The effects of the amino acid, L-tryptophan, alone or with the fatty acid, lauric acid, on energy intake and postprandial glycaemia in health, obesity and type 2 diabetes

A thesis submitted by

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ABSTRACT

The studies reported in this thesis investigated whether specific dietary nutrients, including amino acids and fatty acids, when administered intraduodenally or intragastrically, reduce energy intake and/or blood glucose in healthy people and those with obesity and type 2 diabetes. These studies also investigated whether the effects are associated with modulation in GI functions, i.e. gut hormones, gut motility and gastric emptying. Moreover, they quantified extra-intestinal factors, including circulating amino acid concentrations, that may be surrogate markers for activity in central pathways in the brain.

The key findings of the studies are:

1. Intragastric L-tryptophan, in a dose of 3 g, increased the plasma tryptophan/large neutral amino acids ratio more in lean participants than those with obesity, and in the lean the suppression of energy intake in response to tryptophan was related to the plasma tryptophan/LNAA ratio in the lean (**Chapter 2**).

2. Intragastric L-tryptophan, in a dose of 3 g, potently reduced energy intake, in both lean individuals and those with obesity. Suppression of energy intake was related to circulating tryptophan and the plasma tryptophan/LNAA ratio. L-tryptophan also suppressed appetite for 2 hours after the meal, despite the reduced energy intake. In the lean, appetite suppression was related to circulating tryptophan and the plasma tryptophan/LNAA ratio in lean participants (**Chapter 3**).

3. In individuals with type 2 diabetes, intragastric administration of L-tryptophan, in a dose of 3 g, before a carbohydrate-containing drink delayed the rise in plasma glucose, probably as a

result of slowing of gastric emptying, but did not affect the overall blood glucose response (**Chapter 4**).

4. Intraduodenal infusion of lauric acid in combination with L-tryptophan, and lauric acid alone delayed the rise in postprandial plasma glucose in healthy men, probably by slowing of gastric emptying and GLP-1 stimulation, while L-tryptophan was ineffective (**Chapter 5**).

In conclusion, the research presented in this thesis has established that while L-tryptophan potently suppresses energy intake in lean people and those with obesity, its effect on postprandial blood glucose differs between health, obesity and type 2 diabetes. GI factors, including gastric emptying and gut and pancreatic hormones, contribute to these effects, but apparently to varying extents, and other post-absorptive factors also play a role. Finally, the combination of lauric acid and L-tryptophan, each in doses that individually do not reduce postprandial glycaemia, has a potent effect to delay the early rise in postprandial glucose, reflecting slowing of gastric emptying. Further research is indicated to investigate the sustained effects of L-tryptophan on appetite, energy intake and body weight. Moreover, studies to assess the effect of the combination of lauric acid and L-tryptophan on postprandial glycaemia in obesity and/or type 2 diabetes are also indicated. Broadly, the purpose of these studies would be to determine whether these nutrients have the potential to offer a novel, nutrient-based treatment option for the management of obesity and/or type 2 diabetes.

DECLARATION OF ORIGINALITY

I, Maryam Hajishafiee, the author 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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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Dedicated to my dearest parents, Mahnaz and Mohsen, who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve,

the one and only sister, Nazanin, for having never left my side,

and my beloved husband, Salman, who has been a constant source of support and encouragement during my PhD life.

"You were born with potential. You were born with goodness and trust. You were born with ideals and dreams. You were born with greatness. You were born with wings. You are not meant for crawling, so don't. You have wings. Learn to use them and fly"

Rumi

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Hajishafiee M, Elovaris RA, Jones KL, Heilbronn LK, Horowitz M, Poppitt SD, Feinle-Bisset C. Effects of intragastric administration of L-tryptophan on the glycaemic response to a nutrient drink in men with type 2 diabetes – impacts on gastric emptying, glucoregulatory hormones and glucose absorption. *Nutr Diabetes 2020;11:3*.

Hajishafiee M*, McVeay C*, Lange K, Rehfeld JF, Horowitz M, Feinle-Bisset C. Effects of intraduodenal infusion of lauric acid and L-tryptophan, alone and combined, on glucoregulatory hormones, gastric emptying and glycaemia in healthy men. *Metab Clin Exp 2022;129:155140.*
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ABSTRACTS AND PRESENTATIONS

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ABBREVIATIONS

3-OMG	3-O-methyl-D-glucose
5-HT	5-hydroxy-tryptamine (serotonin)
ANOVA	Analysis of variance
AUC	Area under the curve
BMI	Body mass index
C12	Lauric acid
C18:1	Oleic acid
CaSR	Ca^{+2}-sensing receptors
CCK	Cholecystokinin
CVs	Coefficient variations
DPP-IV	Dipeptidyl peptidase-IV
ELISA	Enzyme-linked immunosorbent assay
FD	Functional dyspepsia
FODMAP	Fermentable oligo-, di- and mono-saccharides and polyols
GI	Gastrointestinal
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide-1
GLUT4	Glucose transporter-4
GPR142	G protein-coupled receptor 142
HbA1c	Haemoglobin A1c
LNAA	Large neutral amino acid
MC4R	Melanocortin-4-receptor
NS	Not significant
POMC	Pro-opiomelanocortin
PYY	Peptide YY
SEM	Standard error of the mean
T50	Gastric half-emptying time
TRP	Tryptophan
VAS	Visual analogue scale
WHO	World Health Organisation

CHAPTER 1: INTRODUCTION

1.1 Epidemiology and aetiology of obesity and type 2 diabetes

Obesity is a chronic progressive disease and a major, and growing, health problem in both children and adults, associated with a reduction in life expectancy of 5-20 years, depending on the severity of the condition and all-cause mortality [1]. The percentage of people with obesity and overweight in the world is increasing at an alarming rate, with the number of adult women with obesity increasing from 69 million in 1975 to 390 million, and the number of men with obesity from 31 million in 1975 to 281 million, in 2016. Moreover, 213 million children and adolescents and 1.3 billion adults were overweight in 2016 [2].

Obesity substantially increases the risk of metabolic diseases, including type 2 diabetes and fatty liver disease, cardiovascular diseases, including hypertension, myocardial infarction and stroke, musculoskeletal disease, Alzheimer disease, depression and a number of cancers, including breast, ovarian, prostate, liver, kidney and colon [1,3,4]. Obesity is also a major risk factor for the development of metabolic syndrome and prediabetes (impaired fasting glucose or impaired glucose tolerance). Central adiposity and excess body weight increase the risk of type 2 diabetes by 4.6 fold in women and 3.5 fold in men [5]. Type 2 diabetes is an important cause of mortality and morbidity accounting for more than 2 million deaths every year and is the seventh leading cause of disability worldwide [6]. Type 2 diabetes complications are largely the consequence of vascular damage, which may be macrovascular (cerebrovascular, cardiovascular and peripheral artery diseases) or microvascular (retinopathy, neuropathy and nephropathy) [7].

1.1.1 Obesity: diagnosis, epidemiology and pathogenesis

The World Health Organisation (WHO) defines obesity as excess body fat that impairs health, increases the risk of long-term medical complications and reduces lifespan [8]. The National

Institutes of Health and WHO have both adopted body mass index (BMI) as a criterion for the definition of obesity, a concept first proposed by Adolphe Quetelet in 1859. BMI is calculated as a person's weight in kilograms divided by the square of their height in meters (kg/m^2) [9]. For adults, obesity is defined as a BMI exceeding 30 kg/m^2 and is sub-classified into generalised obesity class 1 (30-34.9 kg/m^2) and class 2 (35-39.9 kg/m^2), and extreme obesity class 3 (≥40 kg/m^2), while a BMI of 25-29.9 kg/m^2 is considered overweight [10]. Epidemiological data justify a BMI ≥25 kg/m^2 as the definition of obesity in many Southeast Asian populations who are known to be at increased risk of type 2 diabetes and hypertension at lower BMI thresholds compared with non-Asian groups, probably reflecting the predominance of central fat distribution [11]. In children and adolescents, the U.S. Centers for Disease Control and Prevention BMI-for-age growth charts define overweight as a BMI ≥90th percentile of standard weight and obesity as a BMI >95th percentile of standard weight [10,11]. The use of BMI in screening and diagnosis of obesity has advantages and disadvantages. Height and weight are easily measured, and BMI generally correlates with adipose tissue mass in population studies. It is, accordingly, useful for the initial screening to detect excess body fat, and higher BMI levels are associated with increased risk of complications of obesity [4]. However, the inability of BMI to discriminate weight associated with muscle vs. fat represents a major limitation. Population studies have demonstrated a high specificity of BMI cut-off values to diagnose obesity, but a low sensitivity to identify adiposity [12]. Thus, BMI will overestimate adiposity in athletes with high muscle mass and low percent body fat and underestimate adiposity in elderly patients with sarcopenia [13,14]. BMI also does not evaluate body fat distribution. Visceral fat accumulation predicts the development of the metabolic syndrome and cardiovascular mortality risk better than total body fat alone [15]. In contrast to BMI, waist circumference estimates visceral adipose tissue and is the simplest anthropometric measurement of abdominal adiposity in clinical settings. Therefore, integration of both BMI and waist circumference in clinical assessment

may identify the higher-risk phenotype of obesity better than either BMI or waist circumference alone [16]. A waist circumference ≥80 cm for women, and ≥94 cm for men, increases the risk of co-morbidities [8,11].

Epidemiology

Obesity represents a rapidly growing threat to the health of populations in countries worldwide. In 2017-18, an estimated 67% Australians aged 18 and over were overweight or had obesity. The obesity rate in Australia is the fifth highest amongst countries in the Organisation for Economic Co-operation and Development [1]. From 1999-2000 through 2017-2018, a significantly increasing trend in obesity has also been observed among adults in United States, and the age-adjusted prevalence of obesity increased from 30.5% to 42.4% [3]. The World Obesity Federation and other organisations, including the American and Canadian Medical Associations, have declared obesity a chronic progressive disease, clearly distinct from simply being a risk factor for other diseases, and associated with unemployment, social disadvantages and reduced socio-economic productivity, thus, placing a considerable financial burden on individuals, families and nations [1]. The total cost attributable to obesity was $1.72 trillion in 2016 ($480.7 billion in direct costs and $1.24 trillion in indirect costs), equivalent to 9.3% of the gross domestic product, and accounting for 47% of the total cost of chronic diseases, worldwide [17]. Moreover, the cost of obesity in Australia in 2011-2012 was $8.6 billion, including $3.8 billion in direct costs and $4.8 billion in indirect costs [11,18]. Clearly, there is an urgent need to combat the obesity crisis to reverse the concomitant health and economic burden.

Pathogenesis

The pathogenesis of obesity is characterised by a multifactorial interplay between genetic and behavioural and environmental factors, including high availability of energy-dense foods, lack of physical activity due to the industrial environment and psychological conditions. Variation in weight or BMI is mainly attributable to the genetic background [19,20]. This strong genetic contribution has been established through studies of families, twins and adopted children. Some estimates put the heritability of both monogenic and polygenic causes of obesity to be as high as 70% (12). In 1997, Montague et al. described the first single gene defect causing monogenic obesity and reported the first patients with congenital leptin deficiency, a homozygous frameshift mutation in LEP gene, associated with early-onset, severe, obesity and hyperphagia [21]. Mutations in pro-opiomelanocortin (POMC), a complex polypeptide in the pituitary gland which plays a role in the regulation of food intake and energy homeostasis, were described in 1998, and are amongst the leading cause of early-onset severe obesity [22]. Moreover, mutations in melanocortin-4-receptor (MC4R), a regulator of food intake and energy homeostasis in the hypothalamus, are the most common cause of non-syndromic genetic obesity disorders, associated with hyperphagia [19,23].

Environmental "obesogenic factors", including the built environment, city plan, transport and school, TV and screen-related immobility have made lives less physically demanding, therefore, energy expenditure during everyday life has decreased, particularly relative to sustained, or increased, energy intake [24]. The wide availability of energy-dense fast-foods and unhealthy eating-related behaviours, including fast eating, frequent snacking and the consumption of sweetened drinks, are also important contributors to the pathogenesis of obesity [25]. Moreover, some psychosocial factors in childhood, including neglect in care, abuse, anxiety and depression may act as determinants for developing obesity in adulthood [26,27]. While

this information underlines the complexity of body weight regulation, approaches that effectively decrease appetite are likely to be important to encourage weight loss and prevent comorbidities, particularly type 2 diabetes.

1.1.2 Type 2 diabetes: definition, diagnosis, epidemiology and pathogenesis

As outlined, the risk of developing type 2 diabetes is closely linked to BMI. The current classification of diabetes recognises type 2 diabetes, the predominant subtype of diabetes, to be caused by a combination of progressive loss of insulin secretion and insulin resistance [28]. In 1997, an international expert committee re-examined the classification and diagnostic criteria of type 2 diabetes based on the 1979 publication of the National Diabetes Data and WHO study groups. The current criteria separate type 2 diabetes from non-diabetes using fasting plasma glucose ≥140 mg/dL (7.0 mmol/L) (**Table 1.1**). Normal fasting plasma glucose was defined as <110 mg/dL (6.1 mmol/L). Impaired fasting glucose ranges from 110 md/dL to 125 mg/dL. Moreover, the 2-hour blood glucose response to 75 g of glucose (oral glucose tolerance test) and the longer-term marker, haemoglobin A1c (HbA1c), are used [29]. HbA1c is a measure of the degree to which haemoglobin is glycosylated in erythrocytes and is expressed as a percentage of total haemoglobin concentration. HbA1c levels provide an indication of average blood glucose concentration during the preceding 2-3 months. Because blood glucose concentrations may vary widely during a 24-h period and from day to day in type 2 diabetes, an elevated HbA1c indicates a chronic state of hyperglycaemia, while hyperglycaemia as measured by fasting plasma glucose or oral glucose tolerance test may be transient [29]. It is now recognised that in the majority of individuals with type 2 diabetes who have reasonable glycaemic control (i.e. HbA1c <8.0%), the rise in blood glucose after a meal is the major determinant, and, accordingly, represents a specific target for management [30].

Table 1.1. Diagnostic thresholds for type 2 diabetes and lesser degrees of impaired glucose regulation

Category	Test			
	Fasting plasma glucose mg/dL (mmol/L)	2-hour oral glucose tolerance test	HbA1c	
			%	mmol/mol
Normal	<100 (<5.6)	<140 (<7.8)	<6	<42
Impaired fasting plasma glucose	100-125 (5.6-6.9)	-	<6.5	<48
Impaired glucose tolerance	-	149-199 (7.8-11)	<6.5	<48
Diabetes	≥126 (≥7)	≥200 (≥11.1)	≥6.5	≥48

Data from American Diabetes Association [30].

Epidemiology

From 1980 to 2014, worldwide age-standardised adult type 2 diabetes prevalence increased from 4.3% to 9% in men, and from 5% to 7.9% in women, with the greatest increase in low- and middle-income countries [6]. In 2015, the number of adults with type 2 diabetes globally was estimated at 415 million, with a projected increase to 642 million by 2040 [31]. In Australia, the prevalence of type 2 diabetes has more than doubled over the past three decades [32], with an estimate of around one million people with type 2 diabetes in 2011-2012. The prevalence of type 2 diabetes in the United States is projected to increase further markedly during the next 3 decades. In part, this reflects the increasing age of the population, including that people with type 2 diabetes live longer because of decreasing rates of cardiovascular death [33]. Type 2 diabetes is associated with the highest costs to the healthcare system related to overweight and obesity [18].

