCl.

Conclusions: QHCl stimulates insulin secretion and slows gastric emptying to reduce glycaemia, with no difference in the response to IG or ID administration, in healthy men.

4.2 Introduction

Meal ingestion modulates a number of GI functions, including secretion of glucoregulatory hormones and gastric emptying, which are major determinants of postprandial blood glucose concentrations (142, 473). GLP-1 is of particular interest, given that it has been shown to be a physiological modulator of postprandial glycaemia by glucose-dependent stimulation of insulin and suppression of glucagon (490) and slowing gastric emptying (205, 212, 474). Moreover, GLP-1 agonists and DPP-IV inhibitors (which increase endogenous GLP-1) are now used extensively in the management of type 2 diabetes (475, 476). Gastric emptying plays a key role in the postprandial glycaemic response by regulating the delivery, and subsequent absorption, of nutrients in the small intestine, as well as the release of glucoregulatory hormones, including GLP-1 (446).

There has been an increased interest in the therapeutic potential of bitter compounds, subsequent to preclinical observations that they modulate both glucoregulatory hormones and gastric emptying, by stimulating TAS2Rs, present throughout the GI tract (390, 406, 414). For example, berberine, denatonium benzoate and phenylthiourea stimulate the release of GLP-1 from human enteroendocrine cell-lines, NCI-H716 and HuTu-80 (399, 401, 405). Furthermore, both an extract of bitter gourd and denatonium benzoate, administered intragastrically, stimulate GLP-1 secretion and reduce blood glucose in mice (399, 419). In the latter study (399), denatonium benzoate also increased plasma insulin and reduced plasma glucose, following glucose gavage (5 g/kg). The glucose-lowering effect of wild bitter gourd was abolished by pre-treatment with the GLP-1 antagonist, exendin (9-39)amide, suggesting a key role for GLP-1 (421).

There is only limited, as well as inconsistent, information about the effects of bitter compounds in humans. Intragastric administration of 100 mg bitter secoiridoids, an extract from the root of *Gentiana lutea*, was reported to have a small, albeit statistically non-significant, effect to stimulate GLP-1 (430). Intraduodenal infusion of quinine, at doses of 37.5 – 225 mg, over 60 min, had no effects on either plasma GLP-1 or antropyloroduodenal pressures (452, 479), however, the low infusion rate (2 mL/min) may have been insufficient to reach a critical threshold for activation of TAS2Rs. In support of this concept, in our recent study, intragastric administration of 275 or 600 mg quinine as a bolus enhanced the plasma insulin and GLP-1 response to a mixed-nutrient drink consumed 30 min later, associated with a reduction in plasma glucose, but no change in gastric emptying (491). While the latter observation was consistent with the outcome of a study using a much smaller dose of quinine (18 mg) (432), it is also possible that the time interval of 30 min between quinine administration and drink consumption may have been insufficient for quinine to reach intestinal TAS2Rs at a sufficient concentration to induce feedback slowing of gastric emptying, particularly given evidence from studies in mice to indicate a variable regional distribution of TAS2Rs subtypes along the GI tract, with a greater density of receptors in the duodenum than the stomach (390). Accordingly, delivery of quinine to the duodenum, and/or varying the time course of delivery between the stomach and duodenum, may potentially affect the release of glucoregulatory hormones, gastric emptying and postprandial glycaemia differently.

We have now evaluated the effects of intragastric and intraduodenal administration of quinine on the plasma glucose and C-peptide responses to, and gastric emptying of, a mixed-nutrient drink, in healthy men.

4.3 Subjects and methods

4.3.1 Study participants

14 healthy, male participants (mean age: 25 ± 2 years; BMI: 22.5 ± 0.5 kg/m^2) participated in the study. The number of participants was determined using power calculations based on our previous work (491). We calculated that n = 14 participants would allow the detection of a 0.8 mmol/L difference in maximum plasma glucose, which usually occurs approximately 30 min after ingestion of a mixed-nutrient drink, with a standard deviation of 1.1 mmol/L, at $\alpha = 0.05$ and a power of 80%. Participants were recruited through flyers placed around the Royal Adelaide Hospital and local universities, and advertisements in local newspapers. Participants were screened before their inclusion to exclude those with GI symptoms or a history of GI disease or surgery, vegetarians, smokers, an alcohol consumption of > 20 g/day on > 5 days/week, use of medications known to affect appetite, energy intake or GI function, high-performance athletes, unstable body weight (≥ 5% change over the last 3 months before participation) and restrained eaters (score > 12 on the restrained eating component of the 3-factor eating questionnaire (453). The study protocol was approved by The Human Research Ethics Committee of the Central Adelaide Local Health Network and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent before their inclusion. Once participants were enrolled, they were assigned to a treatment order of balanced randomisation that was generated with an online tool (www.randomization.com) by a research officer who was not involved in data analysis. The study was registered as a clinical trial with the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au; ACTRN12619001269123).

4.3.2 Study outline

In a pilot study in 8 healthy males (mean age: 25 ± 3 years; BMI: 21.9 ± 0.6 kg/m^2), we determined the temporal profiles of the plasma insulin and pyloric pressure (as a key determinant for the slowing of gastric emptying) responses to IG and ID bolus administration of 600 mg QHCl, dissolved in 10 mL distilled water ("IG-QHCl" and "ID-QHCl", respectively). Plasma insulin concentrations peaked (means ± SEM) ~ 66 ± 7 min after IG-QHCl, and ~ 33 ± 4 min after ID-QHCl, while the peak number of IPPWs occurred ~ 59 ± 17 min after IG-QHCl, and ~ 29 ± 4 min after ID-QHCl. Thus, the time intervals chosen between IG-QHCl or ID-QHCl administration and subsequent consumption of the nutrient drink were 60 min and 30 min, respectively. We, therefore, evaluated the effects of 600 mg IG-QHCl or ID-QHCl, or IG-control or ID-control, administered 60 min (IG conditions) or 30 min (ID conditions) before a mixed-nutrient drink (**Figure 4.1**).

Due to the disruption resulting from the Covid-19 situation, the analyses of plasma GLP-1 have been delayed.

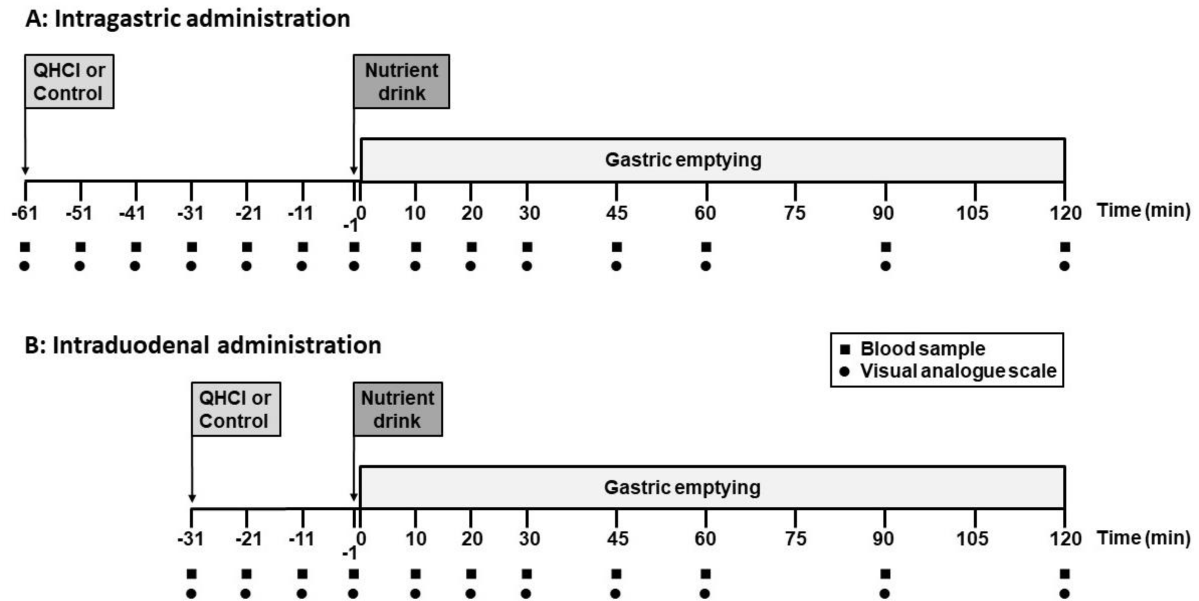

Figure 4.1: Schematic representation of the study design. A, study protocol for intragastric (IG) study days; B, study protocol for intraduodenal (ID) study days. (A) At t = -61 min, after completion of phase III of fasting motor activity, and during phase I, a baseline blood sample and visual analogue scale (VAS) ratings were collected, then either quinine hydrochloride (QHCl) or control was administered intragastrically within 1 min. 60 min later, at t = -1 min, each participant consumed, within 1 min, 350 mL of a mixed-nutrient drink, labelled with 100 mg of ^{13}C-acetate for measurement of gastric emptying by $^{13}CO_2$ breath test. Blood samples, VAS ratings, and breath samples were collected at the indicated time points throughout the study. (B) On ID study days, the protocol was identical, except the mixed-nutrient drink was consumed 30 min after administration of QHCl or control.

4.3.3 Preparation of treatments

The QHCl treatment was prepared by dissolving 600 mg QHCl (Sinkona Indonesia Lestari, Subang, West Java, Indonesia) in 10 mL distilled water. The control treatment consisted of 10 mL distilled water. The treatments were prepared on the morning of each study day, and filled in syringes, by a research officer who had no involvement in data analysis, and administered at a temperature of ~ 30 ºC. QHCl and control treatments were indistinguishable, so that both the study participant and the investigators performing the study were blinded. The 600-mg dose of QHCl was selected as it lowered postprandial blood glucose in our previous study (491).

4.3.4 Study protocol

Participants were studied on four occasions each separated by 3 – 7 days in randomised, double-blind fashion. They were instructed to refrain from vigorous physical activity and alcohol consumption for 24 h prior to each study and provided with a standardised meal (Beef Lasagne; total energy content: 602 kcal; McCain Food, Wendouree, Victoria, Australia) to be consumed by 7 p.m. the night prior to each study visit. The following morning, participants attended the Clinical Research Facility at the Adelaide Medical School, University of Adelaide, at 8.15 a.m. after an overnight fast (from both solids and fluids except water after 7 p.m., and from water after 6.30 a.m.).

Upon arrival, participants were intubated with a manometric catheter (Dentsleeve International, Mui Scientific, Mississauga, Ontario, Canada; total length: 100 cm; external diameter: 3.5 mm), which was inserted through an anaesthetised nostril into the stomach and allowed to pass into the duodenum by peristalsis (196). The catheter included six antral channels, a 4.5-cm pyloric sleeve sensor with two channels situated on the back, and seven duodenal channels, with all side holes positioned at 1.5-cm intervals, measuring pressures in the antrum, pylorus

and duodenum (285, 456). The most proximal antral channel was used for IG administration, and an additional infusion channel, located ~ 14.5 cm distal to the pylorus, for ID administration. The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained by continuous measurement of the transmucosal potential difference between the most distal antral and most proximal duodenal, channels (457). Once the catheter was in position (within ~ 49 ± 8 min (means ± SEM) across study days and participants), an i.v. cannula was placed into a forearm vein and the arm was wrapped in a heat pad for regular sampling of 'arterialised' blood. Immediately following the completion of phase III of the interdigestive MMC (i.e. during phase I, a period of motor quiescence), a baseline blood sample and VAS questionnaire to assess GI symptoms were collected. After fasting motility was monitored for 10 min, QHCl or control were administered into either the stomach (t = -61 min) or duodenum (t = -31 min), and the manometric catheter was then removed. At t = -1 min, participants consumed, within 1 min, a 350-mL mixed-nutrient drink (Resource plus; Nestle, Tongala, Victoria, Australia (325 mL); 500 kcal, 74 g carbohydrates, including maltodextrin and sucrose, 18 g protein, and 15 g fat, plus 25 mL water to make up the final volume), labelled with 100 mg of [^{13}C]acetate for measurement of gastric emptying by breath test (482). The macronutrient composition of the drink reflects National Health and Medical Research Council (NHMRC) dietary guidelines for intake (492). Blood samples for measurement of plasma glucose and C-peptide concentrations, VAS ratings and breath samples were collected at regular time intervals (**Figure 4.1**). At t = 120 min, the i.v. cannula was removed, and each participant was provided with a light lunch, after which they allowed to leave the laboratory.

On a separate day, oral taste detection thresholds for QHCl were quantified to ensure that all participants detected QHCl (491).

4.3.5 Measurements

4.3.5.1 Oral quinine taste-thresholds

Oral detection thresholds for QHCl were quantified using the ascending-series 3-alternative forced-choice technique (460). Participants were asked to abstain from food, beverages and oral care products for a minimum of 2 h before the test, and, to prevent potential confounding from non-taste sensory inputs, all tests were conducted while wearing nose clips. Taste samples were prepared by adding QHCl at varying concentrations (0.00125, 0.0025, 0.0125, 0.025, 0.075, 0.25, 0.75, 1.5, 3, 7.5, 15, 30, and 75 mmol/L) to distilled water (461). Control samples consisted of distilled water. Samples were prepared on the day of testing. Participants were presented with three samples per set, two controls and one containing QHCl, in ascending order from the lowest to the highest concentration, and rinsed their mouth with distilled water before beginning the task and between each sample set. In each set, participants were asked to identify the "odd" sample. If incorrect, they were presented with three samples at the next higher concentration and, if correct, with three more samples at the same concentration. This procedure continued until the participant identified the odd sample at a given concentration three consecutive times, and that concentration was defined as their QHCl detection threshold (491).

4.3.5.2 Plasma glucose and hormone analyses

Arterialised venous blood samples were collected into ice-chilled ethylenediaminetetraacetic acid tubes and centrifuged at ~ 1832 g-force for 1 min at 4 ºC within 15 min of collection, before being stored at -80 ºC until subsequent analysis.

Plasma glucose concentrations (mmol/L) were measured by the glucose oxidase method, using a glucose analyser (YSI 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, Ohio, USA).

Plasma C-peptide concentrations (pmol/L), a measure of insulin secretion (493), were quantified using an ELISA immunoassay (10-1136-01, Mercodia, Uppsala, Sweden). The minimum detectable concentration was 15 pmol/L, and intra- and inter-assay CVs were 8.3% and 3.6%, respectively.

4.3.5.3 Gastric emptying

$^{13}CO_2$ concentrations in end-expiratory breath samples were measured using an isotope ratio mass spectrometer (FANCi; Fischer Analysen Instrumente GmbH, Germany) with an online gas chromatographic purification system. The results were expressed as percentage of $^{13}CO_2$ recovery/hour and profiles plotted as cumulative values over the sampling period (482).

4.3.5.4 GI symptoms

Nausea and bloating were evaluated using a VAS questionnaire (484). Each VAS consisted of a 100-mm horizontal line, indicating each sensation, where 0 mm represented "sensation not felt at all" and 100 mm represented "sensation felt the greatest". Participants rated how they were feeling at a given time-point by placing a vertical stroke at the appropriate point on the line.

4.3.6 Data and statistical analysis

Statistical analysis was performed using SPSS software (version 26.0; SPSS Inc). Plasma glucose and C-peptide concentrations and gastric emptying data were expressed as absolute values, while VAS scores were expressed as changes from baseline.

Effects of QHCl alone (i.e. prior to ingestion of the mixed-nutrient drink) on plasma glucose and C-peptide concentrations and VAS ratings were evaluated by calculating AUCs and then dividing by the number of min (AUC/min), so the two baseline periods could be compared, despite different durations. AUCs/min, as well as the effects at t = -1 min, were analysed using repeated-measures two-way ANOVAs with treatment (QHCl, control) and route of administration (IG, ID) as factors. To evaluate responses to the mixed-nutrient drink, plasma glucose and C-peptide and gastric emptying were analysed using repeated-measures 3-way ANOVAs with treatment, route of administration, and time (t = 10 – 120 min) as factors. Peak plasma glucose, and time to peak concentrations, as well as VAS ratings, expressed as AUCs ($AUC_{10-120\ min}$), were analysed using repeated-measures two-way ANOVAs with treatment and route of administration as factors. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects. Sphericity of the time effect was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P-value was reported. Correlations between plasma glucose concentrations between t = 10 – 30 min (expressed as $AUC_{10-30\ min}$) with gastric emptying between t = 0 – 30 ($AUC_{0-30\ min}$) and C-peptide at t = -1 min and between t = 10 – 30 min ($AUC_{10-30\ min}$), were evaluated, using data across all study days, with the use of linear within-subject correlations (494). All data are reported as means ± SEMs. All tests were two-tailed, and differences were considered statistically significant at $P \leq 0.05$.

4.4 Results

All participants tolerated the study treatments well, and completed all study visits without reporting any adverse effects. All participants were able to detect QHCl orally; the mean detection threshold was 0.15 ± 0.06 mmol/L. Breath test data were unavailable in 2 participants due to technical problems.

4.4.1 Plasma glucose concentrations

There were no differences in baseline concentrations of plasma glucose between study days. (**Figure 4.2A**).

Response to QHCl alone: There were effects of treatment, but not route of administration, on plasma glucose AUCs and plasma glucose at t = -1 min (both $P < 0.05$); ID- and IG-QHCl reduced plasma glucose, albeit modestly, compared with control ($P < 0.05$) (**Figure 4.2A**).

Response to mixed-nutrient drink: Plasma glucose increased on all study days, with maximum concentrations of ~ 7 mmol/L on the control days, and < 6 mmol/L on the QHCl days (**Table 4.1**). There was a treatment × time interaction, but no effect of route of administration, on plasma glucose concentrations ($P < 0.05$); ID- and IG-QHCl reduced plasma glucose between t = 10 – 45 min, compared with control (all $P < 0.05$) (**Figure 4.2A**). There were effects of treatment, but not of route of administration, on both peak plasma glucose and the time to peak plasma glucose (both $P < 0.05$), so that ID- and IG-QHCl lowered peak plasma glucose, and increased the time to peak plasma glucose, compared with control (both $P < 0.05$) (**Table 4.1**).

4.4.2 Plasma C-peptide concentrations

There were no differences in baseline C-peptide concentrations between study days (**Figure 4.2B**).

Response to QHCl alone: There were effects of treatment, but not route of administration, on plasma C-peptide AUCs and concentrations at t = -1 min (both $P < 0.05$); ID- and IG-QHCl increased plasma C-peptide AUCs and concentration at t = -1 min, albeit modestly, compared with control ($P < 0.05$) (**Figure 4.2B**).

Response to the mixed-nutrient drink: Plasma C-peptide concentrations increased markedly on all study days. There was a treatment × time interaction, but no effect of route of administration, on plasma C-peptide concentrations ($P < 0.05$); ID- and IG-QHCl increased C-peptide at t = 10 and 120 min (both $P < 0.05$), and tended to stimulate C-peptide at t = 90 min (P = 0.052), compared with control (**Figure 4.2B**).

4.4.3 Gastric emptying

There was a treatment × time interaction, but no effect of route of administration, on gastric emptying of the drink ($P < 0.05$); ID- and IG-QHCl slowed gastric emptying of the drink modestly between t = 20 – 75 min, compared with control (all $P < 0.05$) (**Figure 4.3**).

4.4.4 Relationships between plasma glucose with gastric emptying or C-peptide

There was a direct correlation between early plasma glucose $AUC_{10-30\ min}$ and early gastric emptying $AUC_{0-30\ min}$ for (r = 0.621, $P < 0.01$) and an inverse correlation with C-peptide at t = -1 min (r = -0.265, $P < 0.05$), but not C-peptide $AUC_{10-30\ min}$ (data not shown).

4.4.5 GI symptoms

There were no effects of treatment or route of administration on ratings of nausea or bloating, either in response to QHCl alone, or the mixed-nutrient drink (**Figure 4.4**).

Table 4.1: Peak, and time to peak, of plasma glucose concentrations, after consumption of a mixed-nutrient drink, following intragastric, or intraduodenal, administration of 600 mg quinine hydrochloride or control[1]

	IG-control	IG-QHCl	ID-control	ID-QHCl	P-value
Peak plasma glucose, mmol/L	7.2 ± 0.3	6.2 ± 0.3*	7.2 ± 0.3	6.4 ± 0.4*	< 0.05
Time to peak plasma glucose, min	43 ± 5	58 ± 8*	45 ± 7	69 ± 9*	< 0.05

[1]Data are means ± SEMs (n = 14). IG-QHCl, intragastric administration of quinine hydrochloride (QHCl); IG-control, intragastric administration of control; ID-QHCl, intraduodenal administration of QHCl; ID-control, intraduodenal administration of control. Data were analysed using repeated-measures two-way ANOVA with treatment and route of administration as factors. There were effects of treatment, but not route of administration, on both peak glucose and the time to peak glucose (both $P < 0.05$); QHCl lowered peak glucose, and increased the time to peak glucose, compared with control (*both $P < 0.05$).

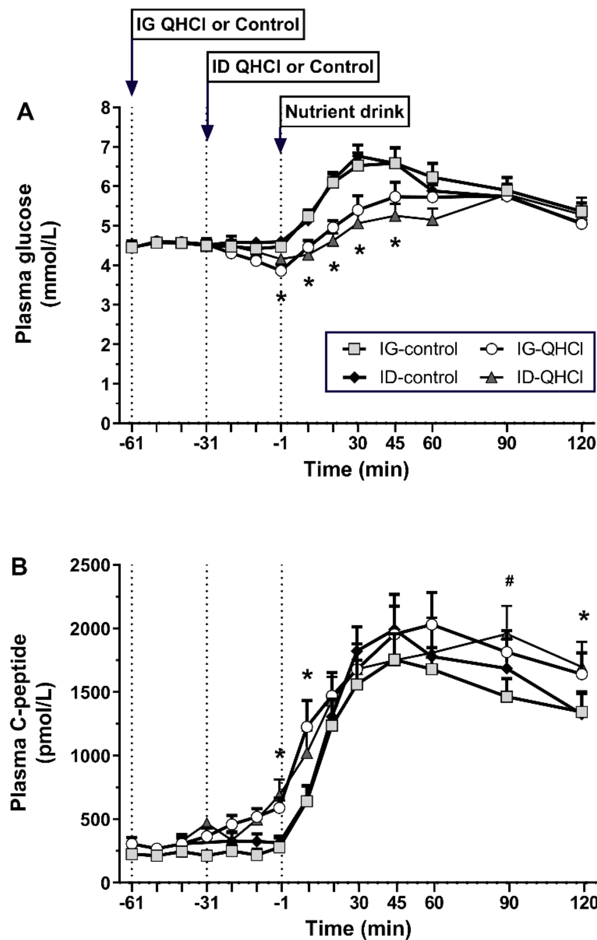

Figure 4.2: Plasma glucose (A) and C-peptide (B) concentrations following to intragastric (IG) quinine hydrochloride (QHCl) or control (t = -61 – 0 min), or intraduodenal (ID) QHCl or control (t = -31 – 0 min), and after a mixed-nutrient drink (t = 0 – 120 min). To evaluate the response to QHCl alone, AUC/min was calculated and analysed using 2-way ANOVA with treatment and route of administration as factors. To evaluate the response to the mixed-nutrient drink, repeated-measures 3-way ANOVA was used, with treatment, route of administration, and time as factors. (A) In response to QHCl alone, there were effects of treatment, but not route of administration, on plasma glucose AUCs and plasma glucose at t = -1 min (both $P < 0.05$); ID- and IG-QHCl reduced plasma glucose, compared with control (* $P < 0.05$). In response to the mixed-nutrient, there was a treatment × time interaction, but no effect of route of administration, on plasma glucose concentrations ($P < 0.05$); ID- and IG-QHCl reduced plasma glucose between t = 10 – 45 min, compared with control (* all $P < 0.05$). (B) In response to QHCl alone, there were effects of treatment, but not route of administration, on plasma C-peptide AUCs and concentrations at t = -1 min (both $P < 0.05$); ID- and IG-QHCl modestly increased plasma C-peptide AUCs and concentrations at t = -1 min, compared with control (* $P < 0.05$). In response to the mixed-nutrient, there was a treatment × time interaction, but no effect of route of administration, on plasma C-peptide ($P < 0.05$); ID- and IG-QHCl increased C-peptide at t = 10 and 120 min (* both $P < 0.05$), and tended to stimulate C-peptide at t = 90 min (# $P = 0.052$), compared with control. Data are expressed as means ± SEMs; n = 14.