Pathogenesis

Glucose homeostasis is maintained by coordinating the production of glucose in the liver through the pathways of glycogenolysis and gluconeogenesis in times of fasting with the disposal during ingestion of glucose into skeletal muscle through glycogen synthesis and glucose metabolism, and to a much lesser extent, with adipose tissue [34]. Insulin, secreted by

the β-cells of the pancreas in response to food consumption, inhibits hepatic glucose output while enhancing glucose uptake into muscle and adipose tissue. A primary defect in β-cells causing hyperresponsiveness to nutrient stimuli can result in chronically elevated plasma insulin concentrations in response to increased food intake and trigger insulin resistance, resulting in hyperinsulinaemia [35]. Once the β-cells can no longer compensate for the insulin resistance, blood glucose levels start to rise and there is transition from a stage of impaired glucose tolerance to type 2 diabetes. At the same time, diminished postprandial glucagon suppression, probably due to hepatic insulin resistance, further elevates postprandial blood glucose. Increased glucagon secretion also elevates fasting blood glucose by increasing hepatic glucose output due to increased gluconeogenesis and glycogenolysis [36,37]. Because insulin action serves different functions in different cell types, insulin resistance has diverse functional ramifications in the various insulin target tissues. Chronically elevated blood glucose levels may lead to a number of changes on a molecular and cellular level, leading to comorbidities, as outlined earlier. Non-enzymatic glycosylation of proteins and lipids may interfere with normal function of enzymes or receptor recognition. Interaction of glycosylated proteins with receptors, on monocyte-derived macrophages or smooth muscle cells, that are important to the development of arteriosclerosis, may cause oxidative stress and inflammation. Tissues that cannot protect themselves from the surplus of glucose, such as cells in the retina, renal glomerulus and in peripheral nerves, are prone to such adverse effects [38]. Due to the pathophysiological changes associated with obesity and type 2 diabetes, it is critical to have effective strategies for their prevention and management.

1.2 Treatment approaches for obesity and type 2 diabetes

It is now increasingly recognised that obesity management should focus on improved health and well-being, and not only weight loss. A modest 5% to 10% weight loss is currently a

standard goal in weight loss interventions [39], and is associated with significant improvements in obesity-related comorbidities, including type 2 diabetes [40-42].

Lifestyle modifications focusing on healthy diet and exercise remain the foundation of obesity management, as they are for other chronic conditions, including type 2 diabetes. However, for the majority of people with obesity, weight loss is difficult to sustain in the long-term. This is due to physiological neuro-hormonal changes that occur in response to weight loss and which increase hunger, associated with increased energy intake, while simultaneously reducing energy expenditure, to defend a "set-point" for body weight. Together these physiological adaptations favour weight regain [10]. When lifestyle modifications are ineffective, pharmacotherapy is generally as a second-line treatment, followed by bariatric devices and surgery [10,33]. This section provides a brief summary of different weight-loss strategies and interventions in the management of obesity and type 2 diabetes.

1.2.1 Dietary and physical activity interventions

Lifestyle modifications - calorie reduction and increased physical activity are the first-line approach to weight reduction in obesity. Although many studies indicate that maximal weight loss is achieved at 6 months and weight loss maintenance is moderately effective up to 24 months, most individuals regain weight thereafter [43,44]. Even with sustained adherence to weight-loss diets, body weight often stabilises over time, or even increases, as a result of adaptive metabolic changes. For example, dietary restriction is associated with a reduction in basal metabolic rate and reducing energy requirements [45]. Moreover, increased hunger, often experienced by people on weight-loss diets, reduces compliance to calorie restriction [46]. Thus, while dietary restriction leads to short-term weight loss, it is often associated with rebound weight gain [47]. In addition to a reduction in energy intake, increasing physical activity is

important for maintenance of weight loss [44]. Numerous epidemiological studies have shown an inverse association between increased physical activity and obesity in adults and children [48,49]. The biological mechanisms by which physical activity prevents weight gain include increasing total energy expenditure, reducing fat mass, maintaining lean body mass and basal metabolic rate [50].

A number of different dietary interventions are used to achieve weight loss, by manipulating the macronutrient proportions and energy content of diets [4,51]. For rapid weight loss, very-low-calorie diets are often used under medical supervision. In the longer-term, adjustments of the recommended macronutrient composition, including consuming 55% carbohydrate, 30% fat (of which no more than 10% is saturated fat) and 15% protein, are common [52]. The following section discusses different dietary interventions for weight loss, including very-low-calorie diets and low-calorie diets with varying macronutrient compositions.

Very-low-calorie diets

Very-low-calorie diets provide 400-800 kcal/day or <50% of resting energy expenditure [53]. They are routinely prescribed to achieve rapid initial weight loss in patients before bariatric surgery to reduce liver size and visceral fat, enabling easier and shorter operations [54]. For example, providing an individualised very-low-calorie diet-based treatment for 14 days prior to surgery resulted in a rapid weight loss of <5% in 87.3% and ≥5% in 12.7% of patients who were referred for bariatric surgery [55]. In a review comparing low-calorie diets with very-low-calorie diets, it was reported that very-low-calorie diets resulted in greater short-term weight loss (16.1%) than did low-calorie diets (9.7%), but similar longer-term weight loss (1 year after maximum weight loss) [56]. However, over the long-term, the probability of weight maintenance is low compared with low-calorie diets, reflecting suboptimal compliance s[57].

Low-calorie diets

Broadly three approaches are used for setting intake goals for low-calorie diets. They are an absolute reduction (e.g. 500 kcal/day) from baseline, a relative reduction from baseline (e.g. 25%), and an intake below that required for weight maintenance (e.g. 1200-1400 kcal/day for women and 1400-1600 kcal/day for men) [58]. A low-calorie diet can be achieved either by lowering of intake with a proportionate reduction in the intake of all macronutrients, or a specific reduction of a particular macronutrient. For example, traditionally, a low-fat diet, consisting of a dietary composition of fat ranging from very low (≤10% of calories from fat) to more moderate (≤30% of calories from fat and <7%-10% from saturated fatty acids), has been the recommended approach [59]. The primary rationale underlying the promotion of low-fat diets is that dietary fat has a higher energy content (9 kcal/g) than either carbohydrates or protein (4 kcal/g) and is positively associated with body fat [60]. Over a duration of 6 months, low-fat diets result in a weight loss of only ~2-4 kg [61,62]. Moreover, the outcome of a meta-analysis indicated that in weight loss trials, low-carbohydrate led to greater weight loss than low-fat, interventions (10-30% of calories from fat), and, accordingly, did not support the use of low-fat diets over other dietary interventions, including low-carbohydrate diets, for long-term (≥1 year) weight loss [63]. In a study including twenty trials, low-density lipoprotein cholesterol was reduced among individuals with obesity who followed a low-fat diet (<30% of calories from fat), however, triglyceride level increased and high-density lipoprotein cholesterol levels decreased [64]. Therefore, it appears that diets which restrict carbohydrate intake are associated with greater weight loss and improvements in cardiovascular risk factors than low-fat diets [65].

A low-carbohydrate diet is defined as a carbohydrate intake below the lower average of the macronutrient distribution range for healthy adults (45%-65% of total daily energy) and

encompasses a range of carbohydrate intakes from 50-130 g/day or 10%-45% total energy. Hence, the optimal range for total fat and protein are 20-35% and 10-35% of energy, respectively [66]. Low-carbohydrate diets are considered to induce weight loss because they promote a reduction in insulin secretion and increased glucagon, resulting in a metabolic shift to increased fat oxidation [67]. For example, in a study with 132 patients with morbid obesity, a low-carbohydrate diet (<30 g carbohydrates/day) induced more weight loss over 6 months than a low-fat diet (<30% of calories from fat) [61]. Low-carbohydrate diets have been widely used not only for weight reduction, but also to manage type 2 diabetes [68]. In a trial in patients with type 2 diabetes, adherence to a low-carbohydrate diet (20% of calories from carbohydrate, 50% of calories from fat) reduced insulin requirement compared with the low-fat diet (55-60% of calories from carbohydrate, 30% of calories from fat) after 6 months [69].

High-protein diets are increasingly popularised as a promising strategy for weight loss with evidence that they are more effective in inducing weight loss compared with diets low in carbohydrates or fat. A high-protein diet refers to an increased protein intake to ~20-35% of total daily calories or 1-1.2 g/kg of the ideal body weight/day, with 20-30% of calories from fat and 30-40% of calories from carbohydrate [70]. Weight loss following a high-protein diet is thought to result from the effects of protein to increase satiety, energy expenditure and suppress appetite, while preserving fat-free mass [71]. The following section discusses effects of high-protein diets on weight loss, appetite and food intake, including any potential adverse effects of high-protein diets.

Effects of high-protein diets on weight loss

The potent effects of a high-protein diet on weight loss have been demonstrated in a number of studies. Wycherley et al. reviewed and summarised findings of 24 randomised controlled

trials, including 1063 individuals with a mean diet duration of 12.1±9.3 weeks, that compared energy-restricted, isocaloric, high-protein diets with standard-protein diets. Individuals in the high-protein diet group consumed 1.07-1.60 g protein/kg body weight/day (27-35% of total energy intake consumed as protein, 22-33% as fat and 35-47% as carbohydrate) with a greater total energy intake <300 kcal/day compared with individuals in the standard-protein diet group who consumed 0.55-0.88 g/kg body weight/day (16-21% of total energy intake consumed as protein, 18-31% as fat and 51-61% as carbohydrate). Individuals in the high-protein group had greater reductions in body weight (mean difference between groups: -0.79 kg; 95% confidence interval (CI), -1.50 to -0.08 kg), fat mass (mean difference between groups: -0.87 kg; 95% CI, -1.26 to -0.48 kg), and blood triglycerides (mean difference between groups: -20.3 mg/dL; 95% CI, -29.2 to -106 mg/dL) and greater increases in fat-free mass (mean difference between groups: 0.43 kg; 95% CI, 0.09-0.78 kg) and resting energy expenditure (mean difference between groups: 142 kcal/day; 95% CI, 16-268 kcal/day) compared with those in the standard-protein diet group [72].

In an individual study by Skov et al. a 6-month randomised dietary intervention trial, participants were divided into two *ad libitum* fat reduced diets, including high-protein diet (25% of total energy, *n*=25) and high-carbohydrate diet (12% of total energy, *n*=25), or control group (*n*=15), with fat intake set to 30% of total energy intake. The diet composition was strictly controlled and participants followed an *ad libitum* diet at designated restaurants. After 6 months, weight loss was 5.1 kg in the high-carbohydrate and 8.9 kg in the high-protein group (mean difference between groups: -3.7 kg; 95% CI, -6.2 to -1.3 kg) and fat loss was 4.3 kg and 7.6 kg (mean difference between groups: -3.3 kg; 95% CI, -5.5 to -1.1 kg) respectively, whereas no changes occurred in the control group. More individuals lost >10 kg in the high-protein group (35%) than in the high-carbohydrate group (9%) [73].

Effects of high-protein diets on appetite and food intake

The effect of protein to reduce body weight may in part be due to the satiating effect of protein. A large number of studies have evaluated the acute effects of protein ingestion and meals high in protein, lasting for 24 hours up to 5 days, on appetite and food intake. For example, in one study using the controlled environment of a respiration chamber, satiety was assessed over 24 hours, comparing high- versus normal-protein diets (protein/carbohydrate/fat: 30/60/10% of energy vs. 10/30/60% of energy), while participants were fed in energy balance. Throughout the day, satiety and fullness were greater on the high-protein diet, while hunger, appetite, desire to eat, and prospective food consumption were lower than on the normal-protein, high-carbohydrate, low-fat diet [74]. Subsequently, in a similar respiration chamber experiment, lean women were fed in energy balance with a normal- or high-protein diet which contained 10/60/30% of energy or 30/40/30% of energy from protein/carbohydrate/fat, providing ~60 g or ~180 g of protein respectively, for 4 days [75]; the high-protein diet increased 24-h satiety over the 4 days and decreased hunger compared with the normal-protein diet. These observations support the hypothesis that protein increases satiety to a greater extent than carbohydrate or fat [74,75]. Weigle et al. [76] investigated the effects of a calorie-fixed, energy-balanced diet with normal or high protein content, followed by an *ad libitum* high-protein diet, on appetite-perceptions, energy intake and body weight, in healthy, normal-weight individuals and those with obesity. Initially, individuals consumed a baseline diet consisting of 15% of daily energy from protein, 50% carbohydrate and 35% fat, followed by an isocaloric, high-protein diet with 30% of daily energy from protein, 50% from carbohydrates and 20% from fat, each for two weeks. For the subsequent 12 weeks, individuals consumed the same high-protein diet, however, under *ad libitum* conditions. During the energy-fixed, high-protein diet, fullness was higher, while hunger was less, compared with the isocaloric adequate-protein diet. Interestingly, during the *ad libitum* phase, participants voluntarily decreased their daily energy

intake by ~441 kcal, which was associated with a weight loss of ~4.9 kg over the 12-week period (67). Hence, high-protein diets appear to result in weight loss, due to the potent effects of protein to suppress appetite and food intake.

Possible detrimental effects of proteins

It is important to appreciate the potential adverse effects of high-protein diets, particularly given that the latter are increasingly popular. High-protein diets have been associated with increased risk of osteoporosis and renal diseases [77]. A potential link with osteoporosis was supported by the observation of increased urinary calcium excretion during a high protein intake. High-protein diets >2 g/kg/day increase bone resorption by increasing acid load in the body, compared with diets of a low- to normal-protein content of 0.7-1.0 g/kg/day [78]. While the source of the increased urinary calcium remains unclear, it has been suggested that high consumption of animal protein, in particular, leads to an acidification of the blood, that may enhance carbonate, and subsequently calcium, release from the skeleton to decrease bone mineral density [79]. However, a study in postmenopausal women reported an increase in intestinal calcium absorption during a high-protein diet (1.6 g/kg/day), compared with a low-protein diet (0.8 g/kg/day), suggesting that a higher excretion may, at least partly, be compensated for by an enhanced absorption of calcium in the small intestine [80]. An epidemiologic study also found that in older men (>60 years), a greater dietary acid load associated with chronic high-protein intake was associated with femoral bone loss only under conditions of very low calcium intake <800 mg/d dietary calcium [81]. Furthermore, the outcomes of a number of studies suggest that a protein intake of ~1.0-1.5 g/kg/day is not associated with adverse effects on calcium or bone metabolism. Accordingly, current evidence indicates that a moderately high protein consumption is not associated with an increased risk for osteoporosis [82,83].

Diets high in protein have also been suggested to lead to increased renal acid load such as the sulfuric acid produced from oxidation of methionine and cysteine, and that may result in kidney stones, and/or an increase in the glomerular filtration rate, which may damage the kidneys over time [84]. In men with obesity, a calorie-restricted, high-protein diet with 35% of daily energy intake from protein (corresponding to ~126 g/day) was not associated with any changes in kidney function over a period of one year, suggesting that high-protein diets are safe in individuals without pre-existing kidney disease [85]. One study in women with mild renal insufficiency reported an association between protein intake and diminished renal function [86]. Hence, the National Kidney Foundation's recommendations for non-dialysed individuals with chronic kidney disease are lower than those for the overall population (0.6-0.75 g/kg/day) [87]. Therefore, high-protein diet should be implemented with caution in individuals with inherited or underlying abnormalities associated with renal disease, including diabetes and cardiovascular disease, including hypertension.

In conclusion, high-protein diets appear to have clinically meaningful, sustained, effects on weight loss and suppression of appetite and energy intake compared with other diets low in carbohydrate or fat. However, it remains controversial as to whether high-protein diets are safe for longer-term consumption, particularly, in individuals with type 2 diabetes and/or cardiovascular disease. Therefore, novel strategies are required that take advantage of the beneficial effects of protein without requiring the consumption of high quantities.

1.2.2 Pharmacotherapy in obesity

Since in many individuals, lifestyle interventions do not lead to sustained weight loss, pharmacotherapeutic agents are applied in combination with lifestyle modifications [47]. There are currently two groups of medications approved by the Food and Drug Administration for

weight management in obesity (BMI ≥30) or overweight individuals (BMI ≥27) with at least one complication, including type 2 diabetes, hypertension or hyperlipidaemia [88]. Medications from the first group are suitable for long-term treatment of obesity. These include orlistat, lorcaserin, liraglutide and semaglutide, the combination of phentermine/topiramate extended release, and the combination of naltrexone and bupropion sustained release. The second group consists of sympathomimetic drugs that are approved for short-term use, usually <12 weeks, including benzphetamine, diethylpropion, phendimetrazine, and phentermine [4].

Orlistat is one of the safest and most commonly used drugs for obesity. It is a potent and selective inhibitor of gastric and pancreatic lipase and reduces intestinal digestion and absorption of fat by about 30% resulting in an enhanced excretion of ingested fat [47,89,90]. In a meta-analysis of 31 studies using orlistat, the maximal weight loss was 6.65 kg, and half of this effect occurred by 35.4 weeks [91]. Due to its mode of action, orlistat is often associated with advance effects, including increased risk of gall stones, fatty stools, faecal incontinence and increased defecation which compromise use [92].

Lorcaserin is a member of the serotonergic class of drugs. It selectively targets serotonin-2c receptors to reduce food intake. In a meta-analysis of five studies using lorcaserin, the maximal weight loss was 5.39 kg, and half the maximal effect occurred by 19 weeks [91]. Adverse effects are common and include headache, dizziness, nausea, dry mouth and hypoglycaemia [93]. There is also a risk of "serotonin syndrome", a high level of serotonin in the body associated with lorcaserin, particularly if used with serotonin reuptake inhibitors [93]. In 2020, the US Food and Drug Administration ordered the withdrawal of lorcaserin from markets, as a clinical trial to assess drug safety showed an increased risk of cancer [94].

Liraglutide, a long-acting agonist of the incretin hormone, glucagon-like peptide-1 (GLP-1) (more detailed description of the incretin hormones is provided in section 1.4.2), has a 97% homology to GLP-1 and mimics the actions of native GLP-1. Liraglutide has been approved for the treatment of type 2 diabetes by increasing glucose-dependent insulin release from the pancreas, decreasing excessive glucagon release and slowing of gastric emptying [93]. Liraglutide also reduces energy intake, at least in part, via central suppression of appetite by stimulating hypothalamic neurons. It has been approved for use in obesity treatment in both adults and children, in doses higher than those need in the management of type 2 diabetes [95]. In a meta-analysis of three studies using liraglutide in adults, the maximal weight loss was 7.68 kg, and half the maximal effect occurred by 12.7 weeks [91]. A disadvantage of liraglutide is the requirement for administration via subcutaneous injection [96], and the high prevalence of adverse effects, including nausea, vomiting, and diarrhea [96]. Semaglutide, injection 2.4 mg once weekly, is also a GLP-1 agonist and has recently been approved for long-term weight management in obesity. The most common side effects of semaglutide include nausea, diarrhea, constipation, dizziness, and hypoglycaemia in patients with type 2 diabetes [96].

Phentermine/topiramate is a combination of two drugs; phentermine acts to reduce appetite through increasing norepinephrine, and topiramate by its effect on gamma aminobutyric acid receptors. In a meta-analysis of six studies using phentermine/topiramate, the maximal weight loss was 15.6 kg, half the maximal effect occurred by 29.8 weeks [91]. Phentermine/topiramate have frequent adverse effects including, dry mouth, insomnia, increased heart rate and blood pressure and kidney stones. This drug is not recommended for patients with depression, kidney or liver dysfunction or hypertension [93].

Naltrexone-bupropion is a combination of naltrexone and bupropion, which have been used independently for different indications. Bupropion is approved for management of depression and smoking cessation [88]. Bupropion also stimulates neurons to produce both α-melanocyte stimulating hormone (which reduces food intake) and β-endorphin (which stimulates feeding). Naltrexone blocks this effect of β-endorphin, allowing the inhibitory effects of α-melanocyte stimulating hormone to reduce food intake [88]. In a meta-analysis of six studies using naltrexone/bupropion, the maximal weight loss was 13.2 kg, and half the maximal effect occurred by 35.2 weeks [93]. Naltrexone-bupropion is not recommended for long-term use due to concerns regarding cardiovascular, psychiatric and cognitive adverse effects [92].

Taken together, all these pharmacotherapies are associated with a meaningful weight loss of ~5-10%, however, they have various adverse effects which limit their long-term use. Therefore, identifying effective treatment strategies with sustained weight loss effects, but lacking adverse effects, is of great importance.

1.2.3 Surgical therapies

Bariatric surgery has proved to be the most effective therapeutic strategy for obesity treatment, achieving ~20-35% of weight loss. However, surgical approaches are only available for people with obesity with BMI ≥40, or BMI ≥35 with at least one comorbidity [88], and are only considered when other treatments are ineffective. The three most common bariatric procedures performed worldwide are Roux-en-Y gastric bypass, sleeve gastrectomy, and adjustable gastric band [97]. Bariatric surgery can be classified as "malabsorptive", "restrictive", or mixed procedures, according to their putative mechanism of action. "Malabsorptive" procedures include jejuno-ileal bypass, which reduces nutrient absorption by shortening the functional small bowel length, and by allowing nutrients to pass directly from the proximal jejunum to

the terminal ileum. “Restrictive” surgery includes the laparoscopic application of an adjustable gastric band.

Roux-en-Y gastric bypass, considered as the “gold-standard”, is a combined restrictive and malabsorptive procedure [98]. It divides the stomach in two parts to create a small gastric pouch of <30 mL volume. The small intestine is then surgically rearranged, so that it bypasses the duodenum and upper jejunum and reconnects to a more distal part of the small intestine [99]. In contrast, the sleeve gastrectomy procedure involves removing the greater portion of the fundus and body of the stomach (reduction in gastric volume by 80%), creating a long, tubular gastric pouch, or sleeve. Compared to other procedures, the adjustable gastric band, which involves laparoscopic placement of an adjustable silicone band around the proximal stomach just below the gastro-oesophageal junction is less invasive and potentially reversible. The band is filled with an isotonic solution, and the amount of fluid can be adjusted via a subcutaneous reservoir fixed to the anterior rectus sheath [100]. All procedures lead to weight loss and improve co-morbidities, including type 2 diabetes or hypertension. While the available data suggest a comparable effect on body weight between gastric bypass surgery and sleeve gastrectomy [101], gastric banding is less effective for long-term weight loss [102]. Importantly, following gastric bypass surgery, as well as sleeve gastrectomy, ~80% of patients with type 2 diabetes experience early improvements in glucose homeostasis, even before major weight loss occurs [103]. Potential underlying mechanisms include a more rapid delivery of nutrients into the 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, which may support weight loss [104]. Following gastric bypass, patients also report a reduced preference for food items high in fat or sugar, which is likely to result in a decrease of overall daily caloric intake [105].

In conclusion, while a number of options exist for the management of obesity, with the exception of bariatric surgery, which is invasive and only available for individuals with morbid obesity in most cases long-term weight loss is not achieved. Novel treatment options that overcome challenges, such as increased hunger, or the adverse effects associated with current weight-loss medications, are required to decrease energy intake and induce long-term weight loss. The success of gastric bypass surgery in weight loss strongly indicates the importance of GI mechanisms, including enhanced release of gut hormones, in the regulation of appetite, energy intake and blood glucose, and point to the upper GI tract represent an important target for novel, effective and adverse effect-free approaches to prevention and management of obesity and type 2 diabetes.

1.2.4 Glucose lowering agents in type 2 diabetes

Glycaemic control represents a major focus in the management of individuals with type 2 diabetes. Given that normalising hyperglycaemia decreases the onset and progression of microvascular complications (i.e. retinopathy, neuropathy and nephropathy) [106]. The impact of glycaemic control on macrovascular complications is more modest and benefits probably only emerge after many years of improved control [107]. There are currently 7 classes of glucose-lowering medications approved in Australia, including biguanides, sulfonylureas, thiazolidinediones, alpha-glucosidase inhibitors, dipeptidyl peptidase-IV (DPP-IV) inhibitors, GLP-1 receptor agonists and sodium-glucose co-transporter 2 inhibitors [108], which target different pathophysiological pathways, including insulin secretion, hepatic glucose production and utilisation, insulin resistance, and gastric emptying [109]. Traditionally, treatment guidelines recommend a monotherapy approach, initially using metformin, in type 2 diabetes. With the addition of other medications, when monotherapy fails to achieve the HbA1c target. The general HbA1c target for most people with type 2 diabetes is ≤7% (53 mmol/mol) [108].

Metformin, the usual first-line treatment, is the only available biguanide in the world. The mechanism of action of metformin is still partly understood, but probably in part related to inhibition of hepatic gluconeogenesis by activating AMP-activated protein kinase and improvement of insulin sensitivity by enhancing peripheral glucose uptake and utilisation [110]. Metformin is also associated with reduced intestinal bile acid resorption, stimulation of GLP-1 secretion, and changes in the composition of the gut microbiota. Gastrointestinal (GI) adverse effects are common [111]. Sulfonylureas, including glibenclamide, gliclazide, glipizide and glimepiride, bind to ATP-sensitive potassium channels of pancreatic β-cells to promote insulin secretion. Adverse effects of sulfonylureas include hypoglycaemia and weight gain [112]. The thiazolidinedione class of drugs, including pioglitazone, increases insulin sensitivity through the activation of peroxisome proliferator-activated receptor gamma [113]. Alpha-glucosidase inhibitors inhibit alpha-glucosidases, enzymes located in the brush border of the small intestine when hydrolyse starch residues and disaccharides to release alpha-glucose molecules. These drugs include acarbose and miglitol, which reduce postprandial glycaemic excursions by delaying the absorption of dietary carbohydrates [114]. DPP-IV inhibitors, including sitagliptin, saxagliptin, linagliptin, alogliptin and vildagliptin, decrease inactivation of GLP-1 by inhibiting the enzyme DPP-IV, thereby increasing its availability. DPP-IV inhibitors have modest glucose-lowering properties and are well-tolerated. They are generally not considered as first-line treatment for type 2 diabetes, but rather as add-on drug therapy for patients who are inadequately controlled with other first-line treatments [115]. GLP-1 receptor agonists, including exenatide, liraglutide, dulaglutide, semaglutide and lixisenatide, are administered by subcutaneous injection (one or two a day, or once a week). They stimulate glucose-dependent insulin release, slow gastric emptying, and reduce postprandial glucagon and food intake [116]. The mechanism of action of sodium-glucose transporter inhibitors is inhibition of glucose transporters in the proximal tubules of the kidneys, which results in the inhibition of renal

glucose reabsorption. Canagliflozin, dapagliflozin, empagliflozin, ipragliflozin, ertugliflozin, and sotagliflozin are compounds of this group [108].

A number of fatty acids, such as lauric acid and the aromatic amino acid, L-tryptophan, have been reported to reduce postprandial glycaemia via stimulation of glucoregulatory hormones, or slowing of gastric emptying (discussed in section 1.5), thus, affecting some of the mechanisms used by the above medications to lower blood glucose.

1.3 GASTROINTESTINAL SENSING OF MEAL-RELATED SIGNALS IN HUMANS AND DYSREGULATIONS IN EATING-RELATED DISORDERS

The regulation of food intake and blood glucose by the GI tract involves a complex interplay of mechanical and neurohumoral signals, arising in anticipation of, during and after a meal. The following section, which has been published as an invited review article in "*Nutrients*" [Hajishafiee M, Bitarafan V, Feinle-Bisset C. Gastrointestinal sensing of meal-related signals in humans, and dysregulations in eating-related disorders. *Nutrients 2019;11:1298*], provides a brief overview of the sensing of intraluminal meal-related signals in humans, specifically, gastric distension and small intestinal nutrients, by describing their effects on GI functions, appetite and energy intake. Dysregulations in the GI responses to these signals in obesity, functional dyspepsia and anorexia of ageing are also examined as examples of eating-related disorders.

Statement of Authorship

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Contribution to the Paper	Wrote, reviewed, and edited the original draft.		
Overall percentage (%)	35%		
Certification:	This paper provides a review of the literature 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.		
Signature		Date	17 / 8 / 2021

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	17 / 8 / 2021

Please cut and paste additional co-author panels here as required.

1.3.1 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 the GI responses to luminal stimuli in obesity, functional dyspepsia 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.

1.3.2 INTRODUCTION

Meal ingestion is associated with well-established changes in GI functions that serve to accommodate food in the stomach and break it down to particles appropriate in 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 [117-120]. 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 [121-123], and gives rise to a sensation of fullness [124]. As gastric emptying progresses, the inputs from mechanical distension are gradually reduced, 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 G protein-coupled receptors, located on enteroendocrine cells, triggering a cascade of intracellular events to increase intracellular calcium, and culminating in the release of gut hormones from the basolateral membrane [118,119,125]. Gut hormones, e.g. cholecystokinin (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 [118,120,122,126]. 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. on the pylorus, to modulate gastropyloroduodenal motility associated with slowing of gastric emptying (**Figure 1.1**). Because the molecular and cellular processes involved in the sensing of these GI luminal signals cannot be investigated readily in humans, the release 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.

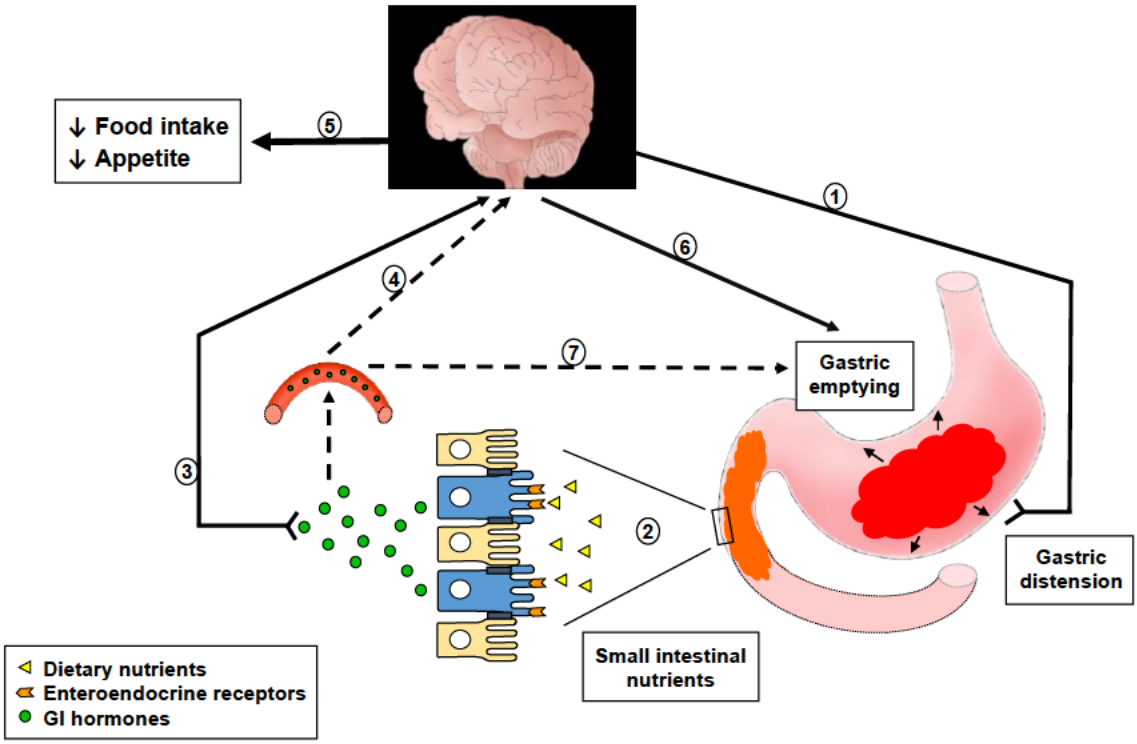

Figure 1.1
Schematic representation of the GI sensing of meal-related stimuli, and effects on GI functions (specifically gut hormone release and slowing of gastric emptying), appetite and energy intake. Meal ingestion initially induces gastric distension, which activates mechanoreceptors on vagal afferents 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 [99,127-129]. For example, in obesity, GI sensitivity to dietary nutrients, particularly fat, appears to be decreased, possibly as a consequence of excess energy intake [130]. 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 [131,132]. GI disorders (including gastro-oesophageal reflux disease, and the functional GI disorders, functional dyspepsia and irritable bowel syndrome), have in common a hypersensitivity to luminal stimuli, particularly fat, triggering postprandial GI symptoms [133]. 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 [128,134]. 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 [135], 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 alluded to 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, functional dyspepsia 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 [136], a discussion of their effects is beyond the scope of this paper.