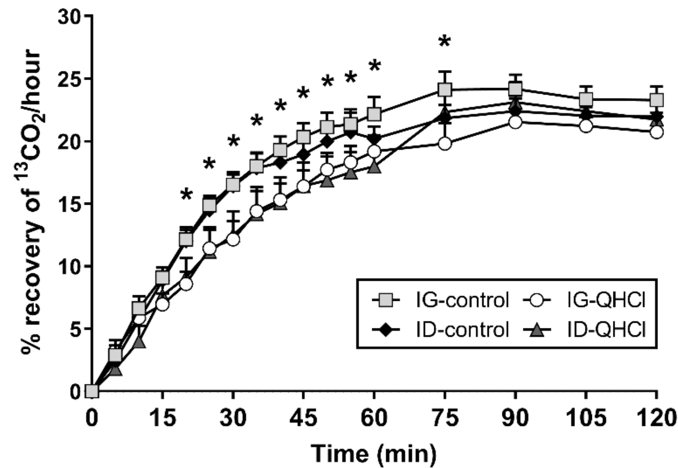

Figure 4.3: Gastric emptying of a mixed-nutrient drink, measured by ^{13}C-acetate breath test, 60 min after intragastric (IG), or 30 min after intraduodenal (ID), administration of either quinine hydrochloride (QHCl; IG-QHCl or ID-QHCl, respectively) or control (IG-control or ID-control, respectively). Data were analysed using repeated-measures 3-way ANOVA, with treatment, route of administration, and time as factors. There was a treatment × time interaction, but no effect of route of administration, on gastric emptying of the drink ($P < 0.05$); ID and IG-QHCl slowed gastric emptying of the drink modestly between t = 20 – 75 min, compared with control (* all $P < 0.05$). Data are expressed as means ± SEMs; n = 12.

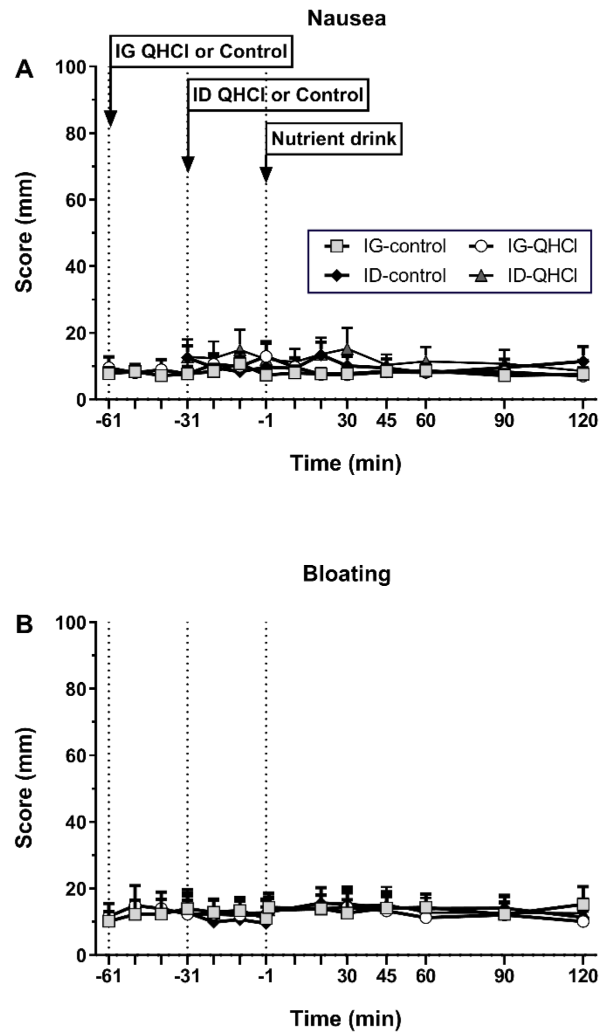

Figure 4.4: Scores for nausea (A) and bloating (B) in response to intragastric (IG) quinine hydrochloride (QHCl) or control (t = -61 – 0 min; IG-QHCl or IG-control, respectively), or intraduodenal (ID) QHCl or control (t = -31 – 0 min; ID-QHCl or ID-control, respectively), and after a mixed-nutrient drink (t = 0 – 120 min). To evaluate the response to QHCl alone, AUC/min was calculated and analysed using 2-way ANOVA with treatment and route of administration as factors. To evaluate the response to the mixed-nutrient drink, repeated-measures 3-way ANOVA was used, with treatment, route of administration, and time as factors. Data are expressed as means ± SEMs; n = 14.

4.5 Discussion

Our study evaluated the comparative effects of IG and ID administration of quinine on the plasma glucose and C-peptide responses to, and gastric emptying of, a mixed-nutrient drink, in healthy men. Our key findings were that quinine markedly stimulated plasma C-peptide and lowered plasma glucose, both alone and following a mixed-nutrient drink, and slowed gastric emptying, without any difference between the IG and ID routes of administration. These observations have important implications for the potential use of bitter compounds to lower postprandial blood glucose.

That quinine lowers plasma glucose confirms our recent findings that 600 mg quinine, administered intragastrically, reduced the plasma glucose response to a mixed-nutrient drink consumed 30 min after quinine (491). In the current study we hypothesised that additional time would be required following IG administration for quinine to reach a sufficient number of TAS2Rs, when compared with ID administration, because a greater number of bitter receptors has been reported in the duodenum, compared with the stomach (390). Accordingly, IG quinine was administered 60 min, and ID quinine 30 min, prior to the mixed-nutrient drink. The plasma glucose-lowering effect of quinine was comparable and substantial in both conditions. Thus, the time difference of 30 min between IG and ID quinine administration appears sufficient for IG quinine to empty into the duodenum to activate small intestinal bitter receptors or, alternatively, to activate a greater number of gastric bitter receptors.

To our knowledge, this is only the second study that has evaluated the effects of a bitter compound, administered into the GI lumen, on insulin secretion in humans. In our recent study in healthy men, IG administration of quinine, at doses of 275 or 600 mg, modestly stimulated insulin immediately before a mixed-nutrient drink, consumed 30 min later, and the insulin

response (within 15 min) to the nutrient drink was also enhanced, associated with a substantial lowering of plasma glucose within 30 min (491). Because the quinine-induced stimulation of insulin before the drink was small, and rising, we hypothesised that the interval of 30 min between IG quinine administration and drink consumption may have been insufficient for more substantive insulin stimulation. In the current study, quinine, in a dose of 600 mg, stimulated C-peptide, within 30 min of ID, and 60 min of IG, administration, modestly, with no difference between the routes of administration, associated with lowering of fasting glucose. C-peptide increased much more after the drink, and this rise was much greater following both IG and ID quinine. An increase in insulin (as indicated by C-peptide) is likely to be primarily responsible for the reduction in plasma glucose. Indeed, we found an inverse relationship between the early rise in plasma glucose post-drink and the rise in C-peptide immediately before the drink. The mechanism(s) underlying the effect of quinine to stimulate insulin secretion are speculative. The absorption of quinine into the bloodstream may stimulate insulin by directly activating receptors on pancreatic beta cells. This is likely, as i.v. quinine, when used to treat malaria patients, markedly increases insulin, associated, not infrequently, with hypoglycaemia (447, 481). Moreover, while it is unclear whether TAS2Rs are present on human pancreatic beta cells, quinine induces insulin secretion from isolated murine pancreatic beta cells in vitro (495). While we did not measure glucagon in the current study, we have shown previously that quinine stimulates glucagon secretion modestly (491), thus, it is possibly that glucagon may have somewhat attenuated the glucose-lowering effect of insulin. A number of bitter compounds, including berberine, 1, 10-phenanthroline and denatonium benzoate, have been reported to stimulate GLP-1 release from enteroendocrine cells (391, 401, 405). In our previous study (491), plasma GLP-1 increased modestly in response to IG administration of quinine at the doses of 275 and 600 mg, in healthy males. While some studies have shown that the insulinotropic effect of GLP-1 requires circulating glucose concentrations of ~ 7 – 8 mml/L

(484, 496), other studies have reported that lower glucose concentrations of ~ 4 – 5 mmol/L are sufficient (497). Since in the current study plasma glucose reached ~ 6 – 7 mmol/L on all study days, the contribution of GLP-1 to the stimulation of insulin was probably modest, and the magnitude of this effect would need to be established with the use of a GLP-1 receptor antagonist. We are currently awaiting analysis of our samples for GLP-1.

Gastric emptying is a key determinant of postprandial blood glucose regulation. In contrast to our previous study, in which quinine was administered intragastrically 30 min before a mixed-nutrient drink, in the current study, administration of the same dose of quinine, either intragastrically 60 min, or intraduodenally 30 min, before the drink, slowed gastric emptying. The underlying mechanisms are unknown, but may potentially involve the release of gut hormones, including CCK and GLP-1. Both have potent effects to slow gastric emptying in humans (142, 147, 209), but the effect of quinine to stimulate these hormones in humans appears to be modest (432, 491). Analysis of our samples for GLP-1 will help to address this question. Nevertheless, the slowing of gastric emptying most likely contributed to postprandial blood glucose lowering, which was apparent for the first postprandial hour. Indeed, we found a correlation between the early lowering of postprandial plasma glucose and slowing of gastric emptying. In contrast, in our previous study (491), in which quinine stimulated insulin, but did not slow gastric emptying, plasma glucose lowering occurred only around 30 min after the meal. Thus, when quinine administration is carefully targeted, a potent effect to lower postprandial blood glucose can be achieved by activating both the secretion of insulin and slowing of gastric emptying. It is also important to point out that slowing of gastric emptying was not due to an aversive effect of quinine, that is, participants did not report any nausea. Studies in patients with type 2 diabetes are now warranted to evaluate potential clinical benefits.

It is appropriate to recognise some limitations of our study. We are still awaiting analysis of our samples for GLP-1. We did not measure circulating quinine concentrations. We only included males to avoid any confounding effect of the menstrual cycle on gastric emptying (487), however, females have been reported to have lower oral detection thresholds for denatonium benzoate (431), suggesting that they may also be more sensitive to intraluminal bitter stimuli. Finally, we cannot exclude the possibility that the effects of quinine observed in our study, or previous studies using similarly high doses, might be due to “non-specific” effects, rather than activation of bitter-receptor subtypes. The use of a specific bitter receptor antagonist(s) would assist in answering this question, however, these are currently not available for use in humans.

In conclusion, our study established that quinine, when allowed to sufficiently interact with small intestinal bitter receptors, reduces postprandial blood glucose by stimulating glucoregulatory functions, including C-peptide secretion, as a measure for insulin release, and slowing of gastric emptying. The role of GLP-1 is uncertain, since the rise in postprandial glucose in response to the nutrient drink was modest. Nevertheless, this study clearly demonstrates the potent blood-glucose lowering effects of quinine in healthy people with good blood glucose control, thus, further research is warranted to investigate the suitability of this bitter compound to improve postprandial blood glucose in people with type 2 diabetes or impaired blood glucose tolerance.

Chapter 5: Effects of intraduodenal and intragastric administration of a bitter extract of hops (*Humulus lupulus L.*) on plasma CCK and PYY concentrations, antropyloroduodenal motility and energy intake in healthy males

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Manuscript in preparation

Statement of Authorship

Title of Paper	Effects of intraduodenal and intragastric administration of a bitter extract of hops (*Humulus lupulus L.*) on plasma CCK and PYY concentrations, antropyloroduodenal motility and energy intake in healthy males
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Bitarafan V, Ingram JR, Poppitt SD, Fitzgerald PCE, Feinle-Bisset C Manuscript in preparation.

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Contribution to the Paper	Conducted the experiments, analysed the data and conducted the statistical analysis, interpreted the data, prepared the manuscript, and contributed to the revision of the manuscript.		
Overall percentage (%)	50%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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5.1 Abstract

Background: The release of gut hormones (e.g. CCK and PYY) and the modulation of gut motility, particularly pyloric stimulation, contribute to the regulation of acute energy intake in humans. In preclinical studies, bitter compounds have been found to potently stimulate gut hormones by activating bitter receptors expressed on enteroendocrine cells. Since there is evidence of variations in the regional distribution of bitter receptor subtypes along the upper GI tract, the administration of bitter compounds in different GI regions may differentially affect these GI functions.

Objective: We evaluated the effects of ID and IG administration of a bitter extract of hops on plasma CCK and PYY concentrations, antropyloroduodenal pressures, appetite perceptions and energy intake in healthy men.

Design: The study consisted of 2 parts: part A included 15 and part B included (to date) 6 healthy, lean men (aged 25 ± 3 yrs). In randomised, double-blind fashion, and on separate occasions, participants received in part A, an ID bolus of hops extract, at doses of either 100 mg ("ID-HE100") or 250 mg ("ID-HE250"), or vehicle (canola oil; "ID-control"), and in part B, an IG bolus at the dose of 250 mg ("IG-HE250"), or vehicle ("IG-control"). Antropyloroduodenal pressures were measured continually (high-resolution manometry), and plasma CCK and PYY (radioimmunoassay), and appetite perceptions/GI symptoms (VAS) at regular time points, for 180 min, after which time energy intake from a standardised buffet-meal was quantified.

Results: ID-HE250 had modest, and transient, effects to stimulate pyloric pressures during the first 90 min ($P < 0.05$), and a significant effect to stimulate PYY after t = 60 min ($P < 0.05$), but did not affect antral or duodenal pressures, plasma CCK or VAS ratings. While part B is ongoing and data have not been analysed statistically, IG-HE250 appeared to stimulate pyloric

pressures during the first 90 min, but did not affect antral and duodenal pressures, VAS ratings or energy intake.

Conclusions: The currently available data appear to suggest that IG administration of hops may have more potent effects to stimulate pyloric pressures, however, the study needs to be completed (i.e. inclusion of further participants, analysis of plasma samples for gut hormones) to enable more definitive conclusions, including on appetite perceptions and energy intake.

5.2 Introduction

The sensing of nutrients in the GI tract modulates GI functions, including the release of GI hormones and gut motility, associated with the slowing of gastric emptying; these changes contribute to the regulation of energy intake and postprandial blood glucose (142, 451, 498). Bitter compounds, traditionally viewed to indicate poison ingestion (381-383), are known to stimulate the TAS2Rs family of G-protein-coupled receptors, located on enteroendocrine cells throughout the GI tract (406, 414, 438, 452); 25 different subtypes have, to date, been identified in humans and have been shown to have various distribution along the GI tract (390, 394, 395). The discovery that TAS2R stimulation is associated with potent activation of GI functions, particularly the stimulation of gut hormones, has generated interest in the effects of bitter compounds in the regulation of energy intake and postprandial blood glucose.

The outcomes of pre-clinical studies suggest potent effects of TAS2R agonists to modulate gut hormone release (393, 399, 415, 418). For example, in STC-1 or HuTu-80 cells, denatonium benzoate stimulated CCK by activating TAS2R108, 137, 138, 144, 135, phenylthiocarbamide via TAS2R35, and steroid glycosides, an extract from *Hoodia gordonii* plant, via TAS2R14 (393, 415, 418), while denatonium benzoate stimulated PYY in NCI-H716 cells by activating TAS2R3-5 (399). These findings suggest that different bitter compounds activate different combinations of TAS2R to stimulate specific gut hormones. Human studies evaluating the effects of bitter compounds on GI hormones have yielded inconsistent results and, if any, often small effects (426-429). For example, a 60-min slow intraduodenal infusion of QHCl, at doses from 37.5 – 225 mg, did not affect CCK (452, 479) and, at the dose of 75 mg, did not affect PYY (452), while, intragastric administration of a lower dose of 18 mg stimulated CCK (432).

Effects of bitter compounds on GI motility have been evaluated in a limited number of both pre- and clinical studies. For example, in mice gastric tissue, denatonium benzoate and chloroquine induced contractions is both fundus and antrum, whereas, phenylthiocarbamide relaxed the fundus and inhibited antral activity, and salicin was ineffective (406). Similarly in studies in humans, while intraduodenal infusion of QHCl (37.5 – 225 mg) did not affect antropyloroduodenal motility, intragastric administration of QHCl (~ 270 mg), or denatonium benzoate (~ 30 mg), impaired fundic relaxation and decreased antral, but not duodenal, motility (406, 431, 438, 479). The observed inconstancies in effects on GI motility and hormones release could be due to the differences in concentrations, or doses, of bitter compounds used in the studies, the specificity of the compounds to particular TAS2R subtypes, which most likely are not all involved in the regulation of a particular function.

Hops flowers, *Humulus lupulus L.*, have a long tradition of use in the brewing of beer, acting as a flavouring, providing bitterness (499). Bitter compounds in hops include mainly α-acids, comprising humulone, and β-acids, comprising lupulone, and trace amounts of xanthohumol, a prenylated chalconoid (500, 501). In human embryonic kidney 293T cells, 15 hops-derived compounds, including α-acids, β-acids, trans/cis-iso-α-acids, xanthohumol, isoxanthohumol and 8-prenylnaringenin, have been shown to activate various combination of three TAS2Rs, including TAS2R1, 14, and 40 (31). In mice STC-1 cells, MHBA, oxidation products from hops α-acid, have been shown to stimulate Ca^{2+}-dependent CCK and PYY release (416), and, in humans, oral administration of isohumulones (48 mg) or MHBA (35 mg) for 12 weeks reduced body weight and fat mass and improved glucose homeostasis (502, 503). However, to date, no study in humans has evaluated the effects of hops extract on GI functions, e.g. gut hormones and/or motility, which contribute to the regulation of acute energy intake.

Therefore, the aim of this study was to investigate the effects of intraduodenal and intragastric administration of a hops extract on plasma CCK and PYY concentrations, antropyloroduodenal pressures, appetite perceptions and energy intake in healthy men.

5.3 Materials and methods

5.3.1 Participants

Healthy, lean men participated in this study: 15 in part A (mean; age: 24 ± 5 yr, body mass index: 22.9 ± 2.0 kg/m²) and (as the study has not been completed because of disruptions due to Covid-19, to date) 6 in part B (mean; age: 27 ± 2 yr, BMI: 22.3 ± 0.1 kg/m²). The study parts were performed consecutively, and 4 of the participants took part in both parts. Participants were recruited through flyers placed around the Royal Adelaide Hospital and local Universities, as well as advertisements placed online, including the University of Adelaide website and a local advertising website, Gumtree. Exclusion criteria were smoking, consumption of > 20 g alcohol/day, any medical condition, surgery, or the use of medications known to affect energy intake, appetite or GI function. All participants had been weight-stable (< 5% change in body weight) for at least 3 months preceding the study. They were unrestrained eaters with a score of ≤ 12 on the eating-restraint section (factor 1) of the Three-Factor Eating Questionnaire (453). The study protocol was approved by the Human Research Ethics Committee of the Central Adelaide Local Health Network and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent before their inclusion. Once a participant was enrolled in the study, they were assigned to a treatment order of balanced randomisation that was generated with an online tool (www.randomization.com) by a research officer who was not involved in data analysis. Both the participant and the investigator who assessed outcomes were blinded to the randomisation. Both study parts were registered as clinical trials with the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au; part A: ACTRN12619000813189, part B: ACTRN12620000503921).

5.3.2 Study design

Study part A evaluated the effects of intraduodenal administration of a hops extract, at doses of 100 mg ("ID-HE100") or 250 mg ("ID-HE250"), or control (canola oil; "ID-control"), on plasma CCK and PYY concentrations, antropyloroduodenal pressures, appetite perceptions, GI symptoms and energy intake in healthy men.

Study part B evaluated the effects of intragastric administration of hops extract, at the dose of 250 mg ("IG-HE250"), or control ("IG-control"), on antropyloroduodenal pressures, appetite perceptions, GI symptoms and energy intake in healthy men. Because the 100-mg dose was found to be ineffective in part A, it was not further studied in study part B.

5.3.3 Study treatments

The hops extract was produced by Pharmalink Extract Ltd. (Appleby, New Zealand) under ISO 9001 certification from hops that were grown in New Zealand (*Humulus lupulus* var 79-32; Pacific Gem, batch # 1226, New Zealand Hops Ltd., Appleby, New Zealand). The treatments comprised of 0.6 mL of either 1) 250 mg of a supercritical CO_2 extract of hops dissolved in 0.342 mL of canola oil (Goodman Fielder, Sydney, New South Wales, Australia), 2) 100 mg of the hops extract dissolved in 0.497 mL of canola oil, or 3) 0.6 mL of canola oil as a vehicle control. The treatments had comparable densities, i.e. 1.034 ± 0.006 g/mL for the hops extract treatments and 0.914 g/mL for canola oil.

All treatments were prepared in the morning of each study day by a research officer who had no involvement in data analysis. Treatments were stored in the dark to minimise photo-oxidation and maintained at 30°C to prevent precipitation of hops extract components prior to use. The treatments were loaded into 1-mL syringes, which were covered to blind both the

study participant and the investigator performing the study as to the nature of the infusate. The higher dose of hops extract was based on what is currently been commercially marketed in New Zealand as a dietary supplement under the brand name “Calocurb” (250 mg; Calocurb Ltd, Auckland, New Zealand), and the 100-mg dose to investigate the effects of a lower dose (504).

5.3.4 Study protocol

Study visits (study part A, 3 visits; study part B, 2 visits) were separated by 3 – 7 days, and performed in randomised, double-blind fashion. Participants were instructed to abstain from strenuous exercise and alcohol consumption for 24 hours prior to each study visit and were provided with a standardised meal (Beef Lasagne; McCain Food, Wendouree, Victoria, Australia; energy content: 602 kcal) to be consumed by 7 p.m. on the night before each visit.

On the morning of each study day, each participant attended our Clinical Research Facility, located in the Adelaide Medical School, University of Adelaide, at 8 a.m. after an overnight fast from both solids and liquids, with the exception of water, after 7 p.m., and from water after 6.30 a.m.. Upon arrival, an i.v. cannula was placed into a forearm vein, and the arm was kept warm with a heat pad for regular sampling of ‘arterialised’ blood. The participant was intubated via an anaesthetised nostril with a small-diameter (external diameter: 3.5 mm), 17-channel manometric catheter (total length: 100 cm; Dentsleeve International, Mui Scientific, Mississauga, Ontario, Canada) into the stomach, and the catheter was then allowed to pass into the duodenum by peristalsis (196). The manometric catheter consisted of six antral channels, a 4.5-cm pyloric sleeve sensor with two channels situated on the back, and seven duodenal channels, with all side holes positioned at 1.5-cm intervals, measuring pressures in the antrum, pylorus and duodenum (285, 456). A dedicated infusion channel, located ~ 14.5 cm distal to

the pylorus, was used for intraduodenal administration (in study part A), and the most proximal antral channel for intragastric administration (in study part B). The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained by continuous measurement of the transmucosal potential difference between the most distal antral and most proximal duodenal, channels (457). Once the catheter was positioned correctly (within 90 ± 13 min across study days and participants), fasting motility was observed until the occurrence of a phase III of the interdigestive MMC. Immediately after the cessation of phase III, during phase I (a period of motor quiescence), a baseline (t = -10 min) blood sample (8.4 mL) was taken for measurement of plasma CCK and PYY concentrations (hormones have yet to be analysed for study part B) and blood glucose, the participant completed a VAS questionnaire to assess appetite perceptions, i.e. hunger, fullness, desire to eat, prospective food consumption, and GI symptoms, i.e. nausea, bloating (458), and fasting motility was monitored continuously for 10 min (t = -10 – -1 min). At t = -1 min, the 0.6-mL bolus of either one of the hops extract doses or control was administered intraduodenally (study part A), or the hops extract at the dose of 250 mg or control was administered intragastrically (part B), within 1 min, after which antropyloroduodenal pressures were recorded continuously, and blood samples and VAS questionnaires were collected at regular time points (t = 10, 20, 30, 45, 60, 90, 120, 150,180 and 210 min) throughout the study. At t = 180 min, the motility recording was terminated and the catheter removed. Participants were then presented with a standardised, cold, buffet-style meal and instructed to consume as much, or as little, food as they desired until they felt comfortably full, for up to 30 min (t = 180 – 210 min) (459). Participants were unaware of the purpose of the meal. At t = 210 min, after completion of the meal, a final blood sample was taken, and the VAS completed, and the participant was then allowed to leave the laboratory.