1.3.3 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.

1.3.3.1 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 [124,137-139]. 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 [124,138]. Gastric distension before, or during, meal ingestion also reduces subsequent food intake [139]. Filling of the antrum also plays a role in the perception of fullness and meal termination [140]. 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 [140]. 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 [141]. 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.

1.3.3.2 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 [142], mediated by gut hormones released in response to nutrients [118,119,126]. Nutrients in the intestinal lumen, particularly lipid and protein, also modulate appetite and subsequent energy intake [143,144], and changes in both motility and gut hormones play critical roles [145,146].

1.3.3.2.1 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 [130]. 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 [143], which underlie the slowing of gastric emptying [142]. Lipid also stimulates the release of GI hormones, including CCK, GLP-1 and PYY, while the release of ghrelin from the stomach is suppressed [126]. These effects occur in a load-dependent manner, and are associated with the suppression of energy intake [143,147]. In fact, the magnitude of the stimulation of pyloric pressures and plasma CCK, as indicators of the GI sensing of lipids, have been identified as independent determinants of energy intake in response to intraduodenal administration of nutrients, particularly fat and protein [145,146].

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 [148,149]. These findings also suggest that 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 [150]. Moreover, even within the group of fatty acids with ≥12 carbon atoms, fatty acids appear to have different potencies [151,152]. For example, only lauric acid (C12), but not oleic acid (C18:1), reduced subsequent energy intake when infused at a load of 0.4 kcal/min [151], while C18:1 was effective at the higher load of ~0.75 kcal/min [152], suggesting that the threshold loads required for luminal detection differ between fatty acids. The sensing of fatty acids in the GI lumen [153,154] is associated with the release of GI hormones, including CCK and GLP-1 [118,119], 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 [155,156].

1.3.3.2.2 Small intestinal sensing of protein

Dietary protein has been recognised to have potent effects to modulate GI functions and suppress appetite and food intake [157,158], and, amongst the proteins, whey protein appears to be particularly potent [159]. 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 [144,160]. 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

[161]. 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 [162-164]. There has, therefore, been an increased interest in evaluating the effects of specific amino acids on these outcomes [164,165]. 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 [166-170]. 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 [167,169,170]. 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 [166,167], 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 [171]. 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 [147].

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.

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

While, as discussed, intraluminal 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 [172], an exaggerated sensitivity to both gastric distension and intestinal lipid in patients with FD, associated with digestive symptoms [133], and reduced GI sensory perception associated with a loss of appetite with ageing [135] (**Table 1.2**).

1.3.4.1 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.

1.3.4.1.1 Sensitivity to gastric distension

Individuals with obesity have been found to have greater fasting gastric volumes [173], 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 [174-176], or consume larger amounts of water or nutrient loads during drink challenges [173]. Thus, individuals with obesity appear to be less sensitive to gastric distension and require larger intragastric volumes to experience fullness. While data relating to gastric meal emptying in obesity have been inconsistent (with studies

reporting slower or faster emptying, or no differences from lean individuals [177]), possibly in part due to differences in study design and methodological approaches [130], a comprehensive recent study of 328 participants found that gastric emptying of both solid and liquid components of a mixed meal was accelerated in people with obesity [173]. 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 [178], 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.

1.3.4.1.2 Small intestinal sensing of fat

Experimental evidence that 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 [179-182]. For example, in normal-weight individuals, consumption of a high-fat diet for 2 weeks accelerated gastric emptying of a high-fat meal [179], and attenuated the pyloric motor response to an intraduodenal lipid infusion, when compared with the low-fat diet [182]. The effect on gastric emptying was fat-specific, since gastric emptying of a high-carbohydrate meal was not accelerated after the high-fat diet [179]. There is, indeed, also evidence that people with obesity are less sensitive to the appetite-suppressant effects of dietary fat [157,158,183,184]. For example, volunteers with obesity consumed a greater amount of food from a high-fat meal than healthy controls [183], and, unlike healthy controls, participants with obesity did not reduce subsequent energy intake after a high-fat meal [157]. Only few studies have specifically evaluated the gut hormone responses to fat ingestion in people with obesity, and findings are somewhat conflicting. For example, male and female people with obesity 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

[185], although gastric emptying of fat was not specifically quantified, thus, may have been faster, resulting potentially in greater CCK stimulation. In contrast, we found no differences in plasma CCK between male with obesity and lean male adults during 3 hours after ingestion of a solid high-fat meal [157]. 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 men with obesity, compared with lean men [186], indicating that the small intestinal response to a standardised fatty acid dose is reduced in people with obesity, 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 individuals with obesity than lean individuals [158], PYY and ghrelin responses to a high-fat meal have also been found to be comparable in the two groups [157]. 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 [187,188], further supporting a contributory role for diet. For example, in volunteers with obesity, 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 [187]. 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 [188].

1.3.4.1.3 Small intestinal sensing of protein

In contrast to lipid, people with obesity 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 [76,189]. For example, energy intake 3 hours 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 [157]. Similarly, a high-protein meal reduced hunger perceptions for 3 hours post-meal substantially more than a high-fat meal, and the response to protein did not differ from those in lean participants [158]. 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 those with obesity, than in the lean, group, while no differences in plasma GLP-1 or ghrelin concentrations were observed between the two groups [158]. In contrast, in the other study [157], the high-protein meal led to sustained CCK stimulation and ghrelin suppression, in both lean and those with obesity, while the PYY response did not differ between the high-protein and high-fat meal in lean or those with obesity. Nevertheless, that these responses are, at least in part, mediated from the small intestine, is supported by a recent study [190] 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 people and those with obesity, although energy intake was non-significantly higher in those with obesity.

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 or obesity. Intraduodenal infusion of tryptophan had comparable effects on pyloroduodenal motility in lean and overweight participants [191], and intragastrically

administered tryptophan slowed gastric emptying and reduced energy intake after a mixed-nutrient drink in ~50% of lean individuals and those with obesity [192]. In contrast, individuals with obesity have been reported to be less able to detect glutamate orally [193], suggesting that individuals with obesity may be less sensitive to palatable umami taste, which may contribute to higher food intakes.

Taken together, people with obesity 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 treat, and ideally prevent, obesity.

1.3.4.2 Altered GI sensing in functional dyspepsia

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 [127,194]. This originally led to the assumption that FD is 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 [195,196], 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 [196].

1.3.4.2.1 Sensitivity to gastric distension

The frequent occurrence of FD symptoms in close temporal association with meal ingestion [197,198] 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 [199,200]. Thus, when either the proximal [199,200] or distal [201] 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.

1.3.4.2.2 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 [127,196], 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 [196,202-205], 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" [206]), sour, acid-secreting or irritant foods, including citrus fruit, spices, coffee and alcohol [203,204,207]. 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 [208-211]. For example, dyspeptic symptoms, including epigastric pain, bloating and nausea, were substantially greater in response to a high-fat soup than a bland soup [211]. Similarly, a palatable high-fat yogurt was associated with significantly greater fullness, nausea and bloating than an equivolaemic fat-free yogurt [208,209]. 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 [210]. This hypersensitivity appears to be fat-specific, since infusion of glucose did not induce symptoms [212]. Moreover, administration of the CCK-A receptor antagonist, dexloxiglumide, reduced nausea, bloating and fullness, induced by duodenal lipid infusion, in patients [213], 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 [213,214], or altered secretion of [209], CCK, or both, and the involvement of other gut hormones, remains unclear and warrants investigation.

Responses to other nutrients: Since protein, similar 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 a 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 [197]. Moreover, some patients report dyspeptic symptoms after consumption of wheat-containing foods [202,203,215], which may be related to gluten [216,217], and a gluten-free diet has been found to reduce dyspeptic symptoms [217]. 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 [197], suggesting that carbohydrates overall play a favourable role. The role of dietary fibre in FD is still uncertain [203,218]. 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 [203].

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.

1.3.4.3 Altered GI sensing in anorexia of ageing

Ageing, even in healthy people, is often associated with a loss of appetite, termed the "anorexia of ageing". Older people consume smaller meals and fewer snacks, and eat more slowly, compared with young adults [219], 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 [220]. While the causes of appetite loss with ageing are incompletely understood, ageing is associated with a gradual decline in metabolically active

tissue, specifically muscle mass [220], 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 [135], 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 [221-223], and changes in the secretion of, and/or sensitivity to, gut hormones [224]. 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 older people receive adequate nutrition.

1.3.4.3.1 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 ingestion of a mixed-nutrient yogurt-based drink, and reported greater fullness after the drink, than young people [141]. Early studies evaluated gastric emptying of meals and most [225-228], but not all [229], found that gastric emptying of both solid and liquid meal phases was slower in older than young people, although observed differences were often modest [226,228]. Increased gastric meal retention may enhance gastric distension in either proximal or distal stomach contributing to fullness, as described in healthy people [138,140]. While proximal and/or distal gastric retention has been found to be greater [141,228], antral filling has also been reported to be less [225], 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 [230], 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.

1.3.4.3.2 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 [221,231-233]. This may, at least in part, be due 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 [234], however, any effects on the digestion of protein and fat, and whether such changes may alter the GI sensing of these nutrients, remain unknown.

Response to protein: In contrast to the use of high-protein diets to achieve weight loss in obesity [76,189], in older people protein supplements are recommended to prevent weight loss and maintain functionality [220]. Given the potent GI and appetite-suppressant effects of protein in young people [157,158,235], 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 [221,222,231]. 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 [221]. 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 [221]. 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 [231]. 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 [222].

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 [236], and either a high-fat or high-carbohydrate mixed-nutrient drink (250 ml, ~250 kcal) consumed after breakfast increased intake over the following 24 hours by ~200 kcal [223], 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 [237]. 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: The available studies consistently report increased fasting plasma CCK concentrations [224,225,238,239], as well as an exaggerated rise in response to oral or intraduodenal nutrients in older people [222,225,238,240]. Furthermore, an inverse relationship

between hunger and plasma CCK has been found in young, but not older people [238], and exogenous administration of CCK-8 suppressed food intake from a meal after the infusion twice as much in older, than young people [224], suggesting that while older people remain responsive to CCK, the sensitivity to the appetite-suppressant effect of CCK may be reduced. It is not known whether the stomach remains sensitive to CCK with ageing; a reduced gallbladder contraction and emptying has been reported previously [225]. 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 [238-240], or lower [160] or greater [222,225] 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 [241]. The effects of ageing on ghrelin secretion are also unclear, with studies reporting greater [242] or lower [243] fasting acyl-ghrelin, greater fasting total ghrelin [239], no difference in fasting or postprandial ghrelin [244], or lower postprandial total ghrelin [222], in older and young people. The strongest evidence of a dysregulation relates to CCK.

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 identify improved management approaches.

Table 1.2. Changes in upper GI luminal sensing that have been described in eating-related disorders.

	GI sensory disturbances		References
Obesity	↓	Sensitivity to gastric distension	157,158,172-177,183-186
	↑	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	129,131,132,245
	↑	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	131,132,246
	↑	Gastric capacity	
	↓	CCK secretion	
	↓ ↔	Gastric emptying	
	↓	Proximal gastric accommodation	
GI disorders			
Gastroesophageal reflux disease	↑	Sensitivity to gastric distension	133,247-249
	↑	Sensitivity to small intestinal lipid	
	↓	Gastric emptying	
	↓	Gastric accommodation	
Functional dyspepsia	↑	Sensitivity to gastric distension	127,133,196,197,199-201,208-214
	↑	Sensitivity to small intestinal lipid	
	? ↑	Sensitivity to CCK	
	↓	Gastric emptying	
	↓	Gastric accommodation	
	↑	Antral distension	
Irritable bowel syndrome	↑	Sensitivity to gastric distension	133,250,251
	↑	Sensitivity to small intestinal lipid	
	↓	Gastric emptying	
	↑ ↓	Gut motility	
Critical illness	↓	Sensitivity to gastric distension	128,134,252
	↑	Sensitivity to small intestinal nutrients	
	↑	CCK, PYY secretion	
	↓	Ghrelin, motilin secretion	
	↓	Gastric emptying	
Anorexia of ageing	↓	Sensitivity to gastric distension	141,221,222,224-228,230,231,237-240,242-244
	↓	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.

1.3.5 Summary and future directions

This chapter 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. The appropriate sensing of these stimuli is altered in a number of eating-related disorders, including obesity, functional dyspepsia 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. Functional dyspepsia, 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 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? With ageing, what is the temporal relationship between the decline in GI

sensitivity to meal-related stimuli and reduced basal metabolic rate? In functional dyspepsia, studies in large cohorts are required to enable much more detailed investigations of the varied responses to different food groups. Further technological advances will be required to investigate the alterations that occur in these disorders at molecular and celluar levels in vivo and to clarify the locations of the dysregulations along the afferent and efferent pathways. 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.

1.4 The role of the gut functions in the regulation of blood glucose

Postprandial glycaemic control is determined by a number of factors, including preprandial glycaemic levels, meal composition, gastric emptying, insulin and glucagon secretion, incretin hormones, small intestinal glucose absorption, and hepatic and peripheral glucose metabolism [253]. Amongst these, gastric emptying is a highly regulated process, which normally ensures a constant delivery of nutrients, including glucose, to the proximal gut. The subsequent digestion and absorption of nutrients is associated with the release of GI hormones, which contribute to the regulation of subsequent gastric emptying and the release of glucoregulatory hormones, resulting in downregulation of hepatic glucose production and deposition of glucose in sensitive tissues. These mechanisms tightly regulate postprandial glucose excursions within a narrow range, irrespective of the load of glucose ingested [254]. The following sections examine the contributions of gastric emptying and glucoregulatory hormones, including incretin hormones, insulin and glucagon, to the regulation of postprandial blood glucose.

1.4.1 Effects of gastric emptying on blood glucose control

Gastric emptying is a major determinant of postprandial glycaemia and accounts for approximately 35% of the variance in the glycaemic response (both peak and total area under the curve) to oral glucose and/or carbohydrate-containing meals in health and type 2 diabetes [254,255]. There is evidence that relationship of glycaemia with small intestinal glucose delivery is nonlinear, based on the outcome of studies in which glucose was infused directly into the duodenum at rates spanning the normal range of gastric emptying (e.g. 1-4 kcal/min) in healthy young [256] and older people [257] and individuals with type 2 diabetes managed by diet alone [258]. Intraduodenal infusion of glucose at 1 kcal/min was associated with only a modest rise in blood glucose, and while the glycaemic responses to loads of 2, 3, and 4 kcal/min were substantially greater, there was little difference between them. The latter probably reflects the much greater plasma insulin response to the higher (e.g. 3 and 4 kcal/min) glucose loads, which is, in turn, may be attributable to the proportionally much greater GLP-1 responses as well as GIP. As well as being a determinant of glycaemia, there is evidence that gastric emptying is itself modulated by acute changes in the blood glucose concentration [259,260]. While there is a lack of consensus in relation to the magnitude of the effect of acute hyperglycaemia and the potential influence of chronic elevation of blood glucose [261], it is clear that acute marked hyperglycaemia (e.g. blood glucose level ~15 mmol/L) delays gastric emptying substantially in both health and type 2 diabetes when compared with euglycaemia (~5 mmol/L) [259]. Emptying is slowed even at physiological degrees of hyperglycaemia (~8 mmol/L) [259] and is accelerated markedly during insulin-induced hypoglycaemia [262].

1.4.2 Effects of glucoregulatory hormones on blood glucose control

The release of the incretin hormones, GLP-1 and glucose-dependent insulinotropic peptide (GIP), in the small intestine, and the pancreatic hormones, insulin and glucagon, play a key role in the regulation of blood glucose.

Incretin hormones

GLP-1 and GIP are known as "incretin" hormones. The incretin effect describes the observation that oral (or enteral) administration of glucose induces a greater insulin response than intravenous infusion which results in comparable blood glucose levels [263]. Hence, in response to oral glucose, the GI tract plays a key role in decreasing blood glucose by increasing insulin release by up to 70%, compared with intravenous glucose [264]. An increase in plasma concentrations of GLP-1 and GIP is observed ~5-15 min after food intake, and they characteristically return to baseline after ~3-4 hours. Both GLP-1 and GIP are inactivated rapidly by DPP-IV, thus, only have a short half-life of 1-4 min in plasma, resulting in only 10-15% of active GLP-1 or GIP reaching the circulation [265].

GLP-1

GLP-1 is released from endocrine L-cells, which are mainly located in the distal small intestine. GLP-1 is post-translationally cleaved from proglucagon, and two bioactive forms have been identified so far, GLP-17-36 and GLP-17-37. DPP-IV cleaves on the N-terminal side, yielding apparently inactive forms, GLP-19-36 and GLP-19-37, respectively. The GLP-1 receptor is G-protein coupled and expressed in pancreatic beta-cells, the GI tract, including the stomach, abdominal vagal afferents, the heart and a number of brain areas [266]. GLP-1 has well-established effects on GI motility and blood glucose. In response to intravenous GLP-1 at 0.4 pmol/kg/min, resulting in plasma concentrations similar to those observed after a meal (~6.2

pmol/L), followed by a higher infusion rate of 1.2 pmol/kg/min, achieving higher concentrations (~13.1 pmol/L), antral and duodenal contraction were inhibited, and while pyloric tone increased dose-dependently, isolated pyloric pressure waves were stimulated to a similar extent by both doses [267]. This suggests that the maximum effect of GLP-1 on pyloric pressures was already reached at postprandial concentrations. GLP-1, administered intravenously or subcutaneously, slows gastric emptying in healthy, normal-weight people [268], and those with obesity [269] and type 2 diabetes [270]. Moreover, intravenous GLP-1 in doses of 0.3 or 0.9 pmol/kg/min dose-dependently results in a relaxation of the fundus, which increases gastric compliance and enables the accommodation of larger volumes [271]. In normal-weight men, intravenous GLP-1 infusion at 0.75 pmol/kg/min over 180 min, resulting in peak GLP-1 plasma concentrations of ~18 pmol/L, slowed gastric emptying of an omelette meal, measured using scintigraphy [268]. In healthy subjects, intravenous infusion of GLP-1 in doses of 0.3 or 0.9 pmol/kg/min, resulting in higher plasma concentrations of ~25 and ~44 pmol/L, slowed gastric emptying of a solid/liquid meal (100 g ground beef patty with chicken liver, 150 mL of a 15% dextrose solution); the magnitude of this slowing was such that gastroparesis was induced in half of the study subjects [272].

The blood glucose-regulatory properties of GLP-1 are critical to achieve blood glucose regulation within the physiological range. Exogenous administration of GLP-1 lowers blood glucose in the presence of glucose by stimulating insulin release [273], inhibiting glucagon secretion, within a glucose-dependent manner [268], and slowing gastric emptying. The latter may outweigh the insulinotropic effects of GLP-1, since a number of studies have reported a decreased postprandial insulin response following GLP-1 treatment [268,272,274]. In healthy volunteers, intravenous infusion of GLP-1 at 0.4, 0.8 and 1.2 pmol/kg/min attenuated the increase in, or even decreased, blood glucose below fasting levels (peak blood glucose: ~6.2

mmol/L for placebo vs ~3.5 mmol/L for GLP-1 at 1.2 pmol/kg/min) and reduced insulin concentrations in response to, and slowed the gastric emptying of a drink (50 g sucrose and 8% amino acids, 327 kcal) [274]. Furthermore, glucagon was reduced in a dose-dependent manner [274]. An antagonist of GLP-1, exendin 9-39, is available for use in humans; studies using exendin 9-39 have established that GLP-1 is a physiological modulator of both gastric emptying and postprandial insulin and glucagon secretion [275]. The insulinotropic and glucagon state properties of GLP-1 are preserved in type 2 diabetes.

GIP

GIP is released by K-cells, which are mainly present in the duodenum. It is a 42-amino acid peptide that circulates in two forms in human plasma: an active form, GIP1-42, and an inactive form, GIP3-42 [276]. The plasma GIP response to oral glucose is biphasic, with the first peak occurring at ~5 min, and the second peak after ~45 min [276]. GIP exerts its effects by binding onto its receptor, which is a member of the 7-transmembrane domain, heterotrimeric G protein-coupled glucagon receptor superfamily, expressed on pancreatic islet cells, the small intestine, stomach and a number of other tissues. It contributes to glucose homeostasis by stimulating the glucose-dependant release of insulin, as well as glucagon. For example, GIP appears to have no effect on glucagon secretion during a physiological increase in blood glucose to ~8 mmol/L [277]. At fasting glycaemia and lower levels of glycaemia, GIP acts to increase glucagon with little effect on insulin release [278]. Unlike GLP-1, GIP has little or no effect on gastric emptying [279], suggesting that it decreases postprandial blood glucose predominantly by stimulation of insulin release. The insulinotropic property of GIP is markedly attenuated in type 2 diabetes for uncertain reasons [280]. GIP may also play an important role in lipid metabolism. It stimulates lipoprotein lipase activity, which promotes fatty acid incorporation into adipocytes, and thereby fat disposition and storage [278].

Insulin

Insulin, a 51-amino acid polypeptide, and C-peptide, a 31-amino acid peptide, are secreted from β-cells of the Langerhans islets of the pancreas [281], primarily in response to a rise in plasma glucose and/or amino acids concentrations. Unlike insulin, C-peptide is not extracted by the liver or other organs, and is therefore often used as a marker for insulin secretion. The major function of insulin is to ensure glucose disposal into almost all tissues, including skeletal muscle and adipose tissue [281]. Insulin binds to the insulin receptor, a tyrosine kinase receptor that activates proteins, including members of the insulin-receptor substrate family. This facilitates glucose uptake into skeletal muscle and adipose tissue by translocation of the glucose transporter, GLUT4, onto the plasma membrane. In health, insulin is released potently by carbohydrates, and also by protein and certain amino acids [282], but to a much lesser extent by lipid [147].

Glucagon

Glucagon, a 29-amino acid peptide, is secreted from α-cells of the islets of the pancreas [281]. Glucagon has a role opposing that of insulin to increase hepatic glucose production. Glucagon promotes glycogenolysis and inhibits glycogenesis primarily through the activation of phosphorylase kinase and its downstream target glycogen phosphorylase [283]. Glucagon increases during hypoglycaemia and long-term fasting, but also in response to amino acids or fatty acids and adrenergic stimulants [283]. It binds to the glucagon receptor, which is expressed in the liver and extrahepatic tissues, including brain, heart, kidney, the GI tract and adipose tissue. Glucagon, when injected intravenously in doses of 0.25-2.0 mg, slowed gastric emptying and inhibited small intestinal motility in humans [284]. However, those doses were very high, and much lower doses of 0.86 pmol/kg/min (~0.05 mg) over 4 hours did not result in slowing of gastric emptying of a liquid mixed-nutrient meal [285].

In conclusion, the rate of gastric emptying and the release of GI and pancreatic hormones play a key role in postprandial glycaemic control, so that rate of gastric emptying regulates the availability of glucose for absorption into the blood stream. Moreover, the glucose load entering the small intestine determines the secretion of the incretin hormones that modulate postprandial insulin and glucagon release.

1.5 Effects of nutrients on gut hormones, energy intake and glycaemia

The sensing of dietary nutrients, particularly lipid and protein, in the GI lumen, and the subsequent effects on GI functions, has been discussed in section 1.3.3. There is substantial evidence that these effects require the digestion of lipid and protein into fatty acids and amino acids, respectively. The role of fatty acids and amino acids in modulating gut functions, including the release of GI hormones, including CCK, PYY, ghrelin, GLP-1, and GIP, and gastric emptying, as well as energy intake and postprandial blood glucose are discussed in the subsequent section.

1.5.1 Role of fat digestion in modulating GI function, energy intake and glycaemia

Lipid ingestion is a potent trigger for the release of GI hormones, including CCK and GLP-1, slows gastric emptying and reduces subsequent energy intake and postprandial glycaemia [148]. It is now well established that these effects are dependent on digestion of fat by intestinal lipases, i.e. the lipolysis of triglycerides to monoacylglycerol and fatty acids [90]. As discussed in section 1.2.2, lipase inhibitors, e.g. tetrahydrolipstatin or orlistat, are used as a strategy to reduce body weight, with the rationale that by preventing fat digestion, fat absorption is reduced, associated with a reduced calorie intake. However, it is now appreciates that by preventing fat digestion, the effects of fat on GI function, including gastric emptying and GI hormones release, energy intake and glycaemic control are diminished. For example, gastric

emptying of both solid and fat phases of a mixed meal was accelerated, and postprandial CCK release was less, when orlistat, in the dose of which is recommended therapeutically, 120 mg, was ingested with the meal [286]. The energy intake-suppressant effect of lipid is also depending on digestion of triglycerides and the release of fatty acids, leading to stimulation of gut hormones. For example, the stimulatory effects of a triglyceride emulsion, infused intraduodenally for 120 min, on antropyloroduodenal motility, CCK, GLP-1 and PYY secretion were abolished, and energy intake at a subsequent lunch was greater, when orlistat (120 mg) was incorporated in the triglyceride solution, in healthy men [148]. Moreover, following a high-fat yoghurt "preload" (70% of energy from fat) containing 120 mg orlistat, energy intake from a standardised meal including foods and drinks, 30 min later, was greater (orlistat: 2441 kcal vs. control: 2246 kcal) and plasma CCK was less, in healthy men and women [90]. The importance of fat digestion to fatty acids in modulating GI functions and energy intake is further underlined in a study comparing the effects of free fatty acids with triglycerides. Thus, in healthy men gastric emptying was slower after intragastric administration of 40 g of the fatty acid, oleic acid (with 18 carbon atoms), compared with 40 g triglycerides (macadamia oil), with greater retention of fatty acid than triglycerides, in the proximal stomach. Stimulation of plasma CCK and PYY levels was also greater, and energy intake tended to be less (free fatty acid: 1002 kcal, triglyceride: 1304 kcal, control: 1135 kcal), after oleic acid compared with macadamia oil [287].

The effects of fat on glycaemic control also depend on fat digestion and the release of fatty acids, associated with slower gastric emptying, particularly of carbohydrate, and an increase in incretin hormones. For example, in patients with type 2 diabetes, co-ingestion of 60 mL of olive oil with 75 g of glucose resulted in greater blood glucose response with 120 mg orlistat than without. This effect could relate to faster gastric emptying leading to a greater increase in

postprandial insulin, although GIP and GLP-1 concentrations were lower [288]. In another study in patients with type 2 diabetes, gastric emptying of a semi-solid meal containing 65 g powdered potato and 20 g glucose was also faster after 120 mg orlistat, associated with an increase in blood glucose and plasma insulin with a reduction in GLP-1 concentrations [289]. These observations indicate that inhibition of fat digestion by orlistat, leading to more rapid gastric emptying, exacerbates postprandial glycaemia in type 2 diabetes after ingestion of meals containing carbohydrate.

1.5.1.1 Effects of fatty acids on GI function, energy intake and glycaemia

Once fatty acids are released in the process of fat digestion, their effects on GI functions have been shown to be acyl chain-length dependent. The classic study by Hunt et al. showed that in humans that salts of fatty acids with 12-18 carbon atoms, administered in the form of solutions or suspensions, emptied from the stomach more slowly than those with up to 10 carbon atoms [150]. The fatty acids with 12-18 carbon atoms were, on average, about 4 times more potent than fatty acids with a chain length of 10 or fewer carbon atoms. The effects of fatty acids on GI motility and hormone release have also been investigated. For example, intraduodenal infusion of lauric acid, at a load of 0.375 kcal/min, stimulated pyloric pressures and suppressed antral and duodenal pressures, motor patterns associated with slowing of gastric emptying, more potently than an isocaloric infusion of decanoic acid (C10) [290,291]. Moreover, intraduodenal lauric acid, at the same load, had more pronounced effects to stimulate CCK, GLP-1 and PYY, and suppress plasma ghrelin than C10 or C8 [290-292]. Intraduodenal infusion of C12, but not C10, also potently decreased energy intake, compared with control (C12: 417 kcal, C10: 981 kcal, control: 1099 kcal), suggesting that the differences between fatty acids with ≤10 and ≥12 carbon atoms on gastroduodenal motility and gut hormones also apply to the regulation of energy intake [291]. Possible mechanism is that fatty acids with ≥12 carbon atoms are transported

from the gut predominantly in lymphatic chylomicrons, a transport process that triggers a variety of gut signals, including satiety and the slowing of gastric emptying [291].

There also appears to be differences in the effects of fatty acids with a chain length ≥12 carbon atoms. For example, while 60-min intraduodenal infusions of both C12 and C18 suppressed antral and duodenal pressure and stimulated pyloric pressures, C12 had a greater effect to stimulate pyloric pressures. Both fatty acids increased plasma CCK and PYY, although C18 had a greater effect on PYY [151]. Furthermore, C12 infusion at a load of 0.4 kcal/min, but not C18, reduced energy intake at a buffet-meal [151]. However, when infused at a higher rate of 0.75 kcal/min, C18 decreased energy intake in healthy individuals [152]. Thus, C12 appears to be more potent in reducing energy intake than C18.

1.5.1.1.1 Effects of lauric acid on GI function, energy intake and blood glucose

The discovery of the potency of lauric acid to modulated GI functions and energy intake, has stimulated research to evaluate its effects on appetite-regulatory hormones and energy intake. For example, intraduodenal infusion of lauric acid, at loads of 0.1, 0.2 and 0.4 kcal/min for 90 min in healthy men, suppressed antral and duodenal pressures, and stimulated pyloric pressure waves, plasma CCK, PYY and GLP-1, in a load-dependent fashion [293]. Moreover, lauric acid suppressed energy intake by ~199 kcal, although the effect was apparent only at the highest load of 0.4 kcal/min, providing 36 kcal, suggesting that the stimulation of antropyloroduodenal motility and gut hormone release have to reach a "threshold" to result in suppression of energy intake [293]. This study also showed that a small dose of lauric acid has the capacity to suppress energy intake well in excess of its own energy content. The effect of lauric acid to suppress energy intake is also maintained when ingested orally, so that doses as small as 2 g or 6 g (providing ~18 or 55 kcal, respectively) decreased energy intake at lunch, 3 hours after

breakfast, by ~135 kcal or ~181 kcal, respectively, without inducing nausea or bloating, in lean men [294].

A study in patients with well-controlled type 2 diabetes investigated the effects of nutrient pellets, including other nutrients and lauric acid (47% C12 by weight), on glucoregulatory hormones and glycaemia. The pellets were ingested orally with a standardised breakfast and lunch meal, 4 hours after breakfast, and provided 2.35 g lauric acid. Ingestion of the pellets reduced blood glucose after both breakfast and lunch, and increased GLP-1, but did not appear to stimulate insulin. Although gastric emptying was not evaluated, the decrease in blood glucose was probably due, at least in part, to the effect of lauric acid to slow gastric emptying of the ingested meals [295]. Taken together, it is clear that lauric acid has the potential to modulate gut motility, stimulate GI and incretin hormones release, regulate energy intake and blood glucose.

1.5.2 Role of protein digestion in modulating GI function, energy intake and glycaemia

As with fat, the effects of protein on GI function, energy intake and glycaemia appear to be mediated by its digestion products, including peptides or amino acids. For example, intraduodenal proteolytic activity and stimulation of plasma CCK and pancreatic enzyme secretion in response to intraduodenal albumin were abolished when the latter was infused concurrently with intraduodenal camostat, a protease inhibitor, in healthy people [162]. However, there is limited information about the role of protein digestion in energy intake and blood glucose control. More research is required to investigate the effects of individual amino acids and their possible contributions to the effects of protein.