5.3.5 Measurements

5.3.5.1 Antropyloroduodenal pressures

Antropyloroduodenal pressures were digitised and recorded using a computer-based system running commercially available software (Solar GI, MMS Data base software, version 8.17; Medical Measurement Systems BV, Enschede, The Netherlands), and stored for subsequent analysis. Data were used to analyse the number and amplitude of antral, duodenal and isolated pyloric pressure waves, as well as basal pyloric pressure, using custom-written software modified to our requirements (kindly provided by Professor Emeritus A Smout, University Medical Centre, Amsterdam, The Netherlands) (174). Antral and phasic pyloric pressure waves were defined by an amplitude of ≥ 10 mmHg with a minimum interval of 10 s between peaks. Duodenal pressure waves were defined by an amplitude of ≥ 10 mmHg with a minimum of 3 s between peaks (505). Basal pyloric pressure was calculated by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral channel from the mean basal pressure recorded at the sleeve (457).

5.3.5.2 Plasma gut hormone and blood glucose concentrations

Blood samples were collected into ice-chilled ethylenediaminetetraacetic acid-treated tubes. Plasma was separated by centrifugation (3200 rpm, 15 min, 4 °C) within 15 min of collection and plasma stored at - 80℃ until assayed.

Plasma CCK-8 concentrations (pmol/L) were measured by radioimmunoassay after ethanol extraction using an adaptation of the method of Santangelo et al (462). The minimum detectable limits were 1 pmol/L. The intra- and inter-assay CVs were ~ 16.1% and 15%, respectively.

Plasma total PYY concentrations (pmol/L) were measured by radioimmunoassay using an antisera (kindly donated by Dr. B Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany) against human PYY (1-36) (Sigma-Aldrich, St Louis, MO) and raised in rabbits. This antiserum showed < 0.001% crossreactivity with human pancreatic polypeptide or sulphated CCK-8 and 0.0025% crossreactivity with human NPY. The minimum detectable limits were 1.5 pmol/L. The intra- and inter-assay CVs were both 12.7%.

Blood glucose was determined with a portable glucometer (Medisense Precision QLD; Abbott Laboratories, North Ryde, New South Wales, Australia), and quantified to ensure that blood glucose concentrations remained within physiological levels.

5.3.5.3 Appetite perceptions and GI symptoms

Appetite perceptions were quantified using validated 100-mm VAS questionnaires (458). Nausea and bloating were also assessed. The strength of each perception was rated on a 100-mm horizontal line, where 0 mm represented ‘sensation not felt at all’ and 100 mm ‘sensation felt the greatest’. Participants were asked to place a vertical stroke at the appropriate point on each 100-mm horizontal line to indicate how they were feeling at each time point. Other perceptions, including happiness and anxiety, were also assessed to distract from the main purpose of the questionnaire, but not evaluated.

5.3.5.4 Food and energy intake

The amount of food and liquids (g) consumed at the buffet meal was obtained by recording the weight of each food item in the buffet meal before being offered to the participant and at the end of the meal. The meal comprised 4 slices (~ 120 g) of whole-meal bread, 4 slices (~ 120 g) of white bread, 100 g sliced ham, 100 g sliced chicken, 85 g sliced cheddar cheese, 100 g

lettuce, 100 g sliced tomato, 100 g sliced cucumber, 22 g mayonnaise, 20 g margarine, 1 apple (~ 170 g), 1 banana (~ 190 g), 175 g strawberry yogurt, 100 g chocolate custard, 120 g fruit salad, 375 mL iced coffee, 300 mL orange juice and 600 mL water, and had a total energy content of ~ 2,300 kcal (~ 27% fat, ~ 52% carbohydrate and ~ 21% protein) and weight of ~ 2,924 g. Energy intake (kcal) was then calculated using commercially available software (Foodworks 9.0; Xyris Software, QLD) (459).

5.3.6 Data and statistical analysis

The number of participants for part A was determined by power calculations based on our previous study (479). We calculated that with 15 participants, we would be able to detect a difference of 17.5 (an effect size of 0.78) in total number of pyloric pressure waves at $\alpha = 0.05$, with power of 80%. The number of participants for part B was determined by power calculations based on the data from the n = 6 completed to date. We calculated that with 12 participants, we would be able to detect a difference of 35.8 (an effect size of 0.89) in total number of pyloric pressure waves at $\alpha = 0.05$, with power of 80%.

Plasma CCK and PYY and blood glucose concentrations were measured in duplicate and means calculated at each time point, and data expressed as absolute values. Basal pyloric pressure, number and amplitude of antral, duodenal, and isolated pyloric pressure, and VAS scores were expressed as change from baseline (i.e., t = -10 min) to account for small variations in baseline values. Mean basal pyloric pressure, total number and mean amplitudes of isolated pyloric pressure waves, antral, and duodenal pressures were calculated over the 180-min period post-treatment administration. The number of isolated pyloric pressure waves was also quantified in 15-min intervals during the 180-min post-treatment period. The total number and mean amplitudes of antral and duodenal pressure waves were used to calculate MI using the

following equation: MI (mmHg·number) = natural logarithm {[sum of amplitudes × number of contractions (pressure waves)] + 1} (463).

In part A, statistical analyses were performed with SPSS software (version 26.0; SPSS Inc.). Plasma CCK and PYY concentrations, number of isolated pyloric pressure waves, VAS scores, and blood glucose concentrations were analysed using repeated-measures two-way ANOVA, with treatment (ID-HE100, ID-HE250 and ID-control) and time (0 – 180 min) as factors. The number of isolated pyloric pressure waves were also analysed from t = 0 – 90 min, using repeated-measures two-way ANOVA, in order to evaluate the early effects of the treatments (506). Total number, mean amplitude, or MI of antral or duodenal pressures, mean basal pyloric pressure, and the total number and mean amplitude of isolated pyloric pressure waves, energy intake (kcal) and amount of food consumed from the test meal (g) were analysed using one-way ANOVA with treatment as factor. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. All data are reported as means ± SEMs. All tests were two-tailed, and differences were considered statistically significant at $P \leq 0.05$.

The data from part B have not be analysed statistically yet, as the study is ongoing.

5.4 Results

All participants completed all study visits of the respective part(s) and tolerated the study conditions well. No adverse effects were reported. In part A, plasma CCK data in one participant was unavailable due to technical problems. In part B, as the recruitment has not been completed, the data have not been analysed statistically, or hormone samples assayed.

5.4.1 Part A: Effects of intraduodenal hops extract

5.4.1.1 Plasma hormone concentrations

5.4.1.1.1 Cholecystokinin

There were no differences in baseline plasma CCK concentrations between study days, and no effect of treatment or time on plasma CCK (**Figure 5.1A**).

5.4.1.1.2 Peptide YY

There were no differences in baseline plasma PYY concentrations between study days. There was a significant treatment × time interaction ($P < 0.01$) for plasma PYY concentrations. At t = 90 min, ID-HE100 increased plasma PYY, compared with ID-control ($P < 0.05$), and from t = 60 min, ID-HE250 stimulated PYY, compared with ID-control ($P < 0.05$) (**Figure 5.1B**).

5.4.1.2 Antropyloroduodenal pressures

Baseline values for antropyloroduodenal pressures did not differ between study days. There was no effect of treatment on the number of isolated pyloric pressure waves over the 180 min post-administration, but there was a significant effect of treatment during the first 90 min ($P < 0.05$); ID-HE250 tended to stimulate number of isolated pyloric pressure waves, compared with ID-control ($P = 0.082$) (**Figure 5.2A**). There was no effect on the amplitude of isolated pyloric

pressure waves, the total number, mean amplitude or MI of antral and duodenal pressures, and mean basal pyloric pressure (**Table 5.1**).

5.4.1.3 Energy intake, appetite perceptions and GI symptoms

There was no effect of treatment on energy intake or the amount consumed from the buffet meal (**Table 5.2**). There were no differences in baseline ratings, or any effect of treatment or time, on ratings of hunger, desire to eat, prospective food consumption, fullness, bloating or nausea (**Figure 5.3A-F**).

5.4.1.4 Blood glucose concentrations

There were no differences in baseline blood glucose concentrations between study days, and no effects of treatment, or time, on blood glucose (data not shown).

5.4.2 Part B: Effects of intragastric hops extract

5.4.2.1 Antropyloroduodenal pressures

Baseline values for antropyloroduodenal pressures did not appear to differ between study days. IG-HE250 appeared to potently stimulate pyloric pressures during the first 90 min (**Figure 5.2B)**, but not to affect the amplitude of isolated pyloric pressure waves, the total number, mean amplitude or MI of antral and duodenal pressures, or mean basal pyloric pressure (**Table 5.1).**

5.4.2.2 Energy intake, appetite perceptions and GI symptoms

There did not appear to be an effect of treatment on energy intake or the amount consumed from the buffet meal (**Table 5.2**). There did not appear to be an effect of treatment or time, on ratings of hunger, desire to eat, prospective food consumption, fullness, nausea, and bloating (**Figure 5.4A-F**).

5.4.2.3 Blood glucose concentrations

There did not appear to be any differences in baseline blood glucose concentrations between study days, or any effect of treatment, or time, on blood glucose (data not shown).

Table 5.1: Total number, mean amplitude, and motility index of antral and duodenal pressure waves, total number, mean amplitude of isolated pyloric pressure waves, and mean basal pyloric pressure over 180-min period after either intraduodenal or intragastric administration of hops extract or control [1].

	control	**HE100**	**HE250**	**P value**
Part A (ID administration)				
Antral pressure waves				
Number	125±23	115±17	132±19	NS
Amplitude, mmHg	36±2	33±2	35±2	NS
Motility index, mmHg × min	13±0	13±0	13±0	NS
Basal pyloric pressure, mmHg	0±0	0±0	0±1	NS
Isolated pyloric pressure waves				
Number	21±6	29±7	37±8	NS
Amplitude, mmHg	15±2	16±2	16±2	NS
Duodenal pressure waves				
Number	842±86	848±122	921±96	NS
Amplitude, mmHg	30±2	26±2	30±2	NS
Motility index, mmHg × min	16±0	17±0	17±0	NS
Part B (IG administration)				
Antral pressure waves				
Number	71±16	–	65±17	NYA
Amplitude, mmHg	40±10	–	59±23	NYA
Motility index, mmHg × min	12±1	–	12±0	NYA
Basal pyloric pressure, mmHg	-2±2	–	-2±1	NYA
Isolated pyloric pressure waves				
Number	23±5	–	44±16	NYA
Amplitude, mmHg	15±2	–	19±1	NYA
Duodenal pressure waves				
Number	758±150	–	787±106	NYA
Amplitude, mmHg	25±1	–	27±2	NYA
Motility index, mmHg × min	16±0	–	17±0	NYA

[1] NS, non-significant; NYA, not yet analysed; HE100, administration of hops extract at the dose of 100 mg; HE250, administration of hops extract at the dose of 250 mg; control, administration of control (canola oil), IG, intragastric; ID, intraduodenal; P values for main treatment effects in part A were determined by one-way ANOVA. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects. Data from part B have not been analysed statistically yet. Data are means ± SEMs; n = 15 for study part A (ID administration) and n = 6 for study part B (IG administration).

Table 5.2: Energy content and amount of food consumed at the buffet meal, 180 min after either intraduodenal or intragastric administration of hops extract or control1.

	control	HE100	HE250	P value
Part A (ID administration)				
Energy intake, kcal	1147±72	1203±93	1141±69	NS
Amount eaten, g	1282±86	1244±73	1293±92	NS
Part B (IG administration)				
Energy intake, kcal	1129±61	–	1094±71	NYA
Amount eaten, g	1064±51	–	1205±124	NYA

[1] NS, non-significant; NYA, not yet analysed; HE100, administration of hops extract at the dose of 100 mg; HE250, administration of hops extract at the dose of 250 mg; control, administration of control (canola oil), IG, intragastric; ID, intraduodenal; in part A, P values for treatment effects were determined by one-way ANOVA. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed where ANOVAs revealed significant effects. Data from part B have not been analysed statistically yet. Data are means ± SEMs; n = 15 for study part A (ID administration) and n = 6 for study part B (IG administration).

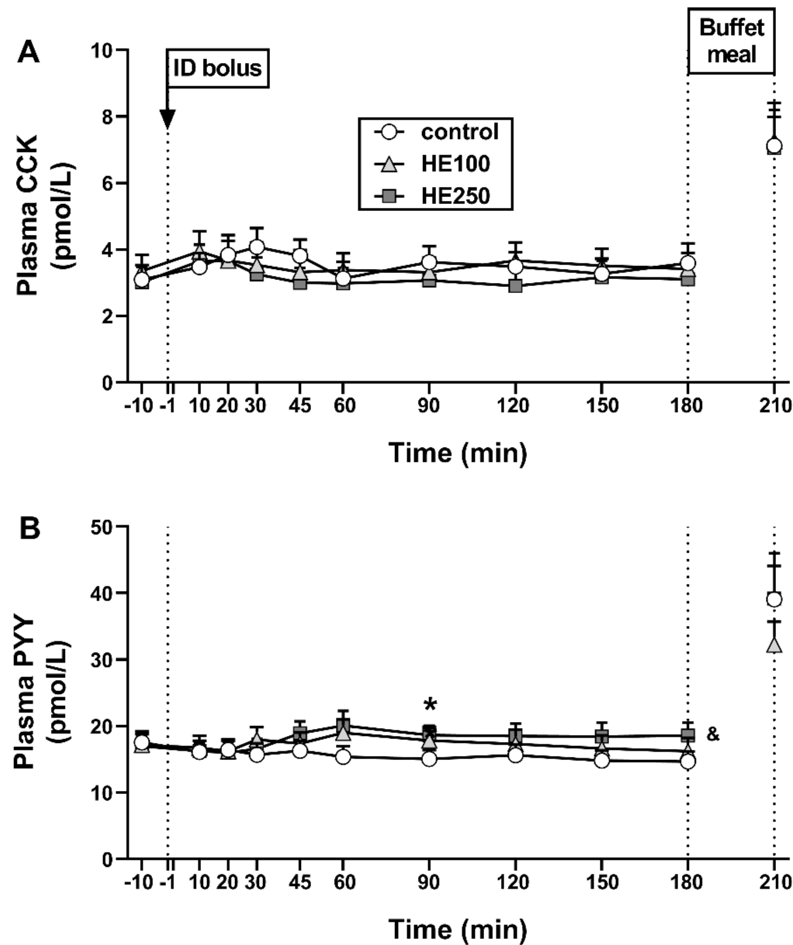

Figure 5.1: Plasma cholecystokinin (CCK) and peptide YY (PYY) concentrations at baseline (t = -10 min) and over 180 min period (t = 0 – 180 min) after intraduodenal (ID) bolus administeration of hops extract, at the doses of either 100 mg (HE100) or 250 mg (HE250), or vehicle (canola oil; control), and after the buffet meal, at t = 210 min. Data were analysed using repeated-measures two-way ANOVA with treatment and time as factors. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted when ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. (B) There was a significant treatment × time interaction ($P < 0.01$) for plasma PYY concentrations. At t = 90 min, HE100 increased plasma PYY, compared with control (* $P < 0.05$), and from t = 60 min, HE250 stimulated PYY, compared with control (& $P < 0.05$). Data are expressed as means ± SEMs; n = 14 for plasma CCK and n = 15 for plasma PYY.

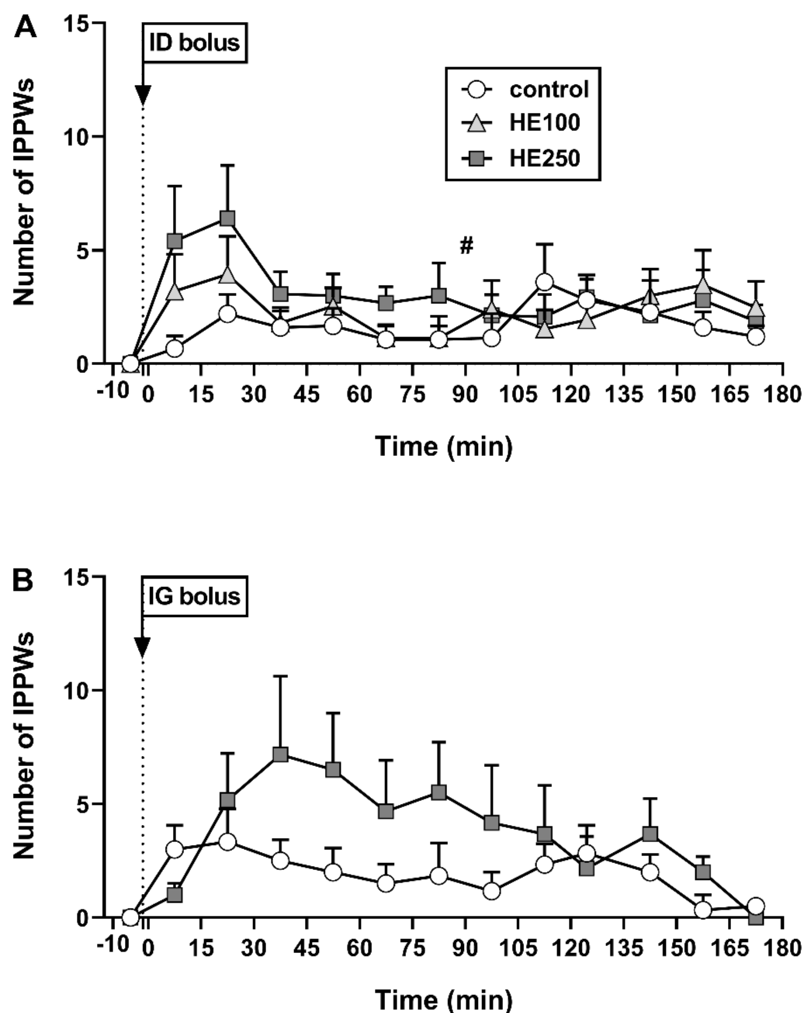

Figure 5.2: Number of isolated pyloric pressure waves during baseline (t = -10 – 0 min) and over 180 min period (t = 0 – 180 min) after (A) intraduodenal (ID) bolus administeration of hops extract, at the doses of either 100 mg (HE100) or 250 mg (HE250), or vehicle (canola oil; control) and (B) intragastric (IG) bolus administeration of either HE100 or control. Data was quantified in 10-min and 15-min intervals pre- and post-administration, respectively. Data from ID study days were analysed using repeated-measures two-way ANOVA with treatment and time as factors. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted when ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. Data from IG study days have not been analysed statistically yet. (A) During the first 90 min post-administration, there was a significant effect of treatment on the number of isolated pyloric pressure waves ($P < 0.05$); HE250 tended to stimulate number of isolated pyloric pressure waves, compared with control (# $P = 0.082$). Data are means ± SEMs; n = 15 for study part A days (A) and n = 6 for study part B (B).

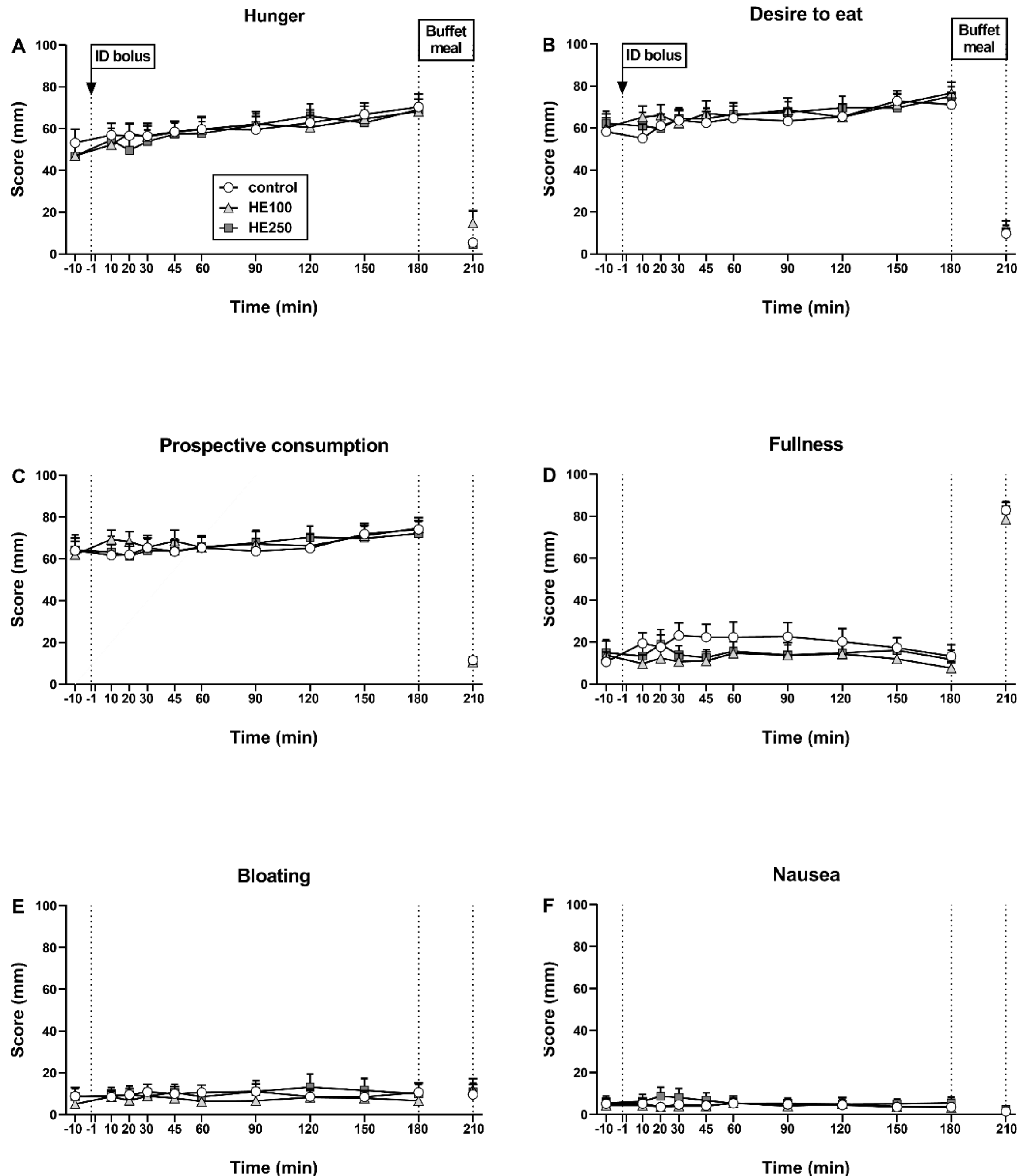

Figure 5.3: Scores for hunger (A), desire to eat (B), prospective food consumption (C), fullness (D), bloating (E), and nausea (F) at baseline (t = -10 min) and over 180 min period (t = 0 – 180 min) after intraduodenal (ID) bolus administeration of hops extract, at the doses of either 100 mg (HE100) or 250 mg (HE250), or vehicle (canola oil; control), and after the buffet meal, at t = 210 min. Data were analysed using repeated-measures two-way ANOVA with treatment and time as factors. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. Data are expressed as means ± SEMs; n = 15.