1.5.2.1 Effects of amino acids on GI function, energy intake and glycaemia

Several studies have evaluated effects of individual amino acids on energy intake and glycaemia in humans and shown that a number of amino acids, including the branched-chain amino acids, i.e. L-leucine, L-isoleucine, and the aromatic amino acids, L-phenylalanine and L-tryptophan, when given in small amounts, have potent effects to lower energy intake and blood glucose [190,296-298]. L-valine is also a branched-chain amino acid, however, it does not appear to play a role in regulating energy intake or blood glucose [299]. Therefore, the following sections will discuss current knowledge about the effects of L-leucine, L-isoleucine, L-phenylalanine and L-tryptophan, on energy intake and blood glucose control via both GI functions and post-absorptive mechanisms.

1.5.2.1.1 Effects of branched-chain amino acids on GI function, energy intake and blood glucose

Leucine, isoleucine and valine are branched-chain amino acids. The side chain in leucine and isoleucine is an isobutyl group and the branching occurs at the γ- and β-carbons, respectively. The side chain of valine is an isopropyl group branched at the β-carbon. The branched-chain amino acids are the most abundant of the essential amino acids and cannot be created from other compounds by the human body; therefore, they must be ingested. Whey protein is particularly rich in branched-chain amino acids [300].

1.5.2.1.1.1 Effect of L-leucine on GI function, energy intake and blood glucose

Based on limited number of studies, leucine has the potential to reduce energy intake and postprandial glycaemia in humans and animals [166,301]. For example, it has been shown that leucine administered intracerebroventricular decreases food intake over 24 hours in rats through hypothalamic activation of the mammalian target of rapamycin (mTOR), which is an

intracellular fuel sensor. MTOR is involved in multiple cellular processes, including protein synthesis, food intake and energy homeostasis [301]. In humans, 90-min intraduodenal infusion of leucine, at a load of 0.45 kcal/min, corresponding to ~10 g, inhibited energy intake by ~170 kcal (13%), and increased plasma CCK. In another study, 90-min intraduodenal leucine, at the same load, tended to reduce energy intake and stimulated CCK [302]. These findings suggest that the energy-suppressant effect by leucine may, at least in part, be related to effects on gut hormones, particularly CCK. In contrast, intragastric leucine, in a dose of 10 g, did not affect energy intake from a standardised meal or CCK release, 60 min after consumption of a mixed-nutrient drink [303]. It is possible that the study design, providing the mixed-nutrient drink 15 min after leucine and 60 min before the buffet lunch, may have interfered with the potential effect of leucine on energy intake by attenuating the GI effects of leucine [304]. Accordingly, additional studies are required to determine whether leucine, when administered at a shorter time-interval to a meal and in the absence of other nutrients would decrease energy intake.

A few studies have also evaluated effects of leucine on blood glucose control. For example, ingestion of leucine in a dose of 1 mmol/kg lean body mass (corresponding to 5-9 g) with 25 g glucose, reduced plasma glucose and increased plasma insulin in healthy people [305]. Leucine, administered intragastrically, in a dose of 10 g, also decreased blood glucose, increased plasma insulin and C-peptide, but did not affect glucagon, GLP-1, GIP, or gastric emptying of a mixed-nutrient drink, in healthy men [303]. These observations suggest that leucine reduces glycaemia probably by stimulating the release of insulin, rather than slowing of gastric emptying. Based on these findings in healthy people, the effects of leucine in people with type 2 diabetes where the glycaemic response is greater, was indicated. In two groups, leucine, administered intragastrically, in a dose of 10 g, did not affect plasma glucose in response to a mixed-nutrient drink [306].

1.5.2.1.1.2 Effect of L-isoleucine on GI function, energy intake and blood glucose

In contrast to leucine, the effects of isoleucine on energy intake have not been studied in humans. Evidence of a possible role for isoleucine in the regulation of energy intake is derived from a study evaluating the effect of a 60-min intraduodenal infusion of whey protein on subsequent energy intake, which reported an inverse relationship between the increase in plasma isoleucine and energy intake suppression [163].

A few studies have evaluated the effects of isoleucine on postprandial blood glucose both in animals and humans. For example, oral administration of isoleucine reduced blood glucose levels, 30 min and 60 min, after glucose administration in rats [307]. In healthy humans, ingestion of isoleucine in a dose of 1 mmol/kg lean body mass (corresponding to 5-9 g) with 25 g glucose, decreased plasma glucose, however, it did not stimulate insulin and had only a small effect to reduce glucagon [308]. Isoleucine, administered intragastrically, in a dose of 10 g, also lowered blood glucose levels and slowed gastric emptying of a mixed-nutrient drink, in healthy men [303]. These observations suggest that isoleucine may reduce glycaemia by slowing of gastric emptying and independent of insulin stimulation. Based on these findings in healthy people, evaluation of the effects of isoleucine in people with type 2 diabetes was indicated. As was the case with leucine, isoleucine, administered intragastrically, in a dose of 10 g, did not reduce postprandial glycaemia in response to a mixed-nutrient drink in type 2 diabetes [306], arguing against the potential role for a preload of isoleucine to be useful in the management of type 2 diabetes.

1.5.2.1.2 Effect of L-phenylalanine on GI function, energy intake and blood glucose

A number of studies have shown that phenylalanine reduces energy intake and lowers blood glucose. For example, oral ingestion of 10 g phenylalanine has been reported to reduce energy

intake from a standardised meal, 20 min later (phenylalanine: 1089±86 kcal, placebo: 1587±174 kcal), and increase both CCK release and the perception of fullness, in normal-weight women and men [168]. Data from our laboratory further showed that intragastric administration of phenylalanine, in a dose of 10 g but not 5 g, reduced energy intake from a standardised meal 30 min later [165]. These observations suggest that the suppression of energy intake by phenylalanine is related to effects on GI hormones, particularly CCK. In contrast, oral ingestion of 10 g phenylalanine, given in capsules, did not suppress energy intake from an *ad libitum* meal served 70 min later [309]. Intraduodenal infusion of phenylalanine (0.45 kcal/min for 90 min, corresponding to ~10 g) also had no effect on energy intake at a subsequent *ad libitum* meal (unpublished data) [310]. The lack of effect on energy intake in these studies may potentially be due to an insufficient delivery of phenylalanine to stimulate receptors in the small intestine.

There is also evidence that phenylalanine has the capacity to lower blood glucose. For example, oral ingestion of 10 g phenylalanine, given in capsules 70 min prior to a pasta meal (3.3 g fat, 14.9 g carbohydrate and 4.5 g protein), reduced postprandial glycaemia and stimulated the release of insulin, glucagon and GIP [309]. In another study, ingestion of phenylalanine in a dose of 1 mmol/kg lean body mass (corresponding to ~10 g) with 25 g glucose attenuated the glucose-induced rise in plasma glucose, stimulated insulin and glucagon in healthy people [311]. Intragastric phenylalanine at both doses of 5 and 10 g also stimulated insulin and glucagon, but only the 10 g dose reduced postprandial plasma glucose, with no effect on GLP-1 or gastric emptying [312]. These observations suggest that phenylalanine has glucose-lowering effects primarily by stimulating insulin.

1.5.2.1.3 Effect of L-tryptophan on GI function, energy intake and blood glucose

L-tryptophan is an essential aromatic large neutral amino acid (LNAA), with an α-amino group, an α-carboxylic acid group, and a side chain indole ring. Tryptophan plays a role in protein synthesis and also serves as a precursor for the metabolites, kynurenine, melatonin and niacin, which are involved in several physiological processes, including GI motility, mood, cognition, memory, sleep/wake cycle, and circadian rhythms [313]. Tryptophan also serves as a precursor for the neurotransmitter, serotonin (5-HT) in the brain [314].

Tryptophan is of particular interest in the search of candidate nutrients to suppress appetite and decrease food intake in animals. For example, daily dietary supplementation of tryptophan at 3.0 g/kg bodyweight reduced food intake in *ad libitum*-fed rats by approximately 25% and led to an overall reduction in bodyweight, associated with alterations in meal patterns, most noticeably an increase in inter-meal interval and a decrease in the number of meals [315]. Studies in humans have also reported that tryptophan, in doses of 2 g and 3 g given in capsules, suppressed energy intake at lunch 45 min later in healthy lean men [316], and 60 min later in participants with obesity [317]. 90-min intraduodenal administration of tryptophan, at a load of 0.15 kcal/min (corresponding to ~3.3 g) also markedly suppressed energy intake at a standardised lunch by ~219 kcal and increased fullness in lean men [167]. Intraduodenal administration of tryptophan also markedly stimulated plasma CCK and increased plasma tryptophan, and energy intake was inversely correlated with pyloric pressures and the plasma CCK concentration [167]. Accordingly, these studies suggest that tryptophan has the capacity to suppress energy intake potently.

Tryptophan has also been shown to lower glycaemia by stimulating pyloric motility and plasma CCK concentrations, underling the slowing of gastric emptying. For example, intragastric

administration of 3 g tryptophan slowed gastric emptying of a mixed-nutrient drink, consumed 15 min later, associated with a reduced glycaemic response in the first 30 min, in both lean men and those with obesity [192]. In addition, solutions containing tryptophan, when administered intragastrically in a dose of 1.56 g emptied from the stomach more slowly than control solutions, or when ingested orally, 3 g in 300 mL, stimulated tonic and phasic pyloric pressures [169,170]. In contrast, 90-min intraduodenal administration of tryptophan, at a load of 0.15 kcal/min (corresponding to ~3.3 g) had only a minimal effect to stimulate plasma insulin [167]. Moreover, intragastric tryptophan had no effect to stimulate insulin [169] and may potentially reduce it [192]. These observations suggest that the attenuation in postprandial blood glucose in response to tryptophan is unlikely to reflect an insulinotropic effect, while slowing of gastric emptying is likely to be fundamental to the reduction in postprandial glycaemia. However, there is no information about the effects of tryptophan in type 2 diabetes. The effects of intragastric administration of tryptophan on the glycaemic response to, as well as glucoregulatory hormones and glucose absorption, and gastric emptying of, a mixed-nutrient drink in people with type 2 diabetes have been evaluated in **Study 3 (described in Chapter 4)**.

Taken together, the above-discussed amino acids have different potencies to suppress energy intake and/or lower blood glucose. These include roles for leucine, phenylalanine and tryptophan in reducing energy intake. Moreover, leucine, isoleucine, phenylalanine and tryptophan appear to reduce blood glucose. The underlying mechanisms for suppressing energy intake include GI motility and the release of gut hormones, including CCK, and post-absorptive pathways. Moreover, effects on glucose lowering differ and are likely to relate to the release of glucoregulatory hormones by leucine and phenylalanine, and slowing of gastric emptying by isoleucine and tryptophan. Although, leucine or isoleucine may not be helpful. There is only

limited information about the potential for phenylalanine and tryptophan in the management of type 2 diabetes.

1.5.3 Relationships between plasma amino acids concentration with energy intake

As discussed, a number of amino acids have potent effects to reduce energy intake and lower postprandial blood glucose. There is substantial evidence that, in addition to GI mechanisms, post-absorptive effects of amino acids play an important role in regulating energy intake. As early as 1956, Mellinkoff et al. observed that elevated plasma amino acid concentrations were related to decreases in appetite and proposed that plasma amino acids may serve as a direct signal to inhibit eating, now often referred to as the "aminostatic theory" [318]. Subsequent studies have confirmed relationships between plasma amino acid concentrations and appetite perceptions [319-321] or energy intake [163,322]. For example, Veldhorst et al. compared the effects of high (% energy from protein/carbohydrates/fat: 25/55/20) with normal (%energy from protein/carbohydrates/fat: 10/55/35) casein-, soy-, or whey-protein breakfast custards on amino acid concentrations and appetite [320]. There was a decrease in hunger after the low-protein whey custard, compared with the other custard, which was associated with higher plasma concentrations of leucine, lysine, tryptophan and isoleucine. After ingestion of the custard with the higher protein content, plasma amino acid concentrations were overall higher, but still differed, but the reductions in hunger were comparable [320]. Relationships between plasma amino acids concentration and energy intake have also been described. For example, following ingestion of 450 mL drinks containing either 30 g or 70 g of pure whey protein, plasma concentration of 19 out of 20 amino acids (with the exception of glycine) increased load-dependently in healthy normal-weight people. Energy intake was also inversely correlated with the increase in 15 out of 20 amino acids (the exceptions being glutamic acid, histidine, cysteine, glutamine and glycine) [323].

There is also evidence to suggest that post-absorptive factors may play the dominant role. For example, in response to intraduodenal administration of tryptophan and leucine, the subsequent suppression of energy intake correlated more strongly with circulating concentrations of tryptophan and leucine, respectively, than GI functions, including pyloric pressures and plasma CCK [166,167]. Thus, an elevated concentration of circulating amino acids may be a dominant post-absorptive signal in mediating the eating-inhibitory effects of amino acids.

1.5.3.1 Plasma tryptophan concentrations and energy-suppressant activity

Post-absorptive pathways which play a role in regulating the responses to tryptophan include increased thermogenesis, glucose signaling and homeostasis via amino-acid induced gluconeogenesis. Moreover, circulating tryptophan affects energy intake potentially via central pathways in the brain [324].

The absorption of tryptophan occurs within 30 min after administration, and tryptophan enters the general circulation from the GI tract in a dose-dependent manner [315,325]. Tryptophan is commonly bound to serum albumin via its indole ring. This contrasts with the other amino acids which typically remain free [314]. Tryptophan serves as a precursor in multiple biochemical and functional pathways. The majority of tryptophan is metabolised via kynurenine pathway, while the remainder is utilised in the synthesis of serotonin, melatonin and niacin [326].

From the general circulation, tryptophan can also cross the blood-brain barriers since it has a higher affinity for the aromatic amino acid transporter than albumin. Tryptophan competes with other LNAAs, which include leucine, and also tyrosine, valine, isoleucine and phenylalanine, for the blood-brain barrier transporter [327]. The uptake and consequent use of tryptophan in the brain is influenced by the ratio of the circulating concentrations of plasma

tryptophan to the sum of LNAAs [327,328]. As discussed earlier, serotonin synthesis is one of the most important tryptophan pathways. Central serotonin is involved in a range of physiological functions, including the regulation of appetite and mood [329,330]. Since central serotonin cannot be measured non-invasively in humans, the plasma tryptophan/LNAA ratio is regarded as an indirect indicator of the synthesis of serotonin in the brain. Therefore, changing the ratio can affect the concentration of brain tryptophan available for serotonin. [331].

Dietary manipulation of the ratio can be achieved experimentally by changing the plasma tryptophan concentration or concentration of the LNAAs in a number of ways. For example, an increase in the consumption of tryptophan or α-lactalbumin, a protein rich in tryptophan increases the plasma tryptophan concentration [332]. Moreover, an increased carbohydrate intake leads to an insulin-mediated decrease in plasma branched-chain amino acid, but a lesser reduction in tryptophan levels, thus, also raising the plasma tryptophan/LNAA ratio [327].

1.5.3.1.1 The plasma tryptophan to LNAA ratio and appetite and energy intake

There is evidence that the plasma tryptophan/LNAA ratio is associated with food intake and eating behaviour. For example, the fasting plasma tryptophan/LNAA ratio was found to be inversely related to hunger scores in both healthy controls and people with type 2 diabetes [333]. Moreover, an increase in the plasma tryptophan/LNAA ratio by dietary manipulation appears to have the potential to suppress appetite and energy intake. For example, a rise in the plasma tryptophan/LNAA ratio 150 min after a high-carbohydrate meal (114 g) by ~60% was found to be inversely related to the desire to binge-eat in food-craving women. Self-reported *ad libitum* intake was also found to be lower in this population, although it did not correlate with the plasma tryptophan/LNAA ratio [334]. In contrast, a rise in the ratio 90 min after a drink containing α-lactalbumin and carbohydrate, by 16% did not affect appetite or food intake in

healthy men [335]. Similar results have been reported following consumption of a high-carbohydrate breakfast (80% carbohydrate) in which the plasma tryptophan/LNAA ratio and food intake did not change compared with a standard diet (60% carbohydrate) [336]. Information relating to the extent to which the plasma tryptophan/LNAA ratio must increase to cause a meaningful increase in brain serotonin to affect appetite is inconclusive. In humans, the outcomes of a study in people with normal pressure hydrocephalus, during which the plasma tryptophan/LNAA ratio was measured, in cerebrospinal fluid through lumbar puncture, following ingestion of a protein or carbohydrate breakfast, suggests that only an increase in the plasma tryptophan/LNAA ratio of 50% or more leads to a meaningful increase in brain serotonin [337].

1.5.3.2 Tryptophan metabolism and energy intake in obesity

A growing body of evidence indicates that obesity is associated with alterations in the metabolism of tryptophan. For example, after consumption of a high-carbohydrate snack together with 0-1000 mg tryptophan, plasma tryptophan and the tryptophan/LNAA ratio were lower in people with obesity (~10% and ~44%, respectively) than lean controls [338]. In people with obesity, the 24-hour plasma tryptophan concentration and the plasma tryptophan/LNAA ratio were also less than in healthy lean controls both before and after weight reduction following a very-low-calorie liquid diet for 6-17 months [339]. The dysregulation in tryptophan metabolism and the plasma tryptophan/LNAA ratio may also be associated with altered energy intake. For example, a previous study reported a relationship between the suppression of energy intake and the tryptophan concentration, in response to acute intragastric tryptophan at a dose of 3 g, in lean men, but not those with obesity which was related to smaller increase in plasma tryptophan (~4- vs. 6-fold increase compared to control in obese vs. lean) in this group [192]. While some of the available data may suggest a role for the plasma tryptophan/LNAA ratio in

appetite regulation, with implications for dysregulated eating in obesity, information about the relationship of acute energy intake with the plasma tryptophan/LNAA ratio, in response to acute administration of tryptophan in lean people and those with obesity, is lacking. Thus, the relationship between energy intake and the plasma tryptophan/LNAA ratio in lean people and those with obesity, and whether the plasma tryptophan/LNAA ratio differs between individuals who reduced their energy intake in response to tryptophan compared with those who did not, have been evaluated in **Study 1 (described in Chapter 2)**. Moreover, the effects of intragastric administration of tryptophan on energy intake from a buffet-meal, pre- and post-meal appetite perceptions, including hunger and fullness, plasma CCK, tryptophan and the tryptophan/LNAA ratio, and whether responses differ in lean and those with obesity, have been examined in **Study 2 (described in Chapter 3)**.

1.5.4 Effects of combination of GI stimuli on GI function, energy intake and glycaemia

There has been recent interest in evaluating the effects of combining different GI stimuli, including gastric distention, gut hormones and nutrients, on the reduction of appetite and energy intake or blood glucose. The primary rationale underlying this concept comes from where the pathways by which these GI stimuli affect energy intake or blood glucose are distinct and can interact. Thus, these stimuli have the potential, even at small loads that alone do not affect energy intake or blood glucose, to do so when combined, resulting in additive, or possibly synergistic, effects. This approach may also have the potential to reduce, or eliminate, any adverse effects [124,139,340-342]. For example, in healthy men the combination of gastric distension, achieved by gradually filling a bag with air (100 mL/min), and intraduodenal 20% lipid infusion (2 kcal/mL) increased the perception of fullness much more than either stimulus alone [124]. Moreover in normal-weight participants, intravenous infusion of a low dose of CCK (CCK-8; 112 ng/min for 23 min) combined with gastric distension induced by a water-filled

balloon (300 ml) resulted in a reduction in intake of a liquid meal, while infusion of CCK and gastric distension individually had little effect on energy intake [139]. The interaction between two potential satiety signals, including GLP-1 and CCK has also been investigated by intravenous infusion of GLP-1 (0.9 pmol/kg/min) and CCK-33 (0.2 pmol/kg/min) for 60 min before a lunch. Although infusion of GLP-1 plus CCK had no effect on calorie consumption, the combination reduced the perception of hungers in the premeal period compared to each stimulus alone in normal-weight participants [340]. Oral administration of GLP-1 (2 mg) in combination with PYY3-36 (1 mg) had an additive inhibitory effect on energy intake 15 min later at a standardised meal in healthy individuals. An increase in fullness was evident at meal onset, when oral GLP-1 and PYY3-36 were given together [342]. These findings suggest that GLP-1 and PYY act in concert to terminate meal consumption.

This concept has also been investigated in relation to blood glucose lowering. For example, in type 2 diabetes, following 100 mg sitagliptin or placebo crossed with a preload drink containing either 50 g D-xylose or 80 mg sucralose (control), followed after 40 min by a mashed potato meal, peak blood glucose and glycaemic excursion was lower after placebo+D-xylose and sitagliptin+control than placebo+control, and was least after sitagliptin+D-xylose, suggesting that acute administration of a D-xylose preload reduces postprandial glycemia and enhances the effect of a DPP-IV inhibitor [343].

The effects of combination of macronutrients on GI functions and energy intake have also been investigated. For example, following 15-min intragastric administration of lipid and a 1:1 lipid/glucose mix infusions (500 mL isocaloric 500 kcal), both infusions suppressed appetite and energy intake compared with saline, with no difference between them in healthy men [344]. Moreover, following 90-min intraduodenal infusions of 3 kcal/min lipid, 2 kcal/min lipid and

1 kcal/min maltodextrin, and 1 kcal/min lipid and 2 kcal/min maltodextrin, a reduction in lipid (thus, increasing the carbohydrate) was associated with reduced stimulation of pyloric pressures, plasma CCK and PYY, and reduced suppression of energy intake [345]. These observations suggest that intraduodenal lipid more potently modulates gut function, associated with greater suppression of energy intake, than combinations of lipid and maltodextrin. However, these effects necessitate administration of relatively large caloric loads, probably because digestion of macronutrients takes time.

1.5.4.1 Combination of fatty acids and amino acids and GI function, energy intake and glycaemia

As discussed earlier, amongst fatty acids and amino acids, lauric acid and L-tryptophan, respectively, at small loads, have potent effects on GI functions, energy intake and blood glucose [167,293]. However, larger loads may not be well tolerated and have adverse effects. 90-min intraduodenal infusion of lauric acid and tryptophan, at loads that individually did not affect energy intake, 0.3 kcal/min for lauric acid and 0.1 kcal/min for tryptophan, markedly reduced energy intake when combined. This effect occurred in the absence of nausea or bloating. Moreover, the combination was also associated with a markedly greater stimulation of CCK and suppression of ghrelin. While, both the combination and lauric acid alone stimulated GLP-1 release [346]. These observations raise questions with regards to potential effects to lower blood glucose. If the effect on CCK was associated with a corresponding effect to slow gastric emptying, given that slowing of gastric emptying is a key determinant of postprandial blood glucose lowering, then the combination will intuitively result in greater blood glucose lowering than each nutrient alone. If, however, the observed effect on GLP-1 was more reflective of the outcome on blood glucose, either via insulin or other mechanisms, then the effect on blood glucose lowering might be greater in the lauric acid conditions.

Therefore, the effects of intraduodenal infusion of lauric acid and tryptophan, alone and combined, on glucoregulatory hormones, gastric emptying and postprandial glycaemia in healthy men have been examined in **Study 4 (described in Chapter 5)**.

1.6 Hypothesis and implications

Obesity and its major co-morbidity, type 2 diabetes, represent an increasing threat to global health as well as a substantial economic burden, and novel, effective, adverse-effect free approaches to treatment and prevention strategies are urgently required. Because of the pivotal role of the GI tract in energy intake and blood glucose regulation, modulation of GI functions, specifically gut hormones and gastric emptying, represent a promising target for decreasing energy intake and lowering postprandial blood glucose. There has been increasing interest in the effects of amino acids and fatty acids, as the building blocks of dietary protein and lipid, respectively, to modulate GI functions, energy intake and blood glucose. However, information about the effects of amino acids and fatty acids, particularly tryptophan and lauric acid, on energy intake and glycaemia is limited. Moreover, studies investigating underlying GI mechanisms and post-absorptive factors in relation to energy intake and blood glucose in lean people, and those with obesity and type 2 diabetes are lacking.

The studies in this thesis, therefore, investigated the following hypotheses:

1. The plasma tryptophan/LNAA ratio is greater in individuals who reduced their energy intake in response to intragastric L-tryptophan than those who did not, in lean men and those with obesity (**Chapter 2**).
2. The effects of intragastric administration of L-tryptophan on energy intake from a buffet-meal, pre- and post-meal appetite perceptions (including hunger and fullness),

plasma CCK, tryptophan and the tryptophan/LNAA ratio are greater in lean men than those with obesity (**Chapter 3**).

3. Intragastric administration of L-tryptophan lowers postprandial glycaemia by slowing of gastric emptying and stimulating glucoregulatory hormones in people with type 2 diabetes (**Chapter 4**).
4. Intraduodenal administration of the combination of lauric acid and L-tryptophan result in greater blood glucose lowering by stimulating glucoregulatory hormones and slowing of gastric emptying, than each nutrient alone, in lean men (**Chapter 5**).

Addressing these hypotheses will enhance current knowledge on the effects of selected amino acids and fatty acids on energy intake and blood glucose, and the relationship with GI functions, such as gut hormones and gastric emptying substantially, and provide evidence as to whether these nutrients are likely to be able to be utilised for the development of novel, nutrient-based treatment strategies for obesity and/or type 2 diabetes.

CHAPTER 2: Effects of intragastric L-tryptophan on acute changes in the plasma tryptophan/large neutral amino acids ratio and relationship with subsequent energy intake in lean and obese men

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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 and aims: Circulating tryptophan/LNAA ratio, an indicator of brain serotonin levels, may be important in appetite regulation, together with GI (gastric emptying, plasma CCK) mechanisms. We have compared effects of intragastric tryptophan on the plasma tryptophan/LNAA ratio in lean men and those with obesity, and the associations of the tryptophan/LNAA ratio, gastric emptying and CCK concentrations with energy intake.

Methods: Lean male participants and those with obesity (*n*=16 each) received 3 g tryptophan or volume-matched control intragastrically, 15 min before a mixed-nutrient drink (300 mL, 400 kcal) (t=0 min) in randomised, double-blind fashion. Plasma amino acid (for calculation of the plasma tryptophan/LNAA ratio) and CCK concentrations were measured from t=-20–60 min. Gastric emptying was assessed from t=0–60 min, and *ad libitum* energy intake from a standardised buffet-style meal from t=60–90 min.

Results: The increase in the plasma tryptophan/LNAA ratio was less in those with obesity, than lean, participants ($P<0.05$), and greater in lean participants who reduced their energy intake (by >0 kcal) after tryptophan compared with those who did not (by ≤0 kcal) ($P<0.05$). Moreover, in participants who reduced their energy intake, the ratio was lower in those with obesity, than in lean ($P<0.05$). There was a trend for an inverse correlation between energy intake with the plasma tryptophan/LNAA ratio in lean participants (r=-0.4, P=0.08), but not in those with obesity. There was no significant difference in gastric emptying or CCK between participants who reduced their energy intake and those who did not.

Conclusion: In conclusion, the plasma tryptophan/LNAA ratio appears to be a determinant of the suppression of energy intake in response to tryptophan in normal-weight people, but not in those with obesity. The role of the plasma tryptophan/LNAA ratio to regulate energy intake, and potential changes in obesity, warrant evaluation in prospective studies.

2.2 INTRODUCTION

Protein, ingested orally or administered into the small intestinal lumen, reduces appetite and suppresses food intake in lean people and those with obesity and type 2 diabetes [144,157,158,235,347,348]. These effects are mediated, at least in part, by acute changes in the release of gut hormones, including CCK, GLP-1 and PYY, and GI motor functions, particularly the stimulation of pyloric pressures [144,145,157,158,190,347]; the magnitude of these effects may be less in people with obesity, than in lean people [158,190].

Amino acids may play an important role in mediating the effects of protein on GI functions and energy intake [162,163,318]. The aromatic amino acid, L-tryptophan, is of particular interest, as tryptophan, given orally, intragastrically or intraduodenally, stimulates plasma CCK and pyloric pressures, slows gastric emptying and suppresses energy intake in lean individuals [167,170,191,192]. However, while energy intake in response to intraduodenal tryptophan is inversely correlated with pyloric pressures and plasma CCK, supporting a contribution of GI functions to energy intake regulation, we reported recently that the relationship with plasma tryptophan levels was much stronger [167], in line with evidence that, beyond gut mechanisms, circulating tryptophan, possibly in the brain, is important in mediating its eating-inhibitory effect [318].

Changes in the availability of tryptophan, a precursor for the anorexigenic neurotransmitter, serotonin, within the brain may affect food intake [331,349]. The uptake and consequent use of tryptophan in the brain is influenced by the ratio of the concentrations of plasma tryptophan to the sum of other LNAAs, including tyrosine, valine, isoleucine, leucine and phenylalanine, which share competitive membrane transporters at the blood-brain barrier [327,328]. In the fasting state, the plasma tryptophan/LNAA ratio has been found to be inversely related to hunger scores in both healthy controls and non-obese people with type 2 diabetes [333]. Moreover, a rise

in the plasma tryptophan/LNAA ratio after a high-carbohydrate meal (which is proposed to increase plasma tryptophan relative to LNAA concentrations) by ~60% was reported to be inversely related to the desire to binge-eat in food-craving women, although there was no correlation with self-reported energy intake from the next meal [334]. In apparent contrast, an increase in the tryptophan/LNAA ratio by 16% following an α-lactalbumin-enriched meal, which is relatively high in tryptophan, did not affect appetite or food intake in healthy men [335]. Since a previous study suggested that a 50-100% rise in the plasma tryptophan/LNAA ratio is required to increase brain serotonin synthesis in healthy men [350], it is possible that the changes achieved in these studies were not sufficient to affect food intake. This is supported by studies in which pure tryptophan (2 - ~3.3 g) was found to potently suppress food intake [167,316], although effects on the plasma tryptophan/LNAA ratio were not evaluated.

There is also evidence that both plasma tryptophan and the tryptophan/LNAA ratio after consumption of a high-carbohydrate snack together with 0–1000 mg tryptophan, are lower in people with obesity (~10% and ~44%, respectively) than lean controls [338], although the relationship with food intake was not evaluated. While some of the available data may suggest a role for the plasma tryptophan/LNAA ratio in appetite regulation, with implications for dysregulated eating in obesity, information on the relationship, if any, between acute energy intake with the plasma tryptophan/LNAA ratio, in response to acute administration of tryptophan in lean people and those with obesity, is lacking.

We recently performed a study, in which tryptophan was administered in doses of 1.5 g and 3 g, directly into the stomach, in lean participants and those with obesity [192]. The primary aim of this study was to determine the effects of tryptophan on the blood glucose response to a mixed-nutrient drink, and the underlying GI mechanisms, particularly gastric emptying and plasma

CCK, but we also assessed ad-libitum energy intake 75 min after tryptophan administration. While tryptophan did not reduce overall energy intake significantly, there was a suppression (defined as >0 kcal) in ~50% of both lean participants and those with obesity (8/16 and 9/19, respectively). Moreover, energy intake was inversely correlated with plasma tryptophan in lean participants, but not those with obesity, suggesting that energy intake regulation by tryptophan may be abnormal in obesity.

In the light of the potential role for the plasma tryptophan/LNAA ratio in the regulation of food intake and the reported differences between lean people and those with obesity [338], we have now further analysed these data to examine the relationship between energy intake and the plasma tryptophan/LNAA ratio in lean people and those with obesity, and whether the plasma tryptophan/LNAA ratio, as well as plasma CCK or gastric emptying, differ between individuals who reduced their energy intake in response to tryptophan compared with those who did not.