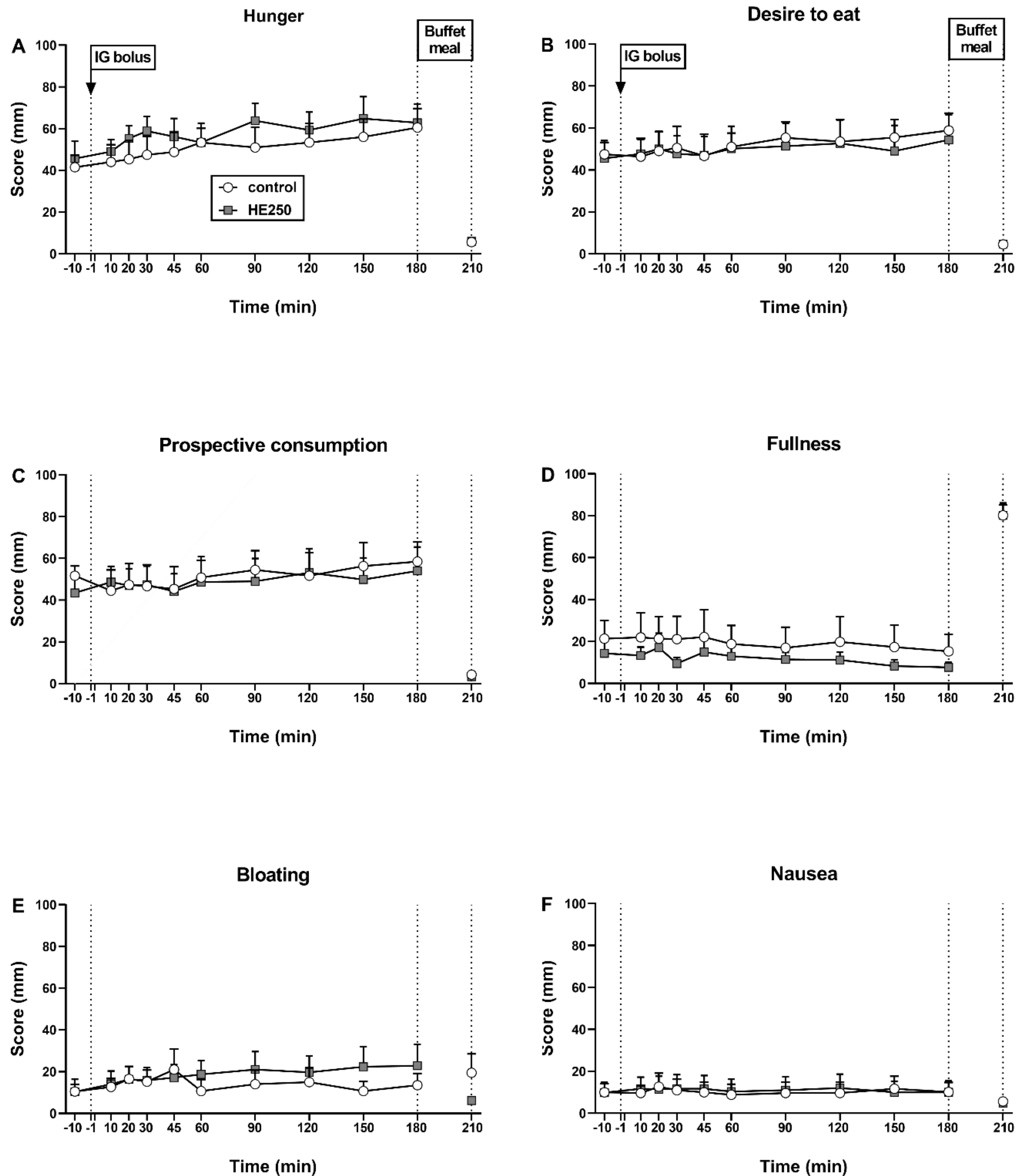

Figure 5.4: Scores for hunger (A), desire to eat (B), prospective food consumption (C), fullness (D), bloating (E), and nausea (F) at baseline (t = -10 min) and over 180 min period (t = 0 – 180 min) after intragastric (IG) bolus administeration of either hops extract, at the dose of 250 mg (HE250), or vehicle (canola oil; control), and after the buffet meal, at t = 210 min. Data have not been analysed statistically yet. Data are expressed as means ± SEMs; n = 6.

5.5. Discussion

Our study evaluated whether a bitter hops extract would stimulate GI functions, e.g. GI hormone release and gut motility, which are associated with the regulation of energy intake. We found that intraduodenal administration of hops extract, at the dose of 250 mg, only had a modest, and transient, effect to stimulate pyloric pressure, and a significant effect to stimulate PYY from ~ 60 min after administration, but did not affect antral or duodenal pressures, CCK, appetite perceptions or GI symptoms or energy intake. While study part B is still ongoing and data have not been analysed statistically, it appears that intragastric administration of hops, at the same dose of 250 mg, may stimulate pyloric pressures more potently and persistently, but may not affect antral and duodenal pressures, VAS ratings or energy intake. Therefore, our data suggest, at this stage, that intragastric delivery of hops may be a more potent stimulus than intraduodenal delivery, as measured by stimulation of pyloric pressures.

A number of studies, in both preclinical and clinical models, have evaluated the role of bitter compounds on the secretion of gut hormones (393, 399, 415, 418, 426-429). In the current study, we were particularly interested in the effects of hops extract on CCK and PYY, because these hormones have well-established roles to reduce energy intake (142, 464). Moreover, there are promising data from a study in mice showing that hops bitter compounds stimulate the release of these hormones (416). We found that intraduodenal hops extract, at the dose of 250 mg, stimulates PYY, but not CCK, from ~ 60 min after administration. Although the lack of effect on CCK is in line with the findings from some previous human studies, which evaluated the effect of slow intraduodenal infusion of QHCl at doses ranging from 37.5 to 225 mg (433, 479), it is in contrast with the outcome from another study, which investigated the effect of intragastric administration of QHCl at a lower dose (18 mg) (432). Since the lack of effect in the first two studies (433, 479) may be due to the low delivery rate, it appears that QHCl and hops may vary

in their CCK-stimulatory effects, although we still need to analyse the data in response to intragastric hops to draw a more definitive conclusion. The later release of PYY in our study is likely to be due to the fact that PYY-secretory cells are predominantly located in the distal small intestine (142), thus, the delay may reflect the time required for the hops extract to reach that region of the small intestine. Since study part B is still ongoing, the effect of intragastric administration of bops extract on PYY is still unclear.

In the current study we found a modest, and transient (lasting only for ~ 30 min), effect on the stimulation of pyloric pressures in response to intraduodenal hops extract, while, the available data from intragastric administration suggest that delivery into the stomach might have a more potent, and long-lasting (for ~ 90 min), effect. This is surprising since a previous study in mice reported the duodenum as the major site for TAS2Rs (390). Moreover, in our recent study (described in chapter 4 of this thesis), intragastric administration of QHCl, 60 min before a mixed-nutrient drink to allow sufficient exposure of duodenal TAS2Rs, had a comparable slowing effect on the emptying of the drink as intraduodenal QHCl given 30 min before the drink; in contrast, in our other study ((491), described in chapter 3), QHCl administered 30 min before the drink did not slow gastric emptying, suggesting that sufficient duodenal exposure of TAS2Rs to quinine is required for slowing of gastric emptying. These findings raise the possibility that hops bitter extract, in contrast to other bitter compounds, including quinine, may stimulate TAS2R subtypes that have a greater distribution in the stomach. In addition, since there was no effect of intraduodenal hops extract on CCK, the modest effect on pyloric pressure was unlikely to be mediated by CCK, which is surprising given the important role for CCK in regulating pyloric contractility (148). While we have currently no information on the effects of intragastric hops extract on CCK, this information will help to elucidate the role of this hormone.

Both modulation of GI motor functions, particularly pyloric pressure and stimulation of CCK, have been identified as key determinants of subsequent energy intake suppression in response to intraluminal nutrients (150-152). In the current study, in line with the small effect of hops extract on these parameters, we found no effect on appetite or energy intake. While the intragastric study part still needs to be completed, it appears that the GI stimulation that intraduodenal hops extract provided may have been insufficient to reduce energy intake. Another possibility could be the long interval of 180 min between 'preload' treatment and the meal, given that it has been shown that the longer such interval to assess energy intake, the less the energy intake-suppressant effect (507). However, energy intake was not a primary outcome for this study, hence the findings should be interpreted with caution. The completion of the intragastric study part will assist in drawing more definitive conclusions.

A few limitations of our study need to be considered. At this stage, the results are incomplete and, therefore, have to be interpreted with caution. We only studied males as they have been shown to be more sensitive to dietary manipulations (472) and also to avoid any confounding effect of the menstrual cycle (487). However, in one study, females have been reported to be more sensitive to bitter taste orally, as shown with denatonium benzoate, and their origin of phase III of fasting motility was more likely to switch from stomach to duodenum and hunger perception was decreased, compared with males (431), thus, future studies should also include females. Given that the GI responses to some nutrients (e.g. oleic acid, glutamate) have been shown to be reduced in obesity (349, 508), the effects of bitter compounds require investigation in people with obesity.

In conclusion, based on the currently available data from this study, it appears that the delivery of hops extract, at the dose of 250 mg, into the stomach more potently, and persistently,

stimulates pyloric pressures than delivery into the duodenum. Completion of the study, i.e. inclusion of further participants and analysis of the samples for gut hormones, will provide important information as to the primary location of action of this compound, which is likely to assist with optimising its effect on appetite and energy intake.

Chapter 6: Conclusions

The studies presented in this thesis have examined the effects of the bitter compound, quinine, and a bitter extract from hops, administered intraduodenally or intragastrically, on GI functions, energy intake and/or postprandial blood glucose concentrations in healthy people. The studies specifically investigated (1) the effects of slow intraduodenal infusion of quinine on antropyloroduodenal motility, plasma CCK and subsequent energy intake; (2) the effect of intragastric bolus administration of quinine on (i) the blood glucose and glucoregulatory hormone (GLP-1, insulin and glucagon) responses to, and gastric emptying of, a mixed-nutrient drink, and (ii) energy intake; (3) the comparative effects of intragastric and intraduodenal bolus administration of quinine, on the blood glucose and C-peptide responses to, and gastric emptying of, a mixed-nutrient drink; and (4) the effects of intragastric and intraduodenal bolus administration of a bitter hops extract on antropyloroduodenal motility, gut hormones (CCK and PYY) and subsequent energy intake.

Obesity and type 2 diabetes are responsible for more than half of the global morbidity and mortality, and represent a substantial economic and social burden. Therefore, the development of novel and side effect-free prevention, management and/or treatment strategies, that are effective in the long-term, are urgently required. The studies presented in this thesis investigated the hypothesis that bitter compounds may reduce energy intake and/or postprandial blood glucose by activating particular aspects of GI functions, including gut hormones release, gut motility and gastric emptying, which are all well-known for their role in the regulation of acute energy intake and/or glycaemia.

The first study, presented in chapter 2, characterised the physiological effects of a wide range of doses (37.5 – 225 mg) of quinine (given as quinine hydrochloride), when infused intraduodenally, on gut hormones, antropyloroduodenal motility and energy intake in healthy

lean males. This study showed that quinine, when infused at a slow rate, even at moderately high doses, did not modulate appetite-related GI functions or energy intake. We concluded that the low infusion rate was probably insufficient to reach a critical threshold required for activation of TAS2Rs.

While intraduodenal delivery of bitter compounds directly into the duodenum allows characterisation of the effects of these compounds in a standardised fashion, it does not provide evidence about the intragastric stimulation of TAS2Rs, and the absence of a meal, particularly containing carbohydrates, does not allow evaluation of the effects on postprandial blood glucose. In order to address these issues, the study in chapter 2 was designed to determine whether intragastric administration of quinine before a standardised meal would enhance the glycaemic response to that meal. In addition, because of the lack of effects in the first study, a higher dose of 600 mg (the recommended therapeutic dose of quinine in the treatment of malaria) was used as a proof of principle, and administered as a bolus with the aim to provide an adequate stimulus for activation of TAS2Rs. We found that intragastric quinine potently reduced postprandial blood glucose, most likely by stimulating insulin, and, to a lesser extent, glucagon and GLP-1, but independent of slowing of gastric emptying, and did not affect energy intake. We concluded that, using this study design, the effect of quinine to lower blood glucose was primarily mediated via post-absorptive mechanisms, i.e. effects of circulating quinine on the pancreas to stimulate insulin, thus, the role of TAS2Rs was unclear. Moreover, the stimulus appeared to be insufficient to reduce energy intake.

The studies presented in chapter 3 were designed based on (1) the outcome from the previous study (chapter 2), from which we hypothesised that probably a longer interval between quinine administration and drink ingestion would lead to greater intraluminal exposure of quinine with

TAS2Rs, resulting in slowing of gastric emptying and stimulation of gut and glucoregulatory hormones, and (2) the evidence from studies in mice indicating a variation in regional distribution of TAS2Rs subtypes along the GI tract, with a greater density of receptors in the duodenum than the stomach, thus, delivery of quinine to the duodenum, may potentially affect the targeted effects more potently that delivery into the stomach. Based on a pilot study, in which intraduodenal quinine (600 mg) resulted in a rise in insulin and stimulation of pyloric pressures (a key determinant for the slowing of gastric emptying) within ~30 min, and intragastric administration within ~60 min, we chose these timings as intervals between quinine administration and consumption of the nutrient drink. Using this study design, intragastric and intraduodenal administration of quinine had a comparable effect to stimulate C-peptide (a measure for insulin secretion) and also slowed gastric emptying of the drink, compared with control, associated with substantial lowering of blood glucose. Thus, it appears that longer luminal exposure of TAS2Rs (either in the stomach or the duodenum) to quinine was required for quinine to slow gastric emptying. While hormone results on GLP-1 are awaited, the role of GLP-1 to the stimulation of insulin is uncertain at this stage; however, such a contribution was likely to be modest as the rise in postprandial glucose was modest (~ 6 – 7 mmol/L) on all study days. Nevertheless, these studies (chapter 2 and 3) clearly demonstrate the potent blood glucose-lowering effects of quinine in healthy subjects, when administered in a targeted fashion.

In the study presented in chapter 5, we evaluated the effects of intraduodenal and intragastric administration of an extract from hops flowers (*Humulus lupulus L.)* on GI functions, which play key roles in the regulation of acute energy intake, specifically gut hormones and pyloric pressures. This hops extract was of interest, as it is available commercially (Calocurb^{TM}, Calocurb Ltd, Auckland, New Zealand) as a treatment to control appetite and body weight.

While the study has not been completed yet, our data suggest, at this stage, that intragastric delivery of hops may stimulate pyloric pressures more potently and persistently than intraduodenal delivery. While intraduodenal delivery, surprisingly, did not affect plasma CCK, it slightly stimulated PYY, while the data from intragastric delivery are not yet available, hence, no firm conclusions can be drawn at this stage. While there did not appear to be effects on energy intake, this was only a secondary outcome, and the period of 180 min between bitter administration and the buffet meal was most likely too long for a meaningful outcome. Nevertheless, the findings relating to pyloric pressures raise the possibility that hops bitter extract, in contrast to quinine, may preferentially stimulate TAS2R subtypes in the stomach. Completion of the study will help to draw more definitive conclusions.

Taken together, the studies presented in chapter 2 – 4 suggest that quinine potently reduces postprandial blood glucose via changes in GI functions, particularly slowing of gastric emptying, but may also act directly on the pancreas to stimulate insulin. The extent of these effect depends on various factors, including (1) the route of administration - given that the duodenum has been reported as the major site for TAS2Rs, intraduodenal delivery of quinine may be a more potent stimulus than intragastric delivery, (2) a sufficient delivery load, or concentration, to reach critical thresholds required for activation of TAS2Rs, and (3) an optimal time interval before the meal for greater intraluminal interaction of quinine with TAS2Rs. These findings also raise the question whether the lack of effect of quinine on energy intake in the previous studies could be addressed in this way and requires further investigation. Moreover, given that the potent blood glucose-lowering effects were seen in healthy individuals with good blood glucose control, studies are also warranted to evaluate these effects in people with type 2 diabetes. Because their postprandial glucose concentrations are much higher, it is possible that smaller doses are sufficient for a substantial glucose-lowering effect.

Finally, the glucose-lowering effects of quinine would need to be studied in long-term settings to determine whether the effects are maintained in the long-term, and safely, and are associated with improvements of long-term indicators of glucose control, e.g. HbA1c. Taken together, whether quinine has the potential to be utilised as a management strategy to regulate blood glucose in people with type 2 diabetes or impaired blood glucose tolerance warrants further investigation.

While our studies on the bitter hops extract (chapter 5) provide preliminary insights into the GI effects of this compound, a number of issues require further investigations, e.g. (1) whether shortening the timing between bitter extract administration and the standardised meal would demonstrate an energy intake-suppressant effect and (2) whether the effect on pyloric pressure is associated with slowing of gastric emptying, and the potential relationship with postprandial blood glucose.

In conclusion, the studies presented in this thesis demonstrate varying effects of bitter compounds on GI functions, including secretion of appetite- or glucoregulatory hormones, modulation of gut motility and gastric emptying. Moreover, the glycaemic effect of bitter compounds also appears to be partly regulated via extra-luminal mechanisms, e.g. pancreatic stimulation of insulin. Among the bitter compounds investigated, quinine had potent postprandial blood glucose lowering effects, while the effects of the hops extract remain less clear. Further research is required to comprehensively characterise the mechanisms underlying the observed effects, such as the involvement of specific bitter-receptor subtypes in the described effects or the role of the circulating bitter compounds, and to investigate the suitability of these compounds as potential management or treatment strategies for obesity and/or type 2 diabetes.

Chapter 7: References

1. World Health Organisation. Obesity and overweight- fact sheet 311. Internet: http://www.who.int/mediacentre/factsheets/fs311/en/ (accessed 30.10.2019).

2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet. 2014;384(9945):766-81.

3. Greenway F. Physiological adaptations to weight loss and factors favouring weight regain. Int J Obes (Lond). 2015;39(8):1188-96.

4. World Health Organisation. Global status report on noncommunicable diseases. WHO Press. 2014.

5. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC Public Health. 2009;9:88.

6. Thompson D, Edelsberg J, Colditz GA, Bird AP, Oster G. Lifetime health and economic consequences of obesity. Arch Intern Med. 1999;159(18):2177-83.

7. Carey VJ, Walters EE, Colditz GA, Solomon CG, Willett WC, Rosner BA, et al. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. Am J Epidemiol. 1997;145(7):614-9.

8. Bogers RP, Bemelmans WJ, Hoogenveen RT, Boshuizen HC, Woodward M, Knekt P, et al. Association of overweight with increased risk of coronary heart disease partly independent of blood pressure and cholesterol levels: a meta-analysis of 21 cohort studies including more than 300 000 persons. Arch Intern Med. 2007;167(16):1720-8.

9. Maggio CA, Pi-Sunyer FX. Obesity and type 2 diabetes. Endocrinol Metab Clin North Am. 2003;32(4):805-22.

10. Eckel RH, Kahn SE, Ferrannini E, Goldfine AB, Nathan DM, Schwartz MW, et al. Obesity and type 2 diabetes: what can be unified and what needs to be individualized? J Clin Endocrinol Metab. 2011;96(6):1654-63.

11. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract. 2017;128:40-50.

12. UK Prospective Diabetes Study (UKPDS). VIII. Study design, progress and performance. Diabetologia. 1991;34(12):877-90.

13. O'Donovan DG, Doran S, Feinle-Bisset C, Jones KL, Meyer JH, Wishart JM, et al. Effect of variations in small intestinal glucose delivery on plasma glucose, insulin, and incretin hormones in healthy subjects and type 2 diabetes. J Clin Endocrinol Metab. 2004;89(7):3431-5.

14. Marathe CS, Rayner CK, Jones KL, Horowitz M. Relationships between gastric emptying, postprandial glycemia, and incretin hormones. Diabetes Care. 2013;36(5):1396-405.

15. Horowitz M, Su YC, Rayner CK, Jones KL. Gastroparesis: prevalence, clinical significance and treatment. Can J Gastroenterol. 2001;15(12):805-13.

16. Group UPDSU. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352(9131):837-53.

17. Organisation for Economic Cooperation and Development (OECD) Directorate for Employment Labour and Social Affairs. OECD obesity update. 2017.

18. Australian Institute of Health and Welfare. Australian Bureau of Statistics Australian Health Survey 2014-15 - overweight and obesity. Internet: http://www.aihw.gov.au/who-is-overweight/ (accessed 21.08.2017).

19. Colagiuri S, Lee CM, Colagiuri R, Magliano D, Shaw JE, Zimmet PZ, et al. The cost of overweight and obesity in Australia. Med J Aust. 2010;192(5):260-4.

20. World Health Organisation. Obesity. Internet: https://www.who.int/topics/obesity/en/ (accessed 25.11.2019).

21. Janesick AS, Shioda T, Blumberg B. Transgenerational inheritance of prenatal obesogen exposure. Mol Cell Endocrinol. 2014;398(1-2):31-5.

22. Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet. 2009;373(9669):1083-96.

23. Freedman DM, Ron E, Ballard-Barbash R, Doody MM, Linet MS. Body mass index and all-cause mortality in a nationwide US cohort. Int J Obes (Lond). 2006;30(5):822-9.

24. Peter RS, Mayer B, Concin H, Nagel G. The effect of age on the shape of the BMI-mortality relation and BMI associated with minimum all-cause mortality in a large Austrian cohort. Int J Obes (Lond). 2015;39(3):530-4.

25. Pingitore R, Spring B, Garfieldt D. Gender differences in body satisfaction. Obes Res. 1997;5(5):402-9.

26. Tillin T, Sattar N, Godsland IF, Hughes AD, Chaturvedi N, Forouhi NG. Ethnicity-specific obesity cut-points in the development of Type 2 diabetes - a prospective study including three ethnic groups in the United Kingdom. Diabet Med. 2015;32(2):226-34.

27. Lean ME, Han TS, Morrison CE. Waist circumference as a measure for indicating need for weight management. BMJ. 1995;311(6998):158-61.

28. Booth A, Magnuson A, Foster M. Detrimental and protective fat: body fat distribution and its relation to metabolic disease. Horm Mol Biol Clin Investig. 2014;17(1):13-27.

29. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. J Clin Endocrinol Metab. 2008;93(11 Suppl 1):S57-63.

30. Sayeed MA, Mahtab H, Latif ZA, Khanam PA, Ahsan KA, Banu A, et al. Waist-to-height ratio is a better obesity index than body mass index and waist-to-hip ratio for predicting diabetes, hypertension and lipidemia. Bangladesh Med Res Counc Bull. 2003;29(1):1-10.

31. Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet. 2009;41(1):25-34.

32. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet. 2009;41(1):18-24.

33. Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien PJ, Theriault G, et al. The response to long-term overfeeding in identical twins. N Engl J Med. 1990;322(21):1477-82.

34. Stunkard AJ, Sørensen TI, Hanis C, Teasdale TW, Chakraborty R, Schull WJ, et al. Adoption study of obesity. N Engl J Med. 1986;314(4):193-8.

35. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007;316(5826):889-94.

36. Dina C, Meyre D, Gallina S, Durand E, Korner A, Jacobson P, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet. 2007;39(6):724-6.

37. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature. 1997;387(6636):903-8.

38. Wasim M, Awan FR, Najam SS, Khan AR, Khan HN. Role of leptin deficiency, inefficiency, and leptin receptors in obesity. Biochem Genet. 2016;54(5):565-72.

39. Scarborough P, Burg MR, Foster C, Swinburn B, Sacks G, Rayner M, et al. Increased energy intake entirely accounts for increase in body weight in women but not in men in the UK between 1986 and 2000. Br J Nutr. 2011;105(9):1399-404.

40. Prentice AM, Jebb SA. Fast foods, energy density and obesity: a possible mechanistic link. Obes Rev. 2003;4(4):187-94.

41. Malik VS, Popkin BM, Bray GA, Despres JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care. 2010;33(11):2477-83.

42. Nielsen SJ, Popkin BM. Patterns and trends in food portion sizes, 1977-1998. JAMA. 2003;289(4):450-3.

43. Kelly MT, Wallace JM, Robson PJ, Rennie KL, Welch RW, Hannon-Fletcher MP, et al. Increased portion size leads to a sustained increase in energy intake over 4 d in normal-weight and overweight men and women. Br J Nutr. 2009;102(3):470-7.

44. World Health Organisation. Global report on diabetes. Internet: https://www.who.int/diabetes/global-report/en/ (accessed 30.10.2019).

45. Diabetes Australia. Internet: https://www.diabetesaustralia.com.au/diabetes-in-australia (accessed 30.10.2019).

46. Seong J, Kang JY, Sun JS, Kim KW. Hypothalamic inflammation and obesity: a mechanistic review. Arch Pharm Res. 2019;42(5):383-92.

47. Brown JC, Harhay MO, Harhay MN. The value of anthropometric measures in nutrition and metabolism: Comment on anthropometrically predicted visceral adipose tissue and blood-based biomarkers: A cross-sectional analysis. Nutr Metab Insights. 2019;12:1178638819831712.

48. Deacon CF. Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes. Front Endocrinol (Lausanne). 2019;10:80.

49. Villena JA, Choi CS, Wang Y, Kim S, Hwang YJ, Kim YB, et al. Resistance to high-fat diet-induced obesity but exacerbated insulin resistance in mice overexpressing preadipocyte factor-1 (Pref-1): a new model of partial lipodystrophy. Diabetes. 2008;57(12):3258-66.

50. Macdonald IA. A review of recent evidence relating to sugars, insulin resistance and diabetes. Eur J Nutr. 2016;55(Suppl 2):17-23.

51. Leon-Acuna A, Alcala-Diaz JF, Delgado-Lista J, Torres-Pena JD, Lopez-Moreno J, Camargo A, et al. Hepatic insulin resistance both in prediabetic and diabetic patients determines postprandial lipoprotein metabolism: from the CORDIOPREV study. Cardiovasc Diabetol. 2016;15:68.

52. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus. N Engl J Med. 1976;295(8):417-20.

53. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000;321(7258):405-12.

54. Royal Australian College of General Practitioners and Diabetes Australia. General practive management of type 2 diabetes. 2016-2018:15.

55. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393-403.

56. Wing RR. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. Arch Intern Med. 2010;170(17):1566-75.

57. Goldstein DJ. Beneficial health effects of modest weight loss. Int J Obes Relat Metab Disord. 1992;16(6):397-415.

58. Maurer S. Weight management in primary care. Visc Med. 2016;32(5):342-6.

59. Bray GA, Frühbeck G, Ryan DH, Wilding JP. Management of obesity. Lancet. 2016;387(10031):1947-56.

60. DeLany JP, Kelley DE, Hames KC, Jakicic JM, Goodpaster BH. Effect of physical activity on weight loss, energy expenditure, and energy intake during diet induced weight loss. Obesity (Silver Spring). 2014;22(2):363-70.

61. Hu G, Lindstrom J, Valle TT, Eriksson JG, Jousilahti P, Silventoinen K, et al. Physical activity, body mass index, and risk of type 2 diabetes in patients with normal or impaired glucose regulation. Arch Intern Med. 2004;164(8):892-6.

62. Ryan D, Heaner M. Preface to the full report. Obesity. 2014;22(S2):S1-S3.

63. Wu T, Gao X, Chen M, Van Dam R. Long-term effectiveness of diet-plus-exercise interventions vs. diet-only interventions for weight loss: a meta-analysis. Obes Rev. 2009;10(3):313-23.

64. Tsai AG, Wadden TA. The evolution of very-low-calorie diets: an update and meta-analysis. Obesity (Silver Spring). 2006;14(8):1283-93.

65. Hemmingsson E, Johansson K, Eriksson J, Sundstrom J, Neovius M, Marcus C. Weight loss and dropout during a commercial weight-loss program including a very-low-calorie diet, a low-calorie diet, or restricted normal food: observational cohort study. Am J Clin Nutr. 2012;96(5):953-61.

66. Seimon RV, Wild-Taylor AL, Keating SE, McClintock S, Harper C, Gibson AA, et al. Effect of weight loss via severe vs moderate energy restriction on lean mass and body composition among postmenopausal women with obesity: The TEMPO diet randomized clinical trial. JAMA Netw Open. 2019;2(10):e1913733.

67. Samaha FF, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, et al. A low-carbohydrate as compared with a low-fat diet in severe obesity. N Engl J Med. 2003;348(21):2074-81.

68. Brehm BJ, Seeley RJ, Daniels SR, D'Alessio DA. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. J Clin Endocrinol Metab. 2003;88(4):1617-23.

69. Skov AR, Toubro S, Ronn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. Int J Obes Relat Metab Disord. 1999;23(5):528-36.

70. Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. Am J Clin Nutr. 2005;81(6):1298-306.

71. Westerterp-Plantenga M, Luscombe-Marsh N, Lejeune M, Diepvens K, Nieuwenhuizen A, Engelen M, et al. Dietary protein, metabolism, and body-weight regulation: dose–response effects. Int J Obes (Lond). 2006;30(S3):S16.

72. Piatti PM, Monti F, Fermo I, Baruffaldi L, Nasser R, Santambrogio G, et al. Hypocaloric high-protein diet improves glucose oxidation and spares lean body mass: comparison to hypocaloric high-carbohydrate diet. Metabolism. 1994;43(12):1481-7.

73. Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, et al. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. Am J Clin Nutr. 2005;82(1):41-8.

74. Brennan IM, Luscombe-Marsh ND, Seimon RV, Otto B, Horowitz M, Wishart JM, et al. Effects of fat, protein, and carbohydrate and protein load on appetite, plasma

cholecystokinin, peptide YY, and ghrelin, and energy intake in lean and obese men. Am J Physiol Gastrointest Liver Physiol. 2012;303(1):G129-40.

75. Barkeling B, Rossner S, Bjorvell H. Effects of a high-protein meal (meat) and a high-carbohydrate meal (vegetarian) on satiety measured by automated computerized monitoring of subsequent food intake, motivation to eat and food preferences. Int J Obes. 1990;14(9):743-51.

76. Marckmann P, Osther P, Pedersen AN, Jespersen B. High-protein diets and renal health. J Ren Nutr. 2015;25(1):1-5.

77. Cuenca-Sanchez M, Navas-Carrillo D, Orenes-Pinero E. Controversies surrounding high-protein diet intake: satiating effect and kidney and bone health. Adv Nutr. 2015;6(3):260-6.

78. Chanoine J-P, Hampl S, Jensen C, Boldrin M, Hauptman J. Effect of orlistat on weight and body composition in obese adolescents: a randomized controlled trial. JAMA. 2005;293(23):2873-83.

79. O'Donovan D, Feinle-Bisset C, Wishart J, Horowitz M. Lipase inhibition attenuates the acute inhibitory effects of oral fat on food intake in healthy subjects. Br J Nutr. 2003;90(5):849-52.

80. O'Donovan D, Horowitz M, Russo A, Feinle-Bisset C, Murolo N, Gentilcore D, et al. Effects of lipase inhibition on gastric emptying of, and on the glycaemic, insulin and cardiovascular responses to, a high-fat/carbohydrate meal in type 2 diabetes. Diabetologia. 2004;47(12):2208-14.

81. Rueda-Clausen CF, Padwal RS. Pharmacotherapy for weight loss. BMJ. 2014;348:g3526.

82. Davies MJ, Bergenstal R, Bode B, Kushner RF, Lewin A, Skjøth TV, et al. Efficacy of liraglutide for weight loss among patients with type 2 diabetes: the SCALE diabetes randomized clinical trial. JAMA. 2015;314(7):687-99.

83. Gadde KM, Allison DB, Ryan DH, Peterson CA, Troupin B, Schwiers ML, et al. Effects of low-dose, controlled-release, phentermine plus topiramate combination on weight and associated comorbidities in overweight and obese adults (CONQUER): a randomised, placebo-controlled, phase 3 trial. Lancet. 2011;377(9774):1341-52.

84. O'Neil PM, Smith SR, Weissman NJ, Fidler MC, Sanchez M, Zhang J, et al. Randomized placebo-controlled clinical trial of lorcaserin for weight loss in type 2 diabetes mellitus: the BLOOM-DM study. Obesity (Silver Spring). 2012;20(7):1426-36.

85. Smith SR, Weissman NJ, Anderson CM, Sanchez M, Chuang E, Stubbe S, et al. Multicenter, placebo-controlled trial of lorcaserin for weight management. N Engl J Med. 2010;363(3):245-56.

86. Berne C. A randomized study of orlistat in combination with a weight management programme in obese patients with Type 2 diabetes treated with metformin. Diabet Med. 2005;22(5):612-8.

87. Torgerson JS, Hauptman J, Boldrin MN, Sjostrom L. XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes Care. 2004;27(1):155-61.

88. Angrisani L, Santonicola A, Iovino P, Formisano G, Buchwald H, Scopinaro N. Bariatric surgery worldwide 2013. Obes Surg. 2015;25(10):1822-32.

89. Yip S, Plank LD, Murphy R. Gastric bypass and sleeve gastrectomy for type 2 diabetes: a systematic review and meta-analysis of outcomes. Obes Surg. 2013;23(12):1994-2003.

90. Tice JA, Karliner L, Walsh J, Petersen AJ, Feldman MD. Gastric banding or bypass? A systematic review comparing the two most popular bariatric procedures. Am J Med. 2008;121(10):885-93.

91. Field BC, Chaudhri OB, Bloom SR. Obesity treatment: novel peripheral targets. Br J Clin Pharmacol. 2009;68(6):830-43.

92. Camilleri M. Integrated upper gastrointestinal response to food intake. Gastroenterology. 2006;131(2):640-58.

93. Richardson CT, Feldman M. Salivary response to food in humans and its effect on gastric acid secretion. Am J Physiol. 1986;250(1 Pt 1):G85-91.

94. Feldman M, Richardson CT. Role of thought, sight, smell, and taste of food in the cephalic phase of gastric acid secretion in humans. Gastroenterology. 1986;90(2):428-33.

95. Katschinski M, Dahmen G, Reinshagen M, Beglinger C, Koop H, Nustede R, et al. Cephalic stimulation of gastrointestinal secretory and motor responses in humans. Gastroenterology. 1992;103(2):383-91.

96. Ohara I, Otsuka S, Yugari Y. Cephalic phase response of pancreatic exocrine secretion in conscious dogs. Am J Physiol. 1988;254(3 Pt 1):G424-8.

97. Bellisle F. The cephalic phase of insulin secretion in man. Diabete Metab. 1987;13(2):144-51.

98. Schafmayer A, Nustede R, Pompino A, Kohler H. Vagal influence on cholecystokinin and neurotensin release in conscious dogs. Scand J Gastroenterol. 1988;23(3):315-20.

99. Furness JB. The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol. 2012;9(5):286.

100. Depoortere I. Taste receptors of the gut: emerging roles in health and disease. Gut. 2014;63(1):179-90.

101. Lindemann B. Taste reception. Physiol Rev. 1996;76(3):719-66.

102. Roper SD. Taste buds as peripheral chemosensory processors. Semin Cell Dev Biol. 2013;24(1):71-9.

103. Cagan R. Allosteric regulation of glutamate taste receptor function. Umami: a basic taste. 1987:155-72.

104. Cartoni C, Yasumatsu K, Ohkuri T, Shigemura N, Yoshida R, Godinot N, et al. Taste preference for fatty acids is mediated by GPR40 and GPR120. J Neurosci. 2010;30(25):8376-82.

105. Laugerette F, Passilly-Degrace P, Patris B, Niot I, Febbraio M, Montmayeur J-P, et al. CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. J Clin Invest. 2005;115(11):3177-84.

106. Kare MR, Mattes RD. A selective overview of the chemical senses. Nutr Rev. 1990;48(2):39-48.

107. Holt S, Reid J, Taylor T, Tothill P, Heading R. Gastric emptying of solids in man. Gut. 1982;23(4):292-6.

108. Sasselli V, Pachnis V, Burns AJ. The enteric nervous system. Dev Biol. 2012;366(1):64-73.

109. Wu T, Rayner CK, Young RL, Horowitz M. Gut motility and enteroendocrine secretion. Curr opin pharmacol. 2013;13(6):928-34.

110. Anvari M, Horowitz M, Fraser R, Maddox A, Myers J, Dent J, et al. Effects of posture on gastric emptying of nonnutrient liquids and antropyloroduodenal motility. Am J Physiol. 1995;268(5 Pt 1):G868-71.

111. Volk N, Lacy B. Anatomy and physiology of the small bowel. Gastrointest Endosc Clin N Am. 2017;27(1):1-13.

112. Kiela PR, Ghishan FK. Physiology of intestinal absorption and secretion. Best Pract Res Clin Gastroenterol. 2016;30(2):145-59.

113. Takahashi T. Mechanism of interdigestive migrating motor complex. J Neurogastroenterol Motil. 2012;18(3):246-57.

114. Code CF, Marlett JA. The interdigestive myo-electric complex of the stomach and small bowel of dogs. J Physiol. 1975;246(2):289-309.

115. Azpiroz F, Malagelada JR. Gastric tone measured by an electronic barostat in health and postsurgical gastroparesis. Gastroenterology. 1987;92(4):934-43.

116. Azpiroz F, Malagelada JR. Vagally mediated gastric relaxation induced by intestinal nutrients in the dog. Am J Physiol. 1986;251(6 Pt 1):G727-35.

117. Read NW. Role of gastrointestinal factors in hunger and satiety in man. Proc Nutr Soc. 1992;51(1):7-11.

118. Hunt J, Smith J, Jiang C. Effect of meal volume and energy density on the gastric emptying of carbohydrates. Gastroenterology. 1985;89(6):1326-30.

119. Burks TF, Galligan JJ, Porreca F, Barber WD. Regulation of gastric emptying. Fed Proc. 1985;44(14):2897-901.

120. Camilleri M, Malagelada JR, Brown ML, Becker G, Zinsmeister AR. Relation between antral motility and gastric emptying of solids and liquids in humans. Am J Physiol. 1985;249(5 Pt 1):G580-5.

121. Collins PJ, Houghton LA, Read NW, Horowitz M, Chatterton BE, Heddle R, et al. Role of the proximal and distal stomach in mixed solid and liquid meal emptying. Gut. 1991;32(6):615-9.

122. Collins PJ, Horowitz M, Cook DJ, Harding PE, Shearman DJ. Gastric emptying in normal subjects--a reproducible technique using a single scintillation camera and computer system. Gut. 1983;24(12):1117-25.

123. Meyer JH, Elashoff J, Porter-Fink V, Dressman J, Amidon GL. Human postprandial gastric emptying of 1-3-millimeter spheres. Gastroenterology. 1988;94(6):1315-25.

124. Meyer JH, Ohashi H, Jehn D, Thomson JB. Size of liver particles emptied from the human stomach. Gastroenterology. 1981;80(6):1489-96.

125. Brener W, Hendrix TR, McHugh PR. Regulation of the gastric emptying of glucose. Gastroenterology. 1983;85(1):76-82.

126. Houghton LA, Read NW, Heddle R, Horowitz M, Collins PJ, Chatterton B, et al. Relationship of the motor activity of the antrum, pylorus, and duodenum to gastric emptying of a solid-liquid mixed meal. Gastroenterology. 1988;94(6):1285-91.

127. Karra E, Batterham RL. The role of gut hormones in the regulation of body weight and energy homeostasis. Mol Cell Endocrinol. 2010;316(2):120-8.

128. Bednarek MA, Feighner SD, Pong SS, McKee KK, Hreniuk DL, Silva MV, et al. Structure-function studies on the new growth hormone-releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor 1a. J Med Chem. 2000;43(23):4370-6.

129. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. Nature. 2001;409(6817):194-8.

130. Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab. 2002;87(6):2988.

131. Cummings DE, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. Am J Physiol Endocrinol Metab. 2004;287(2):E297-304.

132. Callahan HS, Cummings DE, Pepe MS, Breen PA, Matthys CC, Weigle DS. Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. J Clin Endocrinol Metab. 2004;89(3):1319-24.

133. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab. 2001;86(12):5992.

134. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, et al. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab. 2002;87(1):240-4.

135. Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, et al. Ghrelin increases food intake in obese as well as lean subjects. Int J Obes (Lond). 2005;29(9):1130-6.

136. Levin F, Edholm T, Schmidt PT, Gryback P, Jacobsson H, Degerblad M, et al. Ghrelin stimulates gastric emptying and hunger in normal-weight humans. J Clin Endocrinol Metab. 2006;91(9):3296-302.

137. Fujino K, Inui A, Asakawa A, Kihara N, Fujimura M, Fujimiya M. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. J Physiol. 2003;550(Pt 1):227-40.

138. Murray CD, Martin NM, Patterson M, Taylor SA, Ghatei MA, Kamm MA, et al. Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. Gut. 2005;54(12):1693-8.

139. Longo KA, Charoenthongtrakul S, Giuliana DJ, Govek EK, McDonagh T, Qi Y, et al. Improved insulin sensitivity and metabolic flexibility in ghrelin receptor knockout mice. Regul Pept. 2008;150(1-3):55-61.

140. Qi Y, Longo KA, Giuliana DJ, Gagne S, McDonagh T, Govek E, et al. Characterization of the insulin sensitivity of ghrelin receptor KO mice using glycemic clamps. BMC Physiol. 2011;11:1.

141. Tong J, Prigeon RL, Davis HW, Bidlingmaier M, Kahn SE, Cummings DE, et al. Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans. Diabetes. 2010;59(9):2145-51.

142. Steinert RE, Feinle-Bisset C, Asarian L, Horowitz M, Beglinger C, Geary N. Ghrelin, CCK, GLP-1, and PYY(3-36): secretory controls and physiological roles in eating and glycemia in health, obesity, and after RYGB. Physiol Rev. 2017;97(1):411-63.

143. Rehfeld JF, Sun G, Christensen T, Hillingso JG. The predominant cholecystokinin in human plasma and intestine is cholecystokinin-33. J Clin Endocrinol Metab. 2001;86(1):251-8.

144. Rehfeld JF. Accurate measurement of cholecystokinin in plasma. Clin Chem. 1998;44(5):991-1001.

145. Muurahainen N, Kissileff HR, Derogatis AJ, Pi-Sunyer FX. Effects of cholecystokinin-octapeptide (CCK-8) on food intake and gastric emptying in man. Physiol Behav. 1988;44(4-5):645-9.

146. Moran TH, McHugh PR. Cholecystokinin suppresses food intake by inhibiting gastric emptying. Am J Physiol. 1982;242(5):R491-7.

147. Liddle RA, Morita ET, Conrad CK, Williams JA. Regulation of gastric emptying in humans by cholecystokinin. J Clin Invest. 1986;77(3):992-6.

148. Brennan IM, Little TJ, Feltrin KL, Smout AJ, Wishart JM, Horowitz M, et al. Dose-dependent effects of cholecystokinin-8 on antropyloroduodenal motility, gastrointestinal hormones, appetite, and energy intake in healthy men. Am J Physiol Endocrinol Metab. 2008;295(6):E1487-94.

149. Straathof JW, Mearadji B, Lamers CB, Masclee AA. Effect of CCK on proximal gastric motor function in humans. Am J Physiol. 1998;274(5):G939-44.

150. Kissileff HR, Pi-Sunyer FX, Thornton J, Smith GP. C-terminal octapeptide of cholecystokinin decreases food intake in man. Am J Clin Nutr. 1981;34(2):154-60.

151. Pi-Sunyer X, Kissileff HR, Thornton J, Smith GP. C-terminal octapeptide of cholecystokinin decreases food intake in obese men. Physiol Behav. 1982;29(4):627-30.

152. Kraly FS, Carty WJ, Resnick S, Smith GP. Effect of cholecystokinin on meal size and intermeal interval in the sham-feeding rat. J Comp Physiol Psychol. 1978;92(4):697-707.

153. Ballinger A, McLoughlin L, Medbak S, Clark M. Cholecystokinin is a satiety hormone in humans at physiological post-prandial plasma concentrations. Clin Sci (Lond). 1995;89(4):375-81.

154. Lieverse RJ, Jansen JB, Masclee AA, Lamers CB. Satiety effects of a physiological dose of cholecystokinin in humans. Gut. 1995;36(2):176-9.

155. Greenough A, Cole G, Lewis J, Lockton A, Blundell J. Untangling the effects of hunger, anxiety, and nausea on energy intake during intravenous cholecystokinin octapeptide (CCK-8) infusion. Physiol Behav. 1998;65(2):303-10.

156. Fried M, Erlacher U, Schwizer W, Lochner C, Koerfer J, Beglinger C, et al. Role of cholecystokinin in the regulation of gastric emptying and pancreatic enzyme secretion in humans. Studies with the cholecystokinin-receptor antagonist loxiglumide. Gastroenterology. 1991;101(2):503-11.

157. Fried M, Schwizer W, Beglinger C, Keller U, Jansen JB, Lamers CB. Physiological role of cholecystokinin on postprandial insulin secretion and gastric meal emptying in man. Studies with the cholecystokinin receptor antagonist loxiglumide. Diabetologia. 1991;34(10):721-6.

158. Mesquita MA, Thompson DG, Troncon LE, D'Amato M, Rovati LC, Barlow J. Effect of cholecystokinin-A receptor blockade on lipid-induced gastric relaxation in humans. Am J Physiol. 1997;273(1 Pt 1):G118-23.

159. Beglinger C, Degen L, Matzinger D, D'Amato M, Drewe J. Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans. Am J Physiol Regul Integr Comp Physiol. 2001;280(4):R1149-54.

160. Baum F, Nauck MA, Ebert R, Cantor P, Hoffmann G, Choudhury AR, et al. Role of endogenously released cholecystokinin in determining postprandial insulin levels in man: effects of loxiglumide, a specific cholecystokinin receptor antagonist. Digestion. 1992;53(3-4):189-99.

161. Niederau C, Schwarzendrube J, Luthen R, Niederau M, Strohmeyer G, Rovati L. Effects of cholecystokinin receptor blockade on circulating concentrations of glucose, insulin, C-peptide, and pancreatic polypeptide after various meals in healthy human volunteers. Pancreas. 1992;7(1):1-10.

162. Liddle RA, Rushakoff RJ, Morita ET, Beccaria L, Carter JD, Goldfine ID. Physiological role for cholecystokinin in reducing postprandial hyperglycemia in humans. J Clin Invest. 1988;81(6):1675-81.

163. Boguszewski CL, van der Lely AJ. The role of the gastrointestinal tract in the control of energy balance. Transl Gastrointest Cancer. 2014;4(1):3-13.

164. Batterham RL, Bloom SR. The gut hormone peptide YY regulates appetite. Ann N Y Acad Sci. 2003;994(1):162-8.

165. Ekblad E, Sundler F. Distribution of pancreatic polypeptide and peptide YY. Peptides. 2002;23(2):251-61.

166. Lin HC, Chey WY, Zhao X-T. Release of distal gut peptide YY (PYY) by fat in proximal gut depends on CCK. Peptides. 2000;21(10):1561-3.

167. Degen L, Drewe J, Piccoli F, Grani K, Oesch S, Bunea R, et al. Effect of CCK-1 receptor blockade on ghrelin and PYY secretion in men. Am J Physiol Regul Integr Comp Physiol. 2007;292(4):R1391-9.

168. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, et al. Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med. 2003;349(10):941-8.

169. Korner J, Bessler M, Cirilo LJ, Conwell IM, Daud A, Restuccia NL, et al. Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin. J Clin Endocrinol Metab. 2005;90(1):359-65.

170. Morinigo R, Moize V, Musri M, Lacy AM, Navarro S, Marin JL, et al. Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects. J Clin Endocrinol Metab. 2006;91(5):1735-40.

171. Seimon RV, Taylor P, Little TJ, Noakes M, Standfield S, Clifton PM, et al. Effects of acute and longer-term dietary restriction on upper gut motility, hormone, appetite, and energy-intake responses to duodenal lipid in lean and obese men. Am J Clin Nutr. 2014;99(1):24-34.

172. Pappas TN, Debas HT, Chang AM, Taylor IL. Peptide YY release by fatty acids is sufficient to inhibit gastric emptying in dogs. Gastroenterology. 1986;91(6):1386-9.

173. Feinle-Bisset C, Patterson M, Ghatei MA, Bloom SR, Horowitz M. Fat digestion is required for suppression of ghrelin and stimulation of peptide YY and pancreatic polypeptide secretion by intraduodenal lipid. Am J Physiol Endocrinol Metab. 2005;289(6):E948-53.

174. Ryan AT, Feinle-Bisset C, Kallas A, Wishart JM, Clifton PM, Horowitz M, et al. Intraduodenal protein modulates antropyloroduodenal motility, hormone release, glycemia, appetite, and energy intake in lean men. Am J Clin Nutr. 2012;96(3):474-82.

175. Ockander L, Hedenbro JL, Rehfeld JF, Sjolund K. Jejunoileal bypass changes the duodenal cholecystokinin and somatostatin cell density. Obes Surg. 2003;13(4):584-90.

176. Savage AP, Adrian TE, Carolan G, Chatterjee VK, Bloom SR. Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. Gut. 1987;28(2):166-70.

177. Van Citters GW, Lin HC. The ileal brake: a fifteen-year progress report. Curr Gastroenterol Rep. 1999;1(5):404-9.

178. Spiller RC, Trotman IF, Higgins BE, Ghatei MA, Grimble GK, Lee YC, et al. The ileal brake--inhibition of jejunal motility after ileal fat perfusion in man. Gut. 1984;25(4):365-74.

179. Spiller RC, Trotman IF, Adrian TE, Bloom SR, Misiewicz JJ, Silk DB. Further characterisation of the 'ileal brake' reflex in man--effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY. Gut. 1988;29(8):1042-51.

180. Vrang N, Madsen AN, Tang-Christensen M, Hansen G, Larsen PJ. PYY (3–36) reduces food intake and body weight and improves insulin sensitivity in rodent models of diet-induced obesity. Am J Physiol Regul Integr Comp Physiol. 2006;291(2):R367-75.

181. Chelikani PK, Haver AC, Reeve JR, Jr., Keire DA, Reidelberger RD. Daily, intermittent intravenous infusion of peptide YY(3-36) reduces daily food intake and adiposity in rats. Am J Physiol Regul Integr Comp Physiol. 2006;290(2):R298-305.

182. le Roux CW, Batterham RL, Aylwin SJ, Patterson M, Borg CM, Wynne KJ, et al. Attenuated peptide YY release in obese subjects is associated with reduced satiety. Endocrinology. 2006;147(1):3-8.

183. Gura T. Obesity research. Labs fail to reproduce protein's appetite-suppressing effects. Science. 2004;305(5681):158-9.

184. Sloth B, Holst JJ, Flint A, Gregersen NT, Astrup A. Effects of PYY1-36 and PYY3-36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects. Am J Physiol Endocrinol Metab. 2007;292(4):E1062-8.

185. Degen L, Oesch S, Casanova M, Graf S, Ketterer S, Drewe J, et al. Effect of peptide YY3-36 on food intake in humans. Gastroenterology. 2005;129(5):1430-6.

186. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, et al. Gut hormone PYY(3-36) physiologically inhibits food intake. Nature. 2002;418(6898):650-4.

187. Bottcher G, Ahren B, Lundquist I, Sundler F. Peptide YY: intrapancreatic localization and effects on insulin and glucagon secretion in the mouse. Pancreas. 1989;4(3):282-8.

188. Nieuwenhuizen AG, Karlsson S, Fridolf T, Ahren B. Mechanisms underlying the insulinostatic effect of peptide YY in mouse pancreatic islets. Diabetologia. 1994;37(9):871-8.

189. Chandarana K, Gelegen C, Irvine EE, Choudhury AI, Amouyal C, Andreelli F, et al. Peripheral activation of the Y2-receptor promotes secretion of GLP-1 and improves glucose tolerance. Mol Metab. 2013;2(3):142-52.

190. Ahren B, Larsson H. Peptide YY does not inhibit glucose-stimulated insulin secretion in humans. Eur J Endocrinol. 1996;134(3):362-5.

191. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab. 1986;63(2):492-8.

192. Seino Y, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: Similarities and differences. J Diabetes Investig. 2010;1(1-2):8-23.

193. Vilsboll T, Agerso H, Krarup T, Holst JJ. Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. J Clin Endocrinol Metab. 2003;88(1):220-4.

194. Vilsboll T, Agerso H, Lauritsen T, Deacon CF, Aaboe K, Madsbad S, et al. The elimination rates of intact GIP as well as its primary metabolite, GIP 3-42, are similar in type 2 diabetic patients and healthy subjects. Regul Pept. 2006;137(3):168-72.

195. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab. 2013;17(6):819-37.

196. Ryan AT, Luscombe-Marsh ND, Saies AA, Little TJ, Standfield S, Horowitz M, et al. Effects of intraduodenal lipid and protein on gut motility and hormone release, glycemia, appetite, and energy intake in lean men. Am J Clin Nutr. 2013;98(2):300-11.

197. Brennan IM, Feltrin KL, Horowitz M, Smout AJ, Meyer JH, Wishart J, et al. Evaluation of interactions between CCK and GLP-1 in their effects on appetite, energy intake, and antropyloroduodenal motility in healthy men. Am J Physiol Regul Integr Comp Physiol. 2005;288(6):R1477-85.

198. Schirra J, Wank U, Arnold R, Goke B, Katschinski M. Effects of glucagon-like peptide-1(7-36)amide on motility and sensation of the proximal stomach in humans. Gut. 2002;50(3):341-8.

199. Schirra J, Houck P, Wank U, Arnold R, Goke B, Katschinski M. Effects of glucagon-like peptide-1(7-36)amide on antro-pyloro-duodenal motility in the interdigestive state and with duodenal lipid perfusion in humans. Gut. 2000;46(5):622-31.

200. Schirra J, Nicolaus M, Roggel R, Katschinski M, Storr M, Woerle HJ, et al. Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. Gut. 2006;55(2):243-51.

201. Naslund E, Bogefors J, Skogar S, Gryback P, Jacobsson H, Holst JJ, et al. GLP-1 slows solid gastric emptying and inhibits insulin, glucagon, and PYY release in humans. Am J Physiol. 1999;277(3):R910-6.

202. Naslund E, Gutniak M, Skogar S, Rossner S, Hellstrom PM. Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. Am J Clin Nutr. 1998;68(3):525-30.

203. Meier JJ, Gallwitz B, Salmen S, Goetze O, Holst JJ, Schmidt WE, et al. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. J Clin Endocrinol Metab. 2003;88(6):2719-25.

204. Nauck M, Wollschläger D, Werner J, Holst J, Ørskov C, Creutzfeldt W, et al. Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7–36 amide]) in patients with NIDDM. Diabetologia. 1996;39(12):1546-53.

205. Little TJ, Pilichiewicz AN, Russo A, Phillips L, Jones KL, Nauck MA, et al. Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. J Clin Endocrinol Metab. 2006;91(5):1916-23.

206. Edwards CM, Todd JF, Mahmoudi M, Wang Z, Wang RM, Ghatei MA, et al. Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9-39. Diabetes. 1999;48(1):86-93.

207. Turton M, Shea D, Gunn I, Beak S. A role for glucagon-like peptide-1 in the central regulation of feeding. Nature. 1996;379(6560):69-72.

208. Schirra J, Nicolaus M, Roggel R, Katschinski M, Storr M, Woerle HJ, et al. Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. Gut. 2006;55(2):243-51.

209. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, et al. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol. 1997;273(5):E981-8.

210. Naslund E, Barkeling B, King N, Gutniak M, Blundell JE, Holst JJ, et al. Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. Int J Obes Relat Metab Disord. 1999;23(3):304-11.

211. Degen L, Oesch S, Matzinger D, Drewe J, Knupp M, Zimmerli F, et al. Effects of a preload on reduction of food intake by GLP-1 in healthy subjects. Digestion. 2006;74(2):78-84.

212. Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest. 1998;101(3):515-20.

213. Steinert RE, Schirra J, Meyer-Gerspach AC, Kienle P, Fischer H, Schulte F, et al. Effect of glucagon-like peptide-1 receptor antagonism on appetite and food intake in healthy men. Am J Clin Nutr. 2014;100(2):514-23.

214. Murphy KG, Bloom SR. Gut hormones and the regulation of energy homeostasis. Nature. 2006;444(7121):854-9.

215. Andersen DK, Elahi D, Brown JC, Tobin JD, Andres R. Oral glucose augmentation of insulin secretion: interactions of gastric inhibitory polypeptide with ambient glucose and insulin levels. J Clin Invest. 1978;62(1):152-61.

216. Morgan LM. The metabolic role of GIP: physiology and pathology. Biochem Soc Trans. 1996;24(2):585-91.

217. Meier JJ, Kemmeries G, Holst JJ, Nauck MA. Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. Diabetes. 2005;54(7):2212-8.

218. Meier JJ, Goetze O, Anstipp J, Hagemann D, Holst JJ, Schmidt WE, et al. Gastric inhibitory polypeptide does not inhibit gastric emptying in humans. Am J Physiol Endocrinol Metab. 2004;286(4):E621-5.

219. Rijkelijkhuizen JM, McQuarrie K, Girman CJ, Stein PP, Mari A, Holst JJ, et al. Effects of meal size and composition on incretin, alpha-cell, and beta-cell responses. Metabolism. 2010;59(4):502-11.

220. Flatt P. Dorothy Hodgkin Lecture 2008 Gastric inhibitory polypeptide (GIP) revisited: a new therapeutic target for obesity–diabetes? Diabet Med. 2008;25(7):759-64.

221. Fajans SS, Floyd JC, Jr., Knopf RF, Conn FW. Effect of amino acids and proteins on insulin secretion in man. Recent Prog Horm Res. 1967;23:617-62.

222. van Loon LJ, Kruijshoop M, Menheere PP, Wagenmakers AJ, Saris WH, Keizer HA. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. Diabetes Care. 2003;26(3):625-30.

223. Gielkens H, Verkijk M, Frölich M, Lamers C, Masclee A. Is the effect of acute hyperglycaemia on interdigestive antroduodenal motility and small-bowel transit mediated by insulin? Eur J Clin Invest. 1997;27(8):703-10.

224. Thor P, Laskiewicz J, Konturek JW, Konturek SJ, Creutzfeldt W. Role of GIP and insulin in glucose-induced changes in intestinal motility patterns. Am J Physiol. 1987;252(1 Pt 1):G8-12.

225. Bjornsson ES, Urbanavicius V, Eliasson B, Attvall S, Smith U, Abrahamsson H. Effects of insulin and beta-adrenergic blockade on the migrating motor complex in humans. Scand J Gastroenterol. 1995;30(3):219-24.

226. Eliasson B, Bjornsson E, Urbanavicius V, Andersson H, Fowelin J, Attvall S, et al. Hyperinsulinaemia impairs gastrointestinal motility and slows carbohydrate absorption. Diabetologia. 1995;38(1):79-85.

227. Chapman IM, Goble EA, Wittert GA, Morley JE, Horowitz M. Effect of intravenous glucose and euglycemic insulin infusions on short-term appetite and food intake. Am J Physiol. 1998;274(3):R596-603.

228. Verdich C, Toubro S, Buemann B, Lysgard Madsen J, Juul Holst J, Astrup A. The role of postprandial releases of insulin and incretin hormones in meal-induced satiety--effect of obesity and weight reduction. Int J Obes Relat Metab Disord. 2001;25(8):1206-14.

229. Aronoff SL, Berkowitz K, Shreiner B, Want L. Glucose metabolism and regulation: beyond insulin and glucagon. Diabetes Spectr. 2004;17(3):183-90.

230. Komatsu R, Matsuyama T, Namba M, Watanabe N, Itoh H, Kono N, et al. Glucagonostatic and insulinotropic action of glucagonlike peptide I-(7–36)-amide. Diabetes. 1989;38(7):902-5.

231. Chernish SM, Brunelle RR, Rosenak BD, Ahmadzai S. Comparison of the effects of glucagon and atropine sulfate on gastric emptying. Am J Gastroenterol. 1978;70(6):581-6.

232. Dotevall G, Kock NG. The effect of glucagon on intestinal motility in man. Gastroenterology. 1963;45:364-7.

233. Bagger JI, Holst JJ, Hartmann B, Andersen B, Knop FK, Vilsboll T. Effect of oxyntomodulin, glucagon, GLP-1, and combined glucagon +GLP-1 infusion on food intake, appetite, and resting energy expenditure. J Clin Endocrinol Metab. 2015;100(12):4541-52.

234. Penick SB, Hinkle LE, Jr. Depression of food intake induced in healthy subjects by glucagon. N Engl J Med. 1961;264:893-7.

235. Geary N, Kissileff HR, Pi-Sunyer FX, Hinton V. Individual, but not simultaneous, glucagon and cholecystokinin infusions inhibit feeding in men. Am J Physiol. 1992;262(6 Pt 2):R975-80.

236. O'Harte FP, Franklin ZJ, Irwin N. Two novel glucagon receptor antagonists prove effective therapeutic agents in high-fat-fed and obese diabetic mice. Diabetes Obes Metab. 2014;16(12):1214-22.

237. Vella A, Camilleri M. The gastrointestinal tract as an integrator of mechanical and hormonal response to nutrient ingestion. Diabetes. 2017;66(11):2729-37.

238. Latorre R, Sternini C, De Giorgio R, Greenwood-Van Meerveld B. Enteroendocrine cells: a review of their role in brain-gut communication. Neurogastroenterol Motil. 2016;28(5):620-30.

239. Depoortere I. Taste receptors of the gut: emerging roles in health and disease. Gut. 2014;63(1):179-90.

240. de Lartigue G, Diepenbroek C. Novel developments in vagal afferent nutrient sensing and its role in energy homeostasis. Curr Opin Pharmacol. 2016;31:38-43.

241. Grundy D. Signalling the state of the digestive tract. Auton Neurosci. 2006;125(1-2):76-80.

242. Furness JB, Rivera LR, Cho HJ, Bravo DM, Callaghan B. The gut as a sensory organ. Nat Rev Gastroenterol Hepatol. 2013;10(12):729-40.

243. Brookes SJ, Spencer NJ, Costa M, Zagorodnyuk VP. Extrinsic primary afferent signalling in the gut. Nat Rev Gastroenterol Hepatol. 2013;10(5):286-96.

244. Feinle C, Grundy D, Read NW. Effects of duodenal nutrients on sensory and motor responses of the human stomach to distension. Am J Physiol. 1997;273(3 Pt 1):G721-6.

245. Ezcurra M, Reimann F, Gribble FM, Emery E. Molecular mechanisms of incretin hormone secretion. Curr Opin Pharmacol. 2013;13(6):922-7.

246. Enck P, Azpiroz F, Boeckxstaens G, Elsenbruch S, Feinle-Bisset C, Holtmann G, et al. Functional dyspepsia. Nat Rev Dis Primers. 2017;3:17081.

247. Deane A, Chapman MJ, Fraser RJ, Horowitz M. Bench-to-bedside review: the gut as an endocrine organ in the critically ill. Crit Care. 2010;14(5):228.

248. Monteleone P, Castaldo E, Maj M. Neuroendocrine dysregulation of food intake in eating disorders. Regul Pept. 2008;149(1-3):39-50.

249. Suzuki K, Jayasena CN, Bloom SR. Obesity and appetite control. Exp Diabetes Res. 2012;2012:824305.

250. Little TJ, Feinle-Bisset C. Effects of dietary fat on appetite and energy intake in health and obesity--oral and gastrointestinal sensory contributions. Physiol Behav. 2011;104(4):613-20.

251. Hetterich L, Mack I, Giel KE, Zipfel S, Stengel A. An update on gastrointestinal disturbances in eating disorders. Mol Cell Endocrinol. 2019;497:1103318.

252. Tong J, D'Alessio D. Eating disorders and gastrointestinal peptides. Curr Opin Endocrinol Diabetes Obes. 2011;18(1):42-9.

253. Feinle-Bisset C, Azpiroz F. Dietary lipids and functional gastrointestinal disorders. Am J Gastroenterol. 2013;108(5):737-47.

254. Chapman MJ, Deane AM. Gastrointestinal dysfunction relating to the provision of nutrition in the critically ill. Curr Opin Clin Nutr Metab Care. 2015;18(2):207-12.

255. Parker BA, Chapman IM. Food intake and ageing--the role of the gut. Mech Ageing Dev. 2004;125(12):859-66.

256. Kreuch D, Keating DJ, Wu T, Horowitz M, Rayner CK, Young RL. Gut mechanisms linking intestinal sweet sensing to glycemic control. Front Endocrinol (Lausanne). 2018;9:741.

257. Wang GJ, Tomasi D, Backus W, Wang R, Telang F, Geliebter A, et al. Gastric distention activates satiety circuitry in the human brain. Neuroimage. 2008;39(4):1824-31.

258. Distrutti E, Azpiroz F, Soldevilla A, Malagelada JR. Gastric wall tension determines perception of gastric distention. Gastroenterology. 1999;116(5):1035-42.

259. Kissileff HR, Carretta JC, Geliebter A, Pi-Sunyer FX. Cholecystokinin and stomach distension combine to reduce food intake in humans. Am J Physiol Regul Integr Comp Physiol. 2003;285(5):R992-8.

260. Jones KL, Doran SM, Hveem K, Bartholomeusz FD, Morley JE, Sun WM, et al. Relation between postprandial satiation and antral area in normal subjects. Am J Clin Nutr. 1997;66(1):127-32.

261. Sturm K, Parker B, Wishart J, Feinle-Bisset C, Jones KL, Chapman I, et al. Energy intake and appetite are related to antral area in healthy young and older subjects. Am J Clin Nutr. 2004;80(3):656-67.

262. Heddle R, Collins PJ, Dent J, Horowitz M, Read NW, Chatterton B, et al. Motor mechanisms associated with slowing of the gastric emptying of a solid meal by an intraduodenal lipid infusion. J Gastroenterol Hepatol. 1989;4(5):437-47.

263. Pilichiewicz AN, Papadopoulos P, Brennan IM, Little TJ, Meyer JH, Wishart JM, et al. Load-dependent effects of duodenal lipid on antropyloroduodenal motility, plasma CCK and PYY, and energy intake in healthy men. Am J Physiol Regul Integr Comp Physiol. 2007;293(6):R2170-8.

264. Ryan AT, Feinle-Bisset C, Kallas A, Wishart JM, Clifton PM, Horowitz M, et al. Intraduodenal protein modulates antropyloroduodenal motility, hormone release, glycemia, appetite, and energy intake in lean men. Am J Clin Nutr. 2012;96(3):474-82.

265. Schober G, Lange K, Steinert RE, Hutchison AT, Luscombe-Marsh ND, Landrock MF, et al. Contributions of upper gut hormones and motility to the energy intake-suppressant effects of intraduodenal nutrients in healthy, lean men - a pooled-data analysis. Physiol Rep. 2016;4(17):e12943.

266. Seimon RV, Lange K, Little TJ, Brennan IM, Pilichiewicz AN, Feltrin KL, et al. Pooled-data analysis identifies pyloric pressures and plasma cholecystokinin concentrations as major determinants of acute energy intake in healthy, lean men. Am J Clin Nutr. 2010;92(1):61-8.

267. Feinle C, O'Donovan D, Doran S, Andrews JM, Wishart J, Chapman I, et al. Effects of fat digestion on appetite, APD motility, and gut hormones in response to duodenal fat infusion in humans. Am J Physiol Gastrointest Liver Physiol. 2003;284(5):G798-807.

268. Beglinger S, Drewe J, Schirra J, Goke B, D'Amato M, Beglinger C. Role of fat hydrolysis in regulating glucagon-like Peptide-1 secretion. J Clin Endocrinol Metab. 2010;95(2):879-86.

269. Hunt JN, Knox MT. A relation between the chain length of fatty acids and the slowing of gastric emptying. J Physiol. 1968;194(2):327-36.

270. Feltrin KL, Little TJ, Meyer JH, Horowitz M, Rades T, Wishart J, et al. Comparative effects of intraduodenal infusions of lauric and oleic acids on antropyloroduodenal motility, plasma cholecystokinin and peptide YY, appetite, and energy intake in healthy men. Am J Clin Nutr. 2008;87(5):1181-7.

271. Matzinger D, Degen L, Drewe J, Meuli J, Duebendorfer R, Ruckstuhl N, et al. The role of long chain fatty acids in regulating food intake and cholecystokinin release in humans. Gut. 2000;46(5):688-93.

272. Miyauchi S, Hirasawa A, Ichimura A, Hara T, Tsujimoto G. New frontiers in gut nutrient sensor research: free fatty acid sensing in the gastrointestinal tract. J Pharmacol Sci. 2010;112(1):19-24.

273. Cvijanovic N, Isaacs NJ, Rayner CK, Feinle-Bisset C, Young RL, Little TJ. Lipid stimulation of fatty acid sensors in the human duodenum: relationship with gastrointestinal hormones, BMI and diet. Int J Obes (Lond). 2017;41(2):233-9.

274. Feinle C, D'Amato M, Read NW. Cholecystokinin-A receptors modulate gastric sensory and motor responses to gastric distension and duodenal lipid. Gastroenterology. 1996;110(5):1379-85.

275. 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.

276. Brennan IM, Luscombe-Marsh ND, Seimon RV, Otto B, Horowitz M, Wishart JM, et al. Effects of fat, protein, and carbohydrate and protein load on appetite, plasma cholecystokinin, peptide YY, and ghrelin, and energy intake in lean and obese men. Am J Physiol Gastrointest Liver Physiol. 2012;303(1):G129-40.

277. Batterham RL, Heffron H, Kapoor S, Chivers JE, Chandarana K, Herzog H, et al. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab. 2006;4(3):223-33.

278. Anderson GH, Tecimer SN, Shah D, Zafar TA. Protein source, quantity, and time of consumption determine the effect of proteins on short-term food intake in young men. J Nutr. 2004;134(11):3011-5.

279. Giezenaar C, Luscombe-Marsh ND, Hutchison AT, Standfield S, Feinle-Bisset C, Horowitz M, et al. Dose-dependent effects of randomized intraduodenal whey-protein loads on glucose, gut hormone, and amino acid concentrations in healthy older and younger men. Nutrients. 2018;10(1):78.

280. Hall WL, Millward DJ, Long SJ, Morgan LM. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. Br J Nutr. 2003;89(2):239-48.

281. Thimister PW, Hopman WP, Sloots CE, Rosenbusch G, Willems HL, Trijbels FJ, et al. Role of intraduodenal proteases in plasma cholecystokinin and pancreaticobiliary responses to protein and amino acids. Gastroenterology. 1996;110(2):567-75.

282. Luscombe-Marsh ND, Hutchison AT, Soenen S, Steinert RE, Clifton PM, Horowitz M, et al. Plasma free amino acid Responses to intraduodenal whey protein, and relationships with insulin, glucagon-like peptide-1 and energy intake in lean healthy men. Nutrients. 2016;8(1):4.

283. Mellinkoff SM, Frankland M, Boyle D, Greipel M. Relationship between serum amino acid concentration and fluctuations in appetite. 1956. Obes Res. 1997;5(4):381-4.

284. Steinert RE, Ullrich SS, Geary N, Asarian L, Bueter M, Horowitz M, et al. Comparative effects of intraduodenal amino acid infusions on food intake and gut hormone release in healthy males. Physiol Rep. 2017;5(21):e13492.

285. Steinert RE, Landrock MF, Ullrich SS, Standfield S, Otto B, Horowitz M, et al. Effects of intraduodenal infusion of the branched-chain amino acid leucine on ad libitum eating, gut motor and hormone functions, and glycemia in healthy men. Am J Clin Nutr. 2015;102(4):820-7.

286. Steinert RE, Luscombe-Marsh ND, Little TJ, Standfield S, Otto B, Horowitz M, et al. Effects of intraduodenal infusion of L-tryptophan on ad libitum eating, antropyloroduodenal motility, glycemia, insulinemia, and gut peptide secretion in healthy men. J Clin Endocrinol Metab. 2014;99(9):3275-84.

287. Ballinger AB, Clark ML. L-phenylalanine releases cholecystokinin (CCK) and is associated with reduced food intake in humans: evidence for a physiological role of CCK in control of eating. Metabolism. 1994;43(6):735-8.

288. Meyer-Gerspach AC, Hafliger S, Meili J, Doody A, Rehfeld JF, Drewe J, et al. Effect of L-tryptophan and L-leucine on gut hormone secretion, appetite feelings and gastric emptying rates in lean and non-diabetic obese participants: a randomized, double-blind, parallel-group trial. PLoS One. 2016;11(11):e0166758.

289. Carney BI, Jones KL, Horowitz M, Sun WM, Hebbard G, Edelbroek MA. Stereospecific effects of tryptophan on gastric emptying and hunger in humans. J Gastroenterol Hepatol. 1994;9(6):557-63.

290. Potier M, Darcel N, Tome D. Protein, amino acids and the control of food intake. Curr Opin Clin Nutr Metab Care. 2009;12(1):54-8.

291. Stewart JE, Feinle-Bisset C, Keast RS. Fatty acid detection during food consumption and digestion: Associations with ingestive behavior and obesity. Prog Lipid Res. 2011;50(3):225-33.

292. Geliebter A. Gastric distension and gastric capacity in relation to food intake in humans. Physiol Behav. 1988;44(4-5):665-8.

293. Park MI, Camilleri M. Gastric motor and sensory functions in obesity. Obes Res. 2005;13(3):491-500.

294. Acosta A, Camilleri M, Shin A, Vazquez-Roque MI, Iturrino J, Burton D, et al. Quantitative gastrointestinal and psychological traits associated with obesity and response to weight-loss therapy. Gastroenterology. 2015;148(3):537-46.

295. Geliebter A, Schachter S, Lohmann-Walter C, Feldman H, Hashim SA. Reduced stomach capacity in obese subjects after dieting. Am J Clin Nutr. 1996;63(2):170-3.

296. Granstrom L, Backman L. Stomach distension in extremely obese and in normal subjects. Acta Chir Scand. 1985;151(4):367-70.

297. Speechly DP, Buffenstein R. Appetite dysfunction in obese males: evidence for role of hyperinsulinaemia in passive overconsumption with a high fat diet. Eur J Clin Nutr. 2000;54(3):225-33.

298. Rolls BJ, Kim-Harris S, Fischman MW, Foltin RW, Moran TH, Stoner SA. Satiety after preloads with different amounts of fat and carbohydrate: implications for obesity. Am J Clin Nutr. 1994;60(4):476-87.

299. French SJ, Murray B, Rumsey RD, Sepple CP, Read NW. Preliminary studies on the gastrointestinal responses to fatty meals in obese people. Int J Obes Relat Metab Disord. 1993;17(5):295-300.

300. Stewart JE, Seimon RV, Otto B, Keast RS, Clifton PM, Feinle-Bisset C. Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men. Am J Clin Nutr. 2011;93(4):703-11.

301. Heruc GA, Little TJ, Kohn M, Madden S, Clarke S, Horowitz M, et al. Appetite perceptions, gastrointestinal symptoms, ghrelin, peptide YY and state anxiety are disturbed in adolescent females with anorexia nervosa and only partially restored with short-term refeeding. Nutrients. 2018;11(1):59.

302. Geliebter A, Melton PM, McCray RS, Gallagher DR, Gage D, Hashim SA. Gastric capacity, gastric emptying, and test-meal intake in normal and bulimic women. Am J Clin Nutr. 1992;56(4):656-61.

303. Chen J, Brady P. Gastroesophageal reflux disease: pathophysiology, diagnosis, and treatment. Gastroenterol Nurs. 2019;42(1):20-8.

304. Herregods TV, Bredenoord AJ, Smout AJ. Pathophysiology of gastroesophageal reflux disease: new understanding in a new era. Neurogastroenterol Motil. 2015;27(9):1202-13.

305. de Bortoli N, Tolone S, Frazzoni M, Martinucci I, Sgherri G, Albano E, et al. Gastroesophageal reflux disease, functional dyspepsia and irritable bowel syndrome: common overlapping gastrointestinal disorders. Ann Gastroenterol. 2018;31(6):639-48.

306. Barbera R, Feinle C, Read NW. Abnormal sensitivity to duodenal lipid infusion in patients with functional dyspepsia. Eur J Gastroenterol Hepatol. 1995;7(11):1051-7.

307. Feinle-Bisset C, Azpiroz F. Dietary and lifestyle factors in functional dyspepsia. Nat Rev Gastroenterol Hepatol. 2013;10(3):150-7.

308. Pilichiewicz AN, Horowitz M, Holtmann GJ, Talley NJ, Feinle-Bisset C. Relationship between symptoms and dietary patterns in patients with functional dyspepsia. Clin Gastroenterol Hepatol. 2009;7(3):317-22.

309. Bradette M, Pare P, Douville P, Morin A. Visceral perception in health and functional dyspepsia. Crossover study of gastric distension with placebo and domperidone. Dig Dis Sci. 1991;36(1):52-8.

310. Mearin F, Cucala M, Azpiroz F, Malagelada JR. The origin of symptoms on the brain-gut axis in functional dyspepsia. Gastroenterology. 1991;101(4):999-1006.

311. Caldarella MP, Azpiroz F, Malagelada JR. Antro-fundic dysfunctions in functional dyspepsia. Gastroenterology. 2003;124(5):1220-9.

312. Feinle-Bisset C, Meier B, Fried M, Beglinger C. Role of cognitive factors in symptom induction following high and low fat meals in patients with functional dyspepsia. Gut. 2003;52(10):1414-8.

313. Pilichiewicz AN, Feltrin KL, Horowitz M, Holtmann G, Wishart JM, Jones KL, et al. Functional dyspepsia is associated with a greater symptomatic response to fat but not carbohydrate, increased fasting and postprandial CCK, and diminished PYY. Am J Gastroenterol. 2008;103(10):2613-23.

314. Houghton LA, Mangall YF, Dwivedi A, NW R. Sensitivity to nutrients in patients with non-ulcer dyspepsia. Eur J Gastroenterol Hepatol. 1993;5:109-14.

315. Barbera R, Feinle C, Read NW. Nutrient-specific modulation of gastric mechanosensitivity in patients with functional dyspepsia. Dig Dis Sci. 1995;40(8):1636-41.

316. Feinle C, Meier O, Otto B, D'Amato M, Fried M. Role of duodenal lipid and cholecystokinin A receptors in the pathophysiology of functional dyspepsia. Gut. 2001;48(3):347-55.

317. Chua AS, Dinan TG, Rovati LC, Keeling PW. Cholecystokinin hyperresponsiveness in dysmotility-type nonulcer dyspepsia. Ann N Y Acad Sci. 1994;713:298-9.

318. Azpiroz F, Bouin M, Camilleri M, Mayer EA, Poitras P, Serra J, et al. Mechanisms of hypersensitivity in IBS and functional disorders. Neurogastroenterol Motil. 2007;19(1 Suppl):62-88.

319. Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. JAMA. 2015;313(9):949-58.

320. Ukleja A. Altered GI motility in critically Ill patients: current understanding of pathophysiology, clinical impact, and diagnostic approach. Nutr Clin Pract. 2010;25(1):16-25.

321. Giezenaar C, Trahair LG, Rigda R, Hutchison AT, Feinle-Bisset C, Luscombe-Marsh ND, et al. Lesser suppression of energy intake by orally ingested whey protein in healthy older

men compared with young controls. Am J Physiol Regul Integr Comp Physiol. 2015;309(8):R845-54.

322. Giezenaar C, van der Burgh Y, Lange K, Hatzinikolas S, Hausken T, Jones KL, et al. Effects of substitution, and adding of carbohydrate and fat to whey-protein on energy intake, appetite, gastric emptying, glucose, insulin, ghrelin, CCK and GLP-1 in healthy older men-a randomized controlled trial. Nutrients. 2018;10(2):113.

323. MacIntosh CG, Morley JE, Wishart J, Morris H, Jansen JB, Horowitz M, et al. Effect of exogenous cholecystokinin (CCK)-8 on food intake and plasma CCK, leptin, and insulin concentrations in older and young adults: evidence for increased CCK activity as a cause of the anorexia of aging. J Clin Endocrinol Metab. 2001;86(12):5830-7.

324. Di Francesco V, Zamboni M, Dioli A, Zoico E, Mazzali G, Omizzolo F, et al. Delayed postprandial gastric emptying and impaired gallbladder contraction together with elevated cholecystokinin and peptide YY serum levels sustain satiety and inhibit hunger in healthy elderly persons. J Gerontol A Biol Sci Med Sci. 2005;60(12):1581-5.

325. Horowitz M, Maddern GJ, Chatterton BE, Collins PJ, Harding PE, Shearman DJ. Changes in gastric emptying rates with age. Clin Sci (Lond). 1984;67(2):213-8.

326. Wegener M, Borsch G, Schaffstein J, Luth I, Rickels R, Ricken D. Effect of ageing on the gastro-intestinal transit of a lactulose-supplemented mixed solid-liquid meal in humans. Digestion. 1988;39(1):40-6.

327. Clarkston WK, Pantano MM, Morley JE, Horowitz M, Littlefield JM, Burton FR. Evidence for the anorexia of aging: gastrointestinal transit and hunger in healthy elderly vs. young adults. Am J Physiol. 1997;272(1 Pt 2):R243-8.

328. Rayner CK, MacIntosh CG, Chapman IM, Morley JE, Horowitz M. Effects of age on proximal gastric motor and sensory function. Scand J Gastroenterol. 2000;35(10):1041-7.

329. Soenen S, Giezenaar C, Hutchison AT, Horowitz M, Chapman I, Luscombe-Marsh ND. Effects of intraduodenal protein on appetite, energy intake, and antropyloroduodenal motility in healthy older compared with young men in a randomized trial. Am J Clin Nutr. 2014;100(4):1108-15.

330. Cook CG, Andrews JM, Jones KL, Wittert GA, Chapman IM, Morley JE, et al. Effects of small intestinal nutrient infusion on appetite and pyloric motility are modified by age. Am J Physiol. 1997;273(2 Pt 2):R755-61.

331. MacIntosh CG, Andrews JM, Jones KL, Wishart JM, Morris HA, Jansen JB, et al. Effects of age on concentrations of plasma cholecystokinin, glucagon-like peptide 1, and peptide YY and their relation to appetite and pyloric motility. Am J Clin Nutr. 1999;69(5):999-1006.

332. Sturm K, MacIntosh CG, Parker BA, Wishart J, Horowitz M, Chapman IM. Appetite, food intake, and plasma concentrations of cholecystokinin, ghrelin, and other gastrointestinal hormones in undernourished older women and well-nourished young and older women. J Clin Endocrinol Metab. 2003;88(8):3747-55.

333. Giezenaar C, Hutchison AT, Luscombe-Marsh ND, Chapman I, Horowitz M, Soenen S. Effect of age on blood glucose and plasma insulin, glucagon, ghrelin, CCK, GIP, and GLP-1 responses to whey protein ingestion. Nutrients. 2017;10(1):2.

334. Rigamonti AE, Pincelli AI, Corra B, Viarengo R, Bonomo SM, Galimberti D, et al. Plasma ghrelin concentrations in elderly subjects: comparison with anorexic and obese patients. J Endocrinol. 2002;175(1):R1-5.

335. Di Francesco V, Fantin F, Residori L, Bissoli L, Micciolo R, Zivelonghi A, et al. Effect of age on the dynamics of acylated ghrelin in fasting conditions and in response to a meal. J Am Geriatr Soc. 2008;56(7):1369-70.

336. Di Francesco V, Zamboni M, Zoico E, Mazzali G, Dioli A, Omizzolo F, et al. Unbalanced serum leptin and ghrelin dynamics prolong postprandial satiety and inhibit hunger in healthy elderly: another reason for the "anorexia of aging". Am J Clin Nutr. 2006;83(5):1149-52.

337. Wisen O, Johansson C. Gastrointestinal function in obesity: motility, secretion, and absorption following a liquid test meal. Metabolism. 1992;41(4):390-5.

338. Castiglione KE, Read NW, French SJ. Adaptation to high-fat diet accelerates emptying of fat but not carbohydrate test meals in humans. Am J Physiol Regul Integr Comp Physiol. 2002;282(2):R366-71.

339. Park MI, Camilleri M, O'Connor H, Oenning L, Burton D, Stephens D, et al. Effect of different macronutrients in excess on gastric sensory and motor functions and appetite in normal-weight, overweight, and obese humans. Am J Clin Nutr. 2007;85(2):411-8.

340. French SJ, Murray B, Rumsey RD, Fadzlin R, Read NW. Adaptation to high-fat diets: effects on eating behaviour and plasma cholecystokinin. Br J Nutr. 1995;73(2):179-89.

341. Boyd KA, O'Donovan DG, Doran S, Wishart J, Chapman IM, Horowitz M, et al. High-fat diet effects on gut motility, hormone, and appetite responses to duodenal lipid in healthy men. Am J Physiol Gastrointest Liver Physiol. 2003;284(2):G188-96.

342. Brennan IM, Seimon RV, Luscombe-Marsh ND, Otto B, Horowitz M, Feinle-Bisset C. Effects of acute dietary restriction on gut motor, hormone and energy intake responses to duodenal fat in obese men. Int J Obes (Lond). 2011;35(3):448-56.

343. Seimon RV, Taylor P, Little TJ, Noakes M, Standfield S, Clifton PM, et al. Effects of acute and longer-term dietary restriction on upper gut motility, hormone, appetite, and energy-intake responses to duodenal lipid in lean and obese men. Am J Clin Nutr. 2014;99(1):24-34.

344. Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, et al. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body

weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. Am J Clin Nutr. 2005;82(1):41-8.

345. Leidy HJ, Clifton PM, Astrup A, Wycherley TP, Westerterp-Plantenga MS, Luscombe-Marsh ND, et al. The role of protein in weight loss and maintenance. Am J Clin Nutr. 2015;101(6):1320S-9S.

346. Hutchison AT, Feinle-Bisset C, Fitzgerald PC, Standfield S, Horowitz M, Clifton PM, et al. Comparative effects of intraduodenal whey protein hydrolysate on antropyloroduodenal motility, gut hormones, glycemia, appetite, and energy intake in lean and obese men. Am J Clin Nutr. 2015;102(6):1323-31.

347. Edelbroek M, Sun WM, Horowitz M, Dent J, Smout A, Akkermans L. Stereospecific effects of intraduodenal tryptophan on pyloric and duodenal motility in humans. Scand J Gastroenterol. 1994;29(12):1088-95.

348. Ullrich SS, Fitzgerald PCE, Giesbertz P, Steinert RE, Horowitz M, Feinle-Bisset C. Effects of intragastric administration of tryptophan on the blood glucose response to a nutrient drink and energy intake, in lean and obese men. Nutrients. 2018;10(4):463.

349. Pepino MY, Finkbeiner S, Beauchamp GK, Mennella JA. Obese women have lower monosodium glutamate taste sensitivity and prefer higher concentrations than do normal-weight women. Obesity (Silver Spring). 2010;18(5):959-65.

350. Tack J, Talley NJ. Functional dyspepsia--symptoms, definitions and validity of the Rome III criteria. Nat Rev Gastroenterol Hepatol. 2013;10(3):134-41.

351. Barbara G, Feinle-Bisset C, Ghoshal UC, Quigley EM, Santos J, Vanner S, et al. The intestinal microenvironment and functional gastrointestinal disorders. Gastroenterology. 2016;150(6):1305-18.

352. Bisschops R, Karamanolis G, Arts J, Caenepeel P, Verbeke K, Janssens J, et al. Relationship between symptoms and ingestion of a meal in functional dyspepsia. Gut. 2008;57(11):1495-503.

353. Filipovic BF, Randjelovic T, Kovacevic N, Milinic N, Markovic O, Gajic M, et al. Laboratory parameters and nutritional status in patients with functional dyspepsia. Eur J Intern Med. 2011;22(3):300-4.

354. Carvalho RV, Lorena SL, Almeida JR, Mesquita MA. Food intolerance, diet composition, and eating patterns in functional dyspepsia patients. Dig Dis Sci. 2010;55(1):60-5.

355. Cuperus P, Keeling PW, Gibney MJ. Eating patterns in functional dyspepsia: a case control study. Eur J Clin Nutr. 1996;50(8):520-3.

356. Goktas Z, Koklu S, Dikmen D, Ozturk O, Yilmaz B, Asil M, et al. Nutritional habits in functional dyspepsia and its subgroups: a comparative study. Scand J Gastroenterol. 2016;51(8):903-7.

357. Gibson PR, Shepherd SJ. Food choice as a key management strategy for functional gastrointestinal symptoms. Am J Gastroenterol. 2012;107(5):657-66.

358. Mullan A, Kavanagh P, O'Mahony P, Joy T, Gleeson F, Gibney MJ. Food and nutrient intakes and eating patterns in functional and organic dyspepsia. Eur J Clin Nutr. 1994;48(2):97-105.

359. Kaess H, Kellermann M, Castro A. Food intolerance in duodenal ulcer patients, non ulcer dyspeptic patients and healthy subjects. A prospective study. Klin Wochenschr. 1988;66(5):208-11.

360. Elli L, Tomba C, Branchi F, Roncoroni L, Lombardo V, Bardella MT, et al. Evidence for the presence of non-celiac gluten sensitivity in patients with functional gastrointestinal

symptoms: results from a multicenter randomized double-blind placebo-controlled gluten challenge. Nutrients. 2016;8(2):84.

361. Santolaria S, Alcedo J, Cuartero B, Diez I, Abascal M, Garcia-Prats MD, et al. Spectrum of gluten-sensitive enteropathy in patients with dysmotility-like dyspepsia. Gastroenterol Hepatol. 2013;36(1):11-20.

362. Saito YA, Locke GR, 3rd, Weaver AL, Zinsmeister AR, Talley NJ. Diet and functional gastrointestinal disorders: a population-based case-control study. Am J Gastroenterol. 2005;100(12):2743-8.

363. De Castro JM. Age-related changes in spontaneous food intake and hunger in humans. Appetite. 1993;21(3):255-72.

364. Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. J Am Med Dir Assoc. 2013;14(8):542-59.

365. Ryan M, Salle A, Favreau AM, Simard G, Dumas JF, Malthiery Y, et al. Oral supplements differing in fat and carbohydrate content: effect on the appetite and food intake of undernourished elderly patients. Clin Nutr. 2004;23(4):683-9.

366. Kupfer RM, Heppell M, Haggith JW, Bateman DN. Gastric emptying and small-bowel transit rate in the elderly. J Am Geriatr Soc. 1985;33(5):340-3.

367. Rolls BJ, Dimeo KA, Shide DJ. Age-related impairments in the regulation of food intake. Am J Clin Nutr. 1995;62(5):923-31.

368. Roberts SB, Fuss P, Heyman MB, Evans WJ, Tsay R, Rasmussen H, et al. Control of food intake in older men. JAMA. 1994;272(20):1601-6.

369. Milan AM, Cameron-Smith D. Digestion and postprandial metabolism in the elderly. Adv Food Nutr Res. 2015;76:79-124.

370. Poppitt SD, McCormack D, Buffenstein R. Short-term effects of macronutrient preloads on appetite and energy intake in lean women. Physiol Behav. 1998;64(3):279-85.

371. Tylner S, Cederholm T, Faxen-Irving G. Effects on weight, blood lipids, serum fatty acid profile and coagulation by an energy-dense formula to older care residents: a randomized controlled crossover trial. J Am Med Dir Assoc. 2016;17(3):275-e5.

372. Spiller RC, Trotman IF, Adrian TE, Bloom SR, Misiewicz JJ, DB S. Further characterisation of the 'ileal brake' reflex in man--effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY. Gut. 1988;29:1042-51.

373. Kingsbury JM. Poisonous plants of the United States and Canada. Soil Sci. 1964;98(5):349.

374. Drewnowski A, Gomez-Carneros C. Bitter taste, phytonutrients, and the consumer: a review. Am J Clin Nutr. 2000;72(6):1424-35.

375. Murata Y, Sata NU. Isolation and structure of pulcherrimine, a novel bitter-tasting amino acid, from the sea urchin (Hemicentrotus pulcherrimus) ovaries. J Agric Food Chem. 2000;48(11):5557-60.

376. Hofmann T. Taste-active maillard reaction products: the "tasty" world of nonvolatile maillard reaction products. Ann N Y Acad Sci. 2005;1043:20-9.

377. DuBois G, DeSimone J, Lyall V. Chemistry of gustatory stimuli. In: Firestein S, Beauchamp GK, editors. Olfaction and taste. Amsterdam (The Netherlands): Elsevier. 2008:27-74.

378. Belitz HD, Wieser H. Bitter compounds: occurrence and structure-activity relationships. Food Rev Int. 1985;1(2):271-354.

379. Keller KL, Adise S. Variation in the ability to taste bitter thiourea compounds: implications for food acceptance, dietary intake, and obesity risk in children. Annu Rev Nutr. 2016;36:157-82.

380. Meyerhof W, Batram C, Kuhn C, Brockhoff A, Chudoba E, Bufe B, et al. The molecular receptive ranges of human TAS2R bitter taste receptors. Chem Senses. 2010;35(2):157-70.

381. Steiner JE. The gustofacial response: observation on normal and anencephalic newborn infants. Symp Oral Sens Percept. 1973(4):254-78.

382. Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS. The receptors and cells for mammalian taste. Nature. 2006;444(7117):288-94.

383. Beauchamp GK, Mennella JA. Early flavor learning and its impact on later feeding behavior. J Pediatr Gastroenterol Nutr. 2009;48 Suppl 1:S25-30.

384. Glendinning JI. Is the bitter rejection response always adaptive? Physiol Behav. 1994;56(6):1217-27.

385. Lindemann B. Taste reception. Physiol Rev. 1996;76(3):719-66.

386. Roper SD. Signal transduction and information processing in mammalian taste buds. Pflugers Arch. 2007;454(5):759-76.

387. Behrens M, Meyerhof W. Mammalian bitter taste perception. Results Probl Cell Differ. 2009;47:1-18.

388. Calvo SS, Egan JM. The endocrinology of taste receptors. Nat Rev Endocrinol. 2015;11(4):213-27.

389. Lu P, Zhang CH, Lifshitz LM, ZhuGe R. Extraoral bitter taste receptors in health and disease. J Gen Physiol. 2017;149(2):181-97.

390. Wu SV, Rozengurt N, Yang M, Young SH, Sinnett-Smith J, Rozengurt E. Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells. Proc Natl Acad Sci U S A. 2002;99(4):2392-7.

391. Yue X, Liang J, Gu F, Du D, Chen F. Berberine activates bitter taste responses of enteroendocrine STC-1 cells. Mol Cell Biochem. 2018;447(1-2):21-32.

392. Jeon TI, Zhu B, Larson JL, Osborne TF. SREBP-2 regulates gut peptide secretion through intestinal bitter taste receptor signaling in mice. J Clin Invest. 2008;118(11):3693-700.

393. Chen MC, Wu SV, Reeve JR, Jr., Rozengurt E. Bitter stimuli induce Ca2+ signaling and CCK release in enteroendocrine STC-1 cells: role of L-type voltage-sensitive Ca2+ channels. Am J Physiol Cell Physiol. 2006;291(4):C726-39.

394. Shi P, Zhang J. Extraordinary diversity of chemosensory receptor gene repertoires among vertebrates. Results Probl Cell Differ. 2009;47:1-23.

395. Vegezzi G, Anselmi L, Huynh J, Barocelli E, Rozengurt E, Raybould H, et al. Diet-induced regulation of bitter taste receptor subtypes in the mouse gastrointestinal tract. PloS one. 2014;9(9):e107732.

396. Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, et al. T2Rs function as bitter taste receptors. Cell. 2000;100(6):703-11.

397. Bufe B, Breslin PA, Kuhn C, Reed DR, Tharp CD, Slack JP, et al. The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. Curr Biol. 2005;15(4):322-7.

398. Bufe B, Hofmann T, Krautwurst D, Raguse JD, Meyerhof W. The human TAS2R16 receptor mediates bitter taste in response to beta-glucopyranosides. Nat Genet. 2002;32(3):397-401.

399. Kim KS, Egan JM, Jang HJ. Denatonium induces secretion of glucagon-like peptide-1 through activation of bitter taste receptor pathways. Diabetologia. 2014;57(10):2117-25.

400. 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.

401. Pham H, Hui H, Morvaridi S, Cai J, Zhang S, Tan J, et al. A bitter pill for type 2 diabetes? The activation of bitter taste receptor TAS2R38 can stimulate GLP-1 release from enteroendocrine L-cells. Biochem Biophys Res Commun. 2016;475(3):295-300.

402. Park J, Kim KS, Kim KH, Lee IS, Jeong HS, Kim Y, et al. GLP-1 secretion is stimulated by 1,10-phenanthroline via colocalized T2R5 signal transduction in human enteroendocrine L cell. Biochem Biophys Res Commun. 2015;468(1-2):306-11.

403. Shen T, Kaya N, Zhao FL, Lu SG, Cao Y, Herness S. Co-expression patterns of the neuropeptides vasoactive intestinal peptide and cholecystokinin with the transduction molecules alpha-gustducin and T1R2 in rat taste receptor cells. Neuroscience. 2005;130(1):229-38.

404. Iven J, Biesiekierski JR, Zhao D, Deloose E, O'Daly OG, Depoortere I, et al. Intragastric quinine administration decreases hedonic eating in healthy women through peptide-mediated gut-brain signaling mechanisms. Nutr Neurosci. 2018;22(12):850-62.

405. Yu Y, Hao G, Zhang Q, Hua W, Wang M, Zhou W, et al. Berberine induces GLP-1 secretion through activation of bitter taste receptor pathways. Biochem Pharmacol. 2015;97(2):173-7.

406. Avau B, Rotondo A, Thijs T, Andrews CN, Janssen P, Tack J, et al. Targeting extra-oral bitter taste receptors modulates gastrointestinal motility with effects on satiation. Sci Rep. 2015;5:15985.

407. Barrea L, Annunziata G, Muscogiuri G, Arnone A, Tenore GC, Colao A, et al. Could hop-derived bitter compounds improve glucose homeostasis by stimulating the secretion of GLP-1? Crit Rev Food Sci Nutr. 2019;59(3):528-35.

408. Avau B, Depoortere I. The bitter truth about bitter taste receptors: beyond sensing bitter in the oral cavity. Acta Physiol (Oxf). 2016;216(4):407-20.

409. Li J, Xu J, Hou R, Jin X, Wang J, Yang N, et al. Qing-Hua Granule induces GLP-1 secretion via bitter taste receptor in db/db mice. Biomed Pharmacother. 2017;89:10-7.

410. Thompson MD, Cole DE, Capra V, Siminovitch KA, Rovati GE, Burnham WM, et al. Pharmacogenetics of the G protein-coupled receptors. Methods Mol Biol. 2014;1175:189-242.

411. Bartáková V, Kuricová K, Zlámal F, Bělobrádková J, Kaňková K. Differences in food intake and genetic variability in taste receptors between Czech pregnant women with and without gestational diabetes mellitus. Eur J Nutr. 2018;57(2):513-21.

412. Dotson CD, Zhang L, Xu H, Shin YK, Vigues S, Ott SH, et al. Bitter taste receptors influence glucose homeostasis. PLoS One. 2008;3(12):e3974.

413. Keller M, Liu X, Wohland T, Rohde K, Gast MT, Stumvoll M, et al. TAS2R38 and its influence on smoking behavior and glucose homeostasis in the German Sorbs. PLoS One. 2013;8(12):e80512.

414. Xie C, Wang X, Young RL, Horowitz M, Rayner CK, Wu T. Role of intestinal bitter sensing in enteroendocrine hormone secretion and metabolic control. Front Endocrinol (Lausanne). 2018;9:576.

415. Jeon TI, Seo YK, Osborne TF. Gut bitter taste receptor signalling induces ABCB1 through a mechanism involving CCK. Biochem J. 2011;438(1):33-7.

416. Yamazaki T, Morimoto-Kobayashi Y, Koizumi K, Takahashi C, Nakajima S, Kitao S, et al. Secretion of a gastrointestinal hormone, cholecystokinin, by hop-derived bitter components activates sympathetic nerves in brown adipose tissue. J Nutr Biochem. 2019;64:80-7.

417. Intelmann D, Batram C, Kuhn C, Haseleu G, Meyerhof W, Hofmann T. Three TAS2R bitter taste receptors mediate the psychophysical responses to bitter compounds of hops (Humulus lupulus L.) and beer. Chemosens Percept. 2009;2(3):118-32.

418. Le Neve B, Foltz M, Daniel H, Gouka R. The steroid glycoside H.g.-12 from Hoodia gordonii activates the human bitter receptor TAS2R14 and induces CCK release from HuTu-80 cells. Am J Physiol Gastrointest Liver Physiol. 2010;299(6):G1368-75.

419. Kok BP, Galmozzi A, Littlejohn NK, Albert V, Godio C, Kim W, et al. Intestinal bitter taste receptor activation alters hormone secretion and imparts metabolic benefits. Mol Metab. 2018;16:76-87.

420. Suh HW, Lee KB, Kim KS, Yang HJ, Choi EK, Shin MH, et al. A bitter herbal medicine Gentiana scabra root extract stimulates glucagon-like peptide-1 secretion and regulates blood glucose in db/db mouse. J Ethnopharmacol. 2015;172:219-26.

421. Huang TN, Lu KN, Pai YP, Chin H, Huang CJ. Role of GLP-1 in the hypoglycemic effects of wild bitter gourd. Evid Based Complement Alternat Med. 2013;2013:625892.

422. Eiki J, Saeki K, Nagano N, Iino T, Yonemoto M, Takayenoki-Iino Y, et al. A selective small molecule glucagon-like peptide-1 secretagogue acting via depolarization-coupled Ca(2+) influx. J Endocrinol. 2009;201(3):361-7.

423. Janssen S, Laermans J, Verhulst PJ, Thijs T, Tack J, Depoortere I. Bitter taste receptors and alpha-gustducin regulate the secretion of ghrelin with functional effects on food intake and gastric emptying. Proc Natl Acad Sci U S A. 2011;108(5):2094-9.

424. Avau B, Bauters D, Steensels S, Vancleef L, Laermans J, Lesuisse J, et al. The gustatory signaling pathway and bitter taste receptors affect the development of obesity and adipocyte metabolism in mice. PLoS One. 2015;10(12):e0145538.

425. Lossow K, Hubner S, Roudnitzky N, Slack JP, Pollastro F, Behrens M, et al. Comprehensive analysis of mouse bitter taste receptors reveals different molecular receptive ranges for orthologous receptors in mice and humans. J Biol Chem. 2016;291(29):15358-77.

426. Wu T, Rayner CK, Horowitz M. Incretins. Handb Exp Pharmacol. 2016;233:137-71.

427. 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.

428. Amori RE, Lau J, Pittas AG. Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. JAMA. 2007;298(2):194-206.

429. Parker HE, Habib AM, Rogers GJ, Gribble FM, Reimann F. Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. Diabetologia. 2009;52(2):289-98.

430. Mennella I, Fogliano V, Ferracane R, Arlorio M, Pattarino F, Vitaglione P. Microencapsulated bitter compounds (from Gentiana lutea) reduce daily energy intakes in humans. Br J Nutr. 2016:1-10.

431. Deloose E, Janssen P, Corsetti M, Biesiekierski J, Masuy I, Rotondo A, et al. Intragastric infusion of denatonium benzoate attenuates interdigestive gastric motility and hunger scores in healthy female volunteers. Am J Clin Nutr. 2017;105(3):580-8.

432. Andreozzi P, Sarnelli G, Pesce M, Zito FP, Alessandro AD, Verlezza V, et al. The bitter taste receptor agonist quinine reduces calorie intake and increases the postprandial release of cholecystokinin in healthy subjects. J Neurogastroenterol Motil. 2015;21(4):511-9.

433. 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.

434. Ingram J, Walker E, Pahl M, Lo K, Shin H, Lang C, et al. Activation of gastrointestinal bitter taste receptors suppresses food intake and stimulates secretion of gastrointestinal peptide hormones in healthy men. Abstract presented at: EOS - Joint Congress of EASO and IFSO-EC; 2016 June 1-4; Gothenburg, Sweden. Obesity Facts. 9: Suppl. l; p.46.

435. Kimura Y, Sumiyoshi M. Effects of Swertia japonica extract and its main compound swertiamarin on gastric emptying and gastrointestinal motility in mice. Fitoterapia. 2011;82(6):827-33.

436. Wicks D, Wright J, Rayment P, Spiller R. Impact of bitter taste on gastric motility. Eur J Gastroenterol Hepatol. 2005;17(9):961-5.

437. 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.

438. Deloose E, Corsetti M, Van Oudenhove L, Depoortere I, Tack J. Intragastric infusion of the bitter tastant quinine suppresses hormone release and antral motility during the fasting state in healthy female volunteers. Neurogastroenterol Motil. 2018;30(1):e13171.

439. van Heerden FR, Marthinus Horak R, Maharaj VJ, Vleggaar R, Senabe JV, Gunning PJ. An appetite suppressant from Hoodia species. Phytochemistry. 2007;68(20):2545-53.

440. Kratz CM, Levitsky DA, Lustick SL. Long term effects of quinine on food intake and body weight in the rat. Physiol Behav. 1978;21(3):321-4.

441. Yajima H, Noguchi T, Ikeshima E, Shiraki M, Kanaya T, Tsuboyama-Kasaoka N, et al. Prevention of diet-induced obesity by dietary isomerized hop extract containing isohumulones, in rodents. Int J Obes (Lond). 2005;29(8):991-7.

442. Sumiyoshi M, Kimura Y. Hop (Humulus lupulus L.) extract inhibits obesity in mice fed a high-fat diet over the long term. Br J Nutr. 2013;109(1):162-72.

443. Leng SH, Lu FE, Xu LJ. Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion. Acta Pharmacol Sin. 2004;25(4):496-502.

444. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. N Engl J Med. 1995;332(10):621-8.

445. Yajima H, Ikeshima E, Shiraki M, Kanaya T, Fujiwara D, Odai H, et al. Isohumulones, bitter acids derived from hops, activate both peroxisome proliferator-activated receptor alpha and gamma and reduce insulin resistance. J Biol Chem. 2004;279(32):33456-62.

446. Marathe CS, Horowitz M, Trahair LG, Wishart JM, Bound M, Lange K, et al. Relationships of early and late glycemic responses with gastric emptying during an oral glucose tolerance test. J Clin Endocrinol Metab. 2015;100(9):3565-71.

447. Okitolonda W, Delacollette C, Malengreau M, Henquin JC. High incidence of hypoglycaemia in African patients treated with intravenous quinine for severe malaria. Br Med J (Clin Res Ed). 1987;295(6600):716-8.

448. Looareesuwan S, Phillips RE, White NJ, Kietinun S, Karbwang J, Rackow C, et al. Quinine and severe falciparum malaria in late pregnancy. Lancet. 1985;2(8445):4-8.

449. Njomatchoua AC, Tankeu AT, Sobngwi E, Mbanya JC. Glycemic effects of quinine infusion in healthy volunteers. BMC Res Notes. 2017;10(1):423.

450. Seimon RV, Lange K, Little TJ, Brennan IM, Pilichiewicz AN, Feltrin KL, et al. Pooled-data analysis identifies pyloric pressures and plasma cholecystokinin concentrations as major determinants of acute energy intake in healthy, lean men. Am J Clin Nutr. 2010;92(1):61-8.

451. Ma J, Stevens JE, Cukier K, Maddox AF, Wishart JM, Jones KL, et al. Effects of a protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. Diabetes Care. 2009;32(9):1600-2.

452. 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.

453. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res. 1985;29(1):71-83.

454. www.randomization.com (accessed 24.08.2017).

455. www.anzctr.org.au (accessed 18.05.2017).

456. Feltrin KL, Little TJ, Meyer JH, Horowitz M, Smout AJ, Wishart J, et al. Effects of intraduodenal fatty acids on appetite, antropyloroduodenal motility, and plasma CCK and GLP-1 in humans vary with their chain length. Am J Physiol Regul Integr Comp Physiol. 2004;287(3):R524-33.

457. Heddle R, Dent J, Toouli J, Read NW. Topography and measurement of pyloric pressure waves and tone in humans. Am J Physiol. 1988;255(4 Pt 1):G490-7.

458. Parker BA, Sturm K, MacIntosh CG, Feinle C, Horowitz M, Chapman IM. Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. Eur J Clin Nutr. 2004;58(2):212-8.

459. Nair NS, Brennan IM, Little TJ, Gentilcore D, Hausken T, Jones KL, et al. Reproducibility of energy intake, gastric emptying, blood glucose, plasma insulin and cholecystokinin responses in healthy young males. Br J Nutr. 2009;101(7):1094-102.

460. Chale-Rush A, Burgess JR, Mattes RD. Evidence for human orosensory (taste?) sensitivity to free fatty acids. Chem Senses. 2007;32(5):423-31.

461. Park DC, Yeo JH, Ryu IY, Kim SH, Jung J, Yeo SG. Differences in taste detection thresholds between normal-weight and obese young adults. Acta Otolaryngol. 2015;135(5):478-83.

462. Santangelo A, Peracchi M, Conte D, Fraquelli M, Porrini M. Physical state of meal affects gastric emptying, cholecystokinin release and satiety. Br J Nutr. 1998;80(6):521-7.

463. Camilleri M, Malagelada JR. Abnormal intestinal motility in diabetics with the gastroparesis syndrome. Eur J Clin Invest. 1984;14(6):420-7.

464. Steensels S, Depoortere I. Chemoreceptors in the Gut. Annu Rev Physiol. 2018;80:117-41.

465. Fraser R, Fone D, Horowitz M, Dent J. Cholecystokinin octapeptide stimulates phasic and tonic pyloric motility in healthy humans. Gut. 1993;34(1):33-7.

466. Katschinski M, Schirra J, Begliner C, Langbein S, Wank U, D'Amato M, et al. Intestinal phase of human antro-pyloro-duodenal motility: cholinergic and CCK-mediated regulation. Eur J Clin Invest. 1996;26(7):574-83.

467. Schober G, Lange K, Steinert RE, Hutchison AT, Luscombe-Marsh ND, Landrock MF, et al. Contributions of upper gut hormones and motility to the energy intake-suppressant effects of intraduodenal nutrients in healthy, lean men - a pooled-data analysis. Physiol Rep. 2016;4(17):e12943.

468. Latorre R, Huynh J, Mazzoni M, Gupta A, Bonora E, Clavenzani P, et al. Expression of the bitter taste receptor, T2R38, in enteroendocrine cells of the colonic mucosa of overweight/obese vs. lean subjects. PLoS One. 2016;11(2):e0147468.

469. Little TJ, Horowitz M, Feinle-Bisset C. Modulation by high-fat diets of gastrointestinal function and hormones associated with the regulation of energy intake: implications for the pathophysiology of obesity. Am J Clin Nutr. 2007;86(3):531-41.

470. Bell KI, Tepper BJ. Short-term vegetable intake by young children classified by 6-n-propylthoiuracil bitter-taste phenotype. Am J Clin Nutr. 2006;84(1):245-51.

471. Choi SE, Chan J. Relationship of 6-n-propylthiouracil taste intensity and chili pepper use with body mass index, energy intake, and fat intake within an ethnically diverse population. J Acad Nutr Diet. 2015;115(3):389-96.

472. Rolls BJ, Kim-Harris S, Fischman MW, Foltin RW, Moran TH, Stoner SA. Satiety after preloads with different amounts of fat and carbohydrate: implications for obesity. Am J Clin Nutr. 1994;60(4):476-87.

473. Marathe CS, Rayner CK, Jones KL, Horowitz M. Glucagon-like peptides 1 and 2 in health and disease: a review. Peptides. 2013;44:75-86.

474. Deane AM, Nguyen NQ, Stevens JE, Fraser RJ, Holloway RH, Besanko LK, et al. Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. J Clin Endocrinol Metab. 2010;95(1):215-21.

475. Astrup A, Rossner S, Van Gaal L, Rissanen A, Niskanen L, Al Hakim M, et al. Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. Lancet. 2009;374(9701):1606-16.

476. Buse JB, Rosenstock J, Sesti G, Schmidt WE, Montanya E, Brett JH, et al. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). Lancet. 2009;374(9683):39-47.

477. Chaudhari N, Roper SD. The cell biology of taste. J Cell Biol. 2010;190(3):285-96.

478. Behrens M, Meyerhof W. Oral and extraoral bitter taste receptors. Results Probl Cell Differ. 2010;52:87-99.

479. Bitarafan V, Fitzgerald PCE, Little TJ, Meyerhof W, Wu T, Horowitz M, et al. Effects of intraduodenal infusion of the bitter tastant, quinine, on antropyloroduodenal motility, plasma cholecystokinin, and energy intake in healthy men. J Neurogastroenterol Motil. 2019;25(3):413-22.

480. Blount RE, Jr. Acute falciparum malaria. Field experience with quinine-pyrimethamine combined therapy. Ann Intern Med. 1969;70(1):142-7.

481. White NJ, Warrell DA, Chanthavanich P, Looareesuwan S, Warrell MJ, Krishna S, et al. Severe hypoglycemia and hyperinsulinemia in falciparum malaria. N Engl J Med. 1983;309(2):61-6.

482. Mossi S, Meyer-Wyss B, Beglinger C, Schwizer W, Fried M, Ajami A, et al. Gastric emptying of liquid meals measured noninvasively in humans with [13C]acetate breath test. Dig Dis Sci. 1994;39(12 Suppl):107s-9s.

483. Wu T, Rayner CK, Young RL, Horowitz M. Gut motility and enteroendocrine secretion. Curr Opin Pharmacol. 2013;13(6):928-34.

484. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia. 1993;36(8):741-4.

485. Vahl TP, Tauchi M, Durler TS, Elfers EE, Fernandes TM, Bitner RD, et al. Glucagon-like peptide-1 (GLP-1) receptors expressed on nerve terminals in the portal vein mediate the effects of endogenous GLP-1 on glucose tolerance in rats. Endocrinology. 2007;148(10):4965-73.

486. Wang Q, Liszt KI, Deloose E, Canovai E, Thijs T, Farre R, et al. Obesity alters adrenergic and chemosensory signaling pathways that regulate ghrelin secretion in the human gut. FASEB J. 2019;33(4):4907-20.

487. Brennan IM, Feltrin KL, Nair NS, Hausken T, Little TJ, Gentilcore D, et al. Effects of the phases of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in healthy lean women. Am J Physiol Gastrointest Liver Physiol. 2009;297(3):G602-10.

488. Sanaka M, Yamamoto T, Ishii T, Kuyama Y. The Wagner-Nelson method can generate an accurate gastric emptying flow curve from 13CO2 data obtained by a 13C-labeled substrate breath test. Digestion. 2004;69(2):71-8.

489. Stewart JE, Seimon RV, Otto B, Keast RS, Clifton PM, Feinle-Bisset C. Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men. Am J Clin Nutr. 2011;93(4):703-11.

490. Müller TD, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, et al. Glucagon-like peptide 1 (GLP-1). Mol Metab. 2019;30:72-130.

491. Bitarafan V, Fitzgerald PCE, Little TJ, Meyerhof W, Jones KL, Wu T, et al. Intragastric administration of the bitter tastant quinine lowers the glycemic response to a nutrient drink without slowing gastric emptying in healthy men. Am J Physiol Regul Integr Comp Physiol. 2020;318(2):R263-R73.

492. National Health and Medical Research Council. Internet: https://www.nhmrc.gov.au/about-us/publications/australian-dietary-guidelines (accessed 20.05.2020).

493. Polonsky KS, Rubenstein AH. C-peptide as a measure of the secretion and hepatic extraction of insulin. Pitfalls and limitations. Diabetes. 1984;33(5):486-94.

494. Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: Part 1--Correlation within subjects. BMJ. 1995;310(6977):446.

495. Henquin JC, Horemans B, Nenquin M, Verniers J, Lambert AE. Quinine-induced modifications of insulin release and glucose metabolism by isolated pancreatic islets. FEBS Lett. 1975;57(3):280-4.

496. Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87(4):1409-39.

497. Degn KB, Brock B, Juhl CB, Djurhuus CB, Grubert J, Kim D, et al. Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. Diabetes. 2004;53(9):2397-403.

498. Seimon RV, Lange K, Little TJ, Brennan IM, Pilichiewicz AN, Feltrin KL, et al. Pooled-data analysis identifies pyloric pressures and plasma cholecystokinin concentrations as major determinants of acute energy intake in healthy, lean men. Am J Clin Nutr. 2010;92(1):61-8.

499. Liu M, Hansen PE, Wang G, Qiu L, Dong J, Yin H, et al. Pharmacological profile of xanthohumol, a prenylated flavonoid from hops (Humulus lupulus). Molecules. 2015;20(1):754-79.

500. Zhang X, Liang X, Xiao H, Xu Q. Direct characterization of bitter acids in a crude hop extract by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Am Soc Mass Spectrom. 2004;15(2):180-7.

501. Taniguchi Y, Matsukura Y, Taniguchi H, Koizumi H, Katayama M. Development of preparative and analytical methods of the hop bitter acid oxide fraction and chemical properties of its components. Biosci Biotechnol Biochem. 2015;79(10):1684-94.

502. Obara K, Mizutani M, Hitomi Y, Yajima H, Kondo K. Isohumulones, the bitter component of beer, improve hyperglycemia and decrease body fat in Japanese subjects with prediabetes. Clin Nutr. 2009;28(3):278-84.

503. Morimoto-Kobayashi Y, Ohara K, Ashigai H, Kanaya T, Koizumi K, Manabe F, et al. Matured hop extract reduces body fat in healthy overweight humans: a randomized, double-blind, placebo-controlled parallel group study. Nutr J. 2016;15:25.

504. Blatchford PA, Parkar SG, Hopkins W, Ingram JR, Sutton KH. Dose-dependent alterations to in vitro human microbiota composition and butyrate inhibition by a supercritical carbon dioxide hops extract. Biomolecules. 2019;9(9):390.

505. Heddle R, Dent J, Read NW, Houghton LA, Toouli J, Horowitz M, et al. Antropyloroduodenal motor responses to intraduodenal lipid infusion in healthy volunteers. Am J Physiol. 1988;254(5 Pt 1):G671-9.

506. McVeay C, Steinert RE, Fitzgerald PCE, Ullrich SS, Horowitz M, Feinle-Bisset C. Effects of intraduodenal coadministration of lauric acid and leucine on gut motility, plasma cholecystokinin, and energy intake in healthy men. Am J Physiol Regul Integr Comp Physiol. 2020;318(4):R790-R8.

507. Little TJ, Luscombe-Marsh ND, Gentilcore D, Brook EJ, Feinle-Bisset C. Effects of varying the inter-meal interval on relationships between antral area, gut hormones and energy intake following a nutrient drink in healthy lean humans. Physiol Behav. 2014;135:34-43.

508. Stewart JE, Seimon RV, Otto B, Keast RS, Clifton PM, Feinle-Bisset C. Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men–. Am J Clin Nutr. 2011;93(4):703-11.