2.3 PARTICIPANTS AND METHODS

2.3.1 Participants

Sixteen lean men (age: 31±3 years, BMI: 22.1±0.6 kg/m^2) and 16 men with obesity (age: 32±3 years, BMI: 33.2±0.6 kg/m^2, HbA1c: 34±6 mmol/mol (5.3±0.1%)) were recruited into the study as described [192]. Exclusion criteria included use of medications known to affect GI functions and/or appetite, significant GI diseases or surgery, type 2 diabetes, lactose intolerance, consumption of protein supplements or >2 standard drinks (20 g) alcohol on >5 days a week, vegetarianism, smoking and eating restraint (score >12 on the restrained eating component of the 3-factor eating questionnaire) [351]. In people with obesity, the latter was not used as an exclusion criterion, as many people with obesity exhibit some degree of eating restraint [190]. After inclusion, each participant was allocated to a treatment order of balanced

randomisation (www.randomization.com) by a research officer who was not involved in data analysis. The study protocol was approved by the Central Adelaide Local Health Network Human Research Ethics Committee, and performed in accordance with the Declaration of Helsinki. All participants provided written, informed consent prior to their inclusion. The study was registered as a clinical trial with the Australia and New Zealand Clinical Trials Registry (www.anzctr.org.au, trial number: 12613000899741).

2.3.2 Study outline

Study design and protocol

Information about the design of the study has been published [192]. The primary aim of the original study was to evaluate the effects of intragastric administration of two doses (1.5 g and 3 g) of tryptophan ("TRP"), or control, on the blood glucose, plasma hormone and tryptophan responses to, and gastric emptying of, a mixed-nutrient drink, consumed 15 min after TRP administration, in lean participants and those with obesity. TRP in a dose of 3 g, but not 1.5 g, suppressed energy intake (defined as >0 kcal) in 8/16 lean participants and 9/16 participants with obesity when compared with control. The doses were selected based on previous studies [12,15]. We have now assessed the effect of 3 g TRP on the plasma tryptophan/LNAA ratio, and the association with energy intake in lean participants and those with obesity. We have also assessed potential differences in the plasma tryptophan concentrations and the tryptophan/LNAA ratio, as well as plasma CCK and gastric emptying, between individuals who exhibited a reduction in energy intake in response to TRP compared with those who did not (defined as ≤0 kcal).

As described [192], after an overnight fast, participants attended our clinical laboratory at 9:30 am, when an intravenous cannula was placed into a forearm vein for blood sampling. A fasting

blood sample for subsequent measurement of plasma amino acids and CCK, and a baseline breath sample, as a reference prior to the measurement of gastric emptying, were taken. Participants were then seated in an upright position and intubated with a soft-silicone feeding tube, which was inserted through an anaesthetised nostril into the stomach. Immediately thereafter, participants received either TRP or control within 2 min. The tube was then removed and 15 min later participants consumed, within 1 min, 300 mL of a mixed-nutrient drink (Ensure plus®; Abbott, Macquarie Park, New South Wales, Australia; 400 kcal, 56 g carbohydrates, including corn syrup, maltodextrin and sucrose, 15 g protein and 12 g fat) labelled with 100 mg of ^{13}C-acetate for measurement of gastric emptying by breath test [352]. Immediately after the drink (t=0 min), and for the following hour (t=0–60 min), breath samples were collected every 5 min, and blood samples every 15 min. At t=60 min, participants were presented with a standardised, cold, buffet-style meal from which energy intake was quantified, as described previously [192]. After the buffet-meal (t=90 min), the cannula was removed and participants were free to leave the laboratory.

Study treatments

3 g L-tryptophan (PureBulk Inc., Roseburg, OR, USA), 58 mg $CaCl_2xH_2O$ and 1.65 g NaCl were dissolved in 200 mL water for irrigation. The control solution consisted of 58 mg $CaCl_2xH_2O$ and 1.85 g NaCl in 200 mL water. The solutions were approximately isotonic (mOsm, control: 295, TRP: 340) and had a pH of ~7 and a temperature of ~23 °C. Solutions were prepared by a research officer on the morning of each study and administered via a nasogastric catheter directly into the stomach. Syringes were covered so that both the investigators performing the study and analysing the samples, as well as participants, were blinded to the nature of the treatments.

2.3.3 Measurements

Blood sample analysis

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

Plasma amino acids concentrations (mmol/L) were analysed using targeted LC-MS/MS based on the method described by Harder et al. [353].

Plasma CCK-8 concentrations (pmol/L) were measured by radioimmunoassay using an adaptation of the method of Santangelo et al. [354]. The sensitivity of the assay was 1 pmol/L, and intra- and inter-assay CVs were 5.3% and 10.9%, respectively.

Gastric emptying

$^{13}CO_2$ concentrations in end-expiratory breath samples were analysed using an Automated Breath ^{13}Carbon Isotope Ratio Mass Spectrometer (ABCA IRMS, Sercon, Crewe, UK). Data were expressed as % recovery of $^{13}CO_2$ in the breath per hour [352].

Energy intake

The amount of food consumed was quantified by weighing each food item in the buffet-meal before and after it was presented to the participant. Energy intake (kcal) was calculated using commercially available software (Foodworks 8.0, Xyris Software, Highgate Hill, Queensland, Australia) [355].

2.3.4 Statistical analysis

Power calculations were performed as part of the design of the original study [192], indicating that *n*=11 participants would allow detection of a 1.0 mmol/L reduction in postprandial blood glucose, and *n*=16 participants a 170-kcal difference in energy intake, between TRP and control treatments, both at α=0.05, with a power of 80%. Moreover, we used the primary tryptophan data since it has been reported in the previous study to calculate power calculation, and it indicated that the study with *n*=16 participants was powered to detect a difference of 8342 areas under the curve (AUC) units in plasma tryptophan, with an effect size of 0.75, between TRP and control treatments, at α=0.05, with a power of 80%.

At each time point, plasma tryptophan/LNAA ratios were calculated by dividing plasma tryptophan concentration by the sum of the concentrations of other LNAAs (i.e. valine, isoleucine, leucine, phenylalanine and tyrosine). AUCs for plasma tryptophan/LNAA ratios, as well as plasma tryptophan and CCK concentrations, were calculated, using the trapezoidal rule, from t=-20–60 min. AUCs of gastric emptying profiles were calculated from t=0–60 min. To take into account any potential effect of blood volume due to differences between lean people and those with obesity (particularly on circulating amino acids, we corrected AUCs for plasma tryptophan/LNAA ratios and tryptophan for blood volume using the equation by Nadler in both lean group and those with obesity [356].

Statistical analysis was performed using SPSS software (version 25, IBM, Chicago, Illinois). General linear model mixed-model ANOVA was used to analyse effects of treatment (control, TRP) and group (lean, obese) on the plasma tryptophan/LNAA ratio, as well as differences in the plasma tryptophan/LNAA ratio, plasma tryptophan (both total AUC and corrected AUC for blood volume by Nadler equation) and CCK and gastric emptying, between participants

who had a reduction in energy intake after consumption of TRP (reduction defined as >0 kcal) and those who did not (defined as ≤0 kcal). Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed when significant ANOVA effects were found. Differences were considered statistically significant at P≤0.05. All data are reported as means±SEM.

2.4 RESULTS

As reported [192], all participants completed the study and tolerated the study conditions well. A reduction in energy intake after TRP was observed in 8/16 lean (change from control: -311±29 kcal) and 9/16 obese (change from control: -180±52 kcal) participants, with no significant difference between lean and obese participants (**Figure 2.1.A**). There were no differences in baseline fasting plasma tryptophan/LNAA ratios or tryptophan concentrations between study days in lean and obese groups.

2.4.1 Plasma tryptophan/LNAAs ratio

Comparison between obese and lean participants: The plasma tryptophan/LNAA increased rapidly within the first 15 min after TRP administration, and then declined gradually, in both lean and obese participants **(Figure 2.1.B)**. There was a significant treatment-by-group interaction for the plasma tryptophan/LNAA ratio (P<0.05). TRP increased the plasma tryptophan/LNAA ratio in both lean and obese participants compared with control (P<0.001), with no differences between obese and lean in response to control. Moreover, the plasma tryptophan/LNAA ratio was lower in obese, than in lean, participants (P<0.05) (AUC (min); lean, control 9±3, TRP: 52±3; obese, control: 8±3, TRP: 35±3). The observed differences remained even after correcting values for the blood volume between obese and lean participants

(P<0.01) (AUC (min/L blood volume); lean, control: 2±0, TRP: 11±0; obese, control: 1±0, TRP: 5±0).

Comparison between participants with and without a reduction in energy intake with TRP: The plasma tryptophan/LNAA ratio in response to TRP was greater in lean participants who reduced their energy intake compared with those who did not (P<0.05) (**Figure 2.1.C**). Moreover, in participants who reduced their energy intake in response to TRP, the plasma tryptophan/LNAA ratio was lower in obese, than in lean participants (P<0.05). However, there was no difference in obese participants who reduced their energy intake in response to TRP compared with those who did not, or between obese and lean participants who did not reduce their energy intake (AUC (min); lean, with energy intake suppression: 61±5, without energy intake suppression: 43±5; obese, with energy intake suppression: 32±5, without energy intake suppression: 38±5). The observed differences remained even after correcting values for the blood volume between obese and lean participants (P<0.01) (AUC (min/L blood volume); lean, with energy intake suppression: 11±1, without energy intake suppression: 7±1; obese, with energy intake suppression: 4±1, without energy intake suppression: 5±1).

Relationships between energy intake with plasma tryptophan/LNAA ratio: There was no significant overall correlation between energy intake with the plasma tryptophan/LNAA ratio. However, there was a trend for an inverse correlation between energy intake with the plasma tryptophan/LNAA ratio in lean (r=-0.4, P=0.08), but not obese (r=0.3, P=0.3), participants (**Figure 2.1.D**).

2.4.2 Plasma tryptophan concentrations

Comparison between obese and lean participants: As reported [192], TRP increased plasma tryptophan concentrations, as expressed by the tryptophan AUC, in both lean and obese participants compared with control (P<0.001), and tryptophan AUC tended to be lower in obese, compared with lean, participants (P=0.06) (AUC (μmol/L*min); lean, control: 5437±1662, TRP: 30383±1662; obese, control: 5648±1662, TRP: 23961±1662). The observed differences altered to significant after correcting values for the blood volume between obese and lean participants (P<0.01) (AUC (μmol/L*min/L blood volume); lean, control: 1099±352, TRP: 6214±352; obese, control: 904±352, TRP: 3856±352).

Comparison between participants with and without reduction in energy intake with TRP: Tryptophan AUC in response to TRP was greater in lean participants who reduced their energy intake compared with those who did not (P<0.05). In addition, in participants who reduced their energy intake in response to TRP, tryptophan AUC was lower in obese, compared with lean, participants (P<0.05). There was no difference in tryptophan AUC between obese participants who reduced their energy intake and those who did not, or between obese and lean participants who did not reduce their energy intake (AUC (μmol/L*min); lean, with energy intake suppression: 35231±3072, without energy intake suppression: 25534±3072; obese, with energy intake suppression: 21328±2896, without energy intake suppression: 27348±3284) (**Figure 2.2.A**). The observed differences remained even after correcting values for the blood volume between obese and lean participants (P<0.01) (AUC (μmol/L*min/L blood volume); lean, with energy intake suppression: 6301±603, without energy intake suppression: 3929±603; obese, with energy intake suppression: 2515±569, without energy intake suppression: 3511±645).

2.4.3 Gastric emptying

Comparison between obese and lean participants: As reported, TRP slowed gastric emptying in lean and obese participants ($P<0.05$), with no differences between the two groups (AUC (%recovery of $^{13}CO_2$/hour*min); lean, control: 960±70, TRP: 768±70, obese, control: 969±70, TRP: 780±70).

Comparison between participants with and without a reduction in energy intake with TRP: There were no differences in gastric emptying between individuals who reduced their energy intake after TRP compared with those who did not, although the mean emptying profile appeared to be somewhat lower in lean participants who reduced their energy intake (AUC (%recovery of $^{13}CO_2$/hour*min); lean, with energy intake suppression: 682±112, without energy intake suppression: 854±112, obese, with energy intake suppression: 770±107, without energy intake suppression: 792±120) (**Figure 2.2.B**).

2.2.4 Plasma CCK concentrations

Comparison between obese and lean participants: As reported [192], there were no differences in CCK between TRP and control, or between lean and obese participants (AUC (pmol/L*min); lean, control: 287±29, TRP: 290±29, obese, control: 296±29, TRP: 279±29).

Comparison between participants with and without a reduction in energy intake with TRP: CCK AUC in response to TRP did not differ between participants who reduced their energy intake and those who did not in either lean or obese groups, or between obese and lean participants who did and did not reduce their energy intake (AUC (pmol/L*min); lean, with energy intake suppression: 299±45, without energy intake suppression: 281±45; obese, with

energy intake suppression: 306±42, without energy intake suppression: 244±48) (**Figure 2.2.C**).

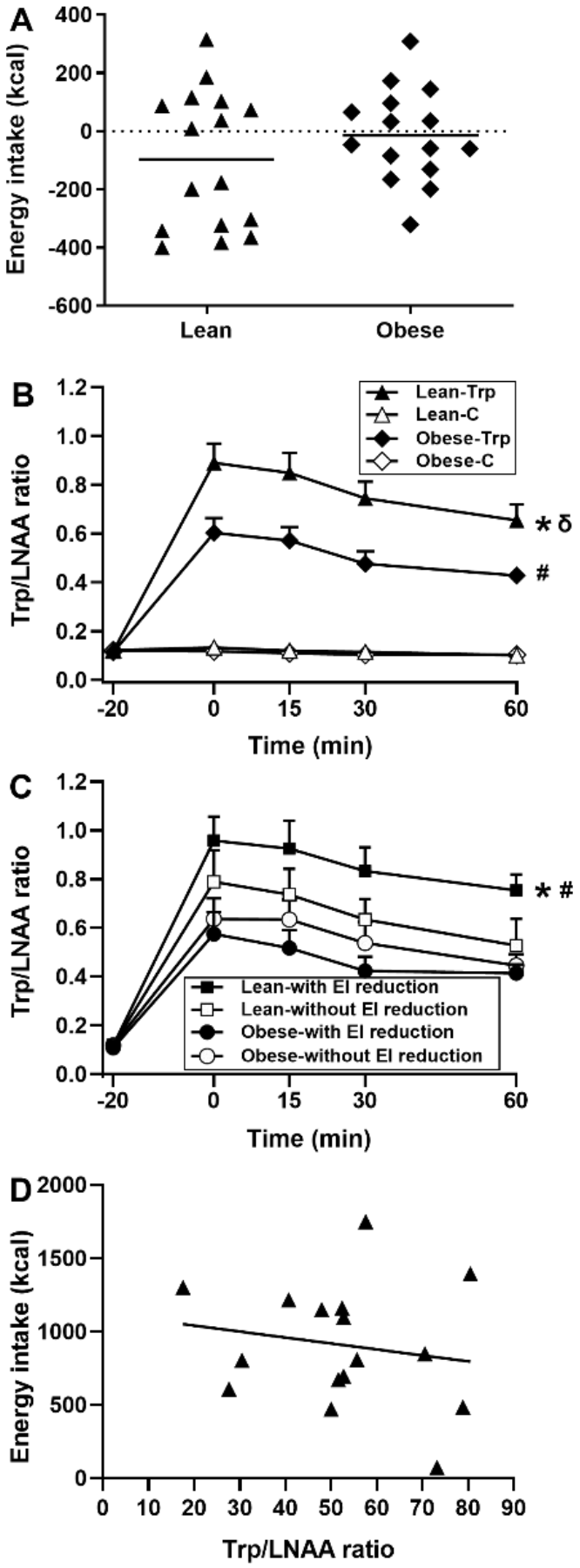
A
Energy intake (kcal)
400
200
0
-200
-400
-600
Lean
Obese
B
Trp/LNAA ratio
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Lean-Trp
Lean-C
Obese-Trp
Obese-C
* δ
#
-20
0
15
30
60
Time (min)
C
Trp/LNAA ratio
* #
Lean-with EI reduction
Lean-without EI reduction
Obese-with EI reduction
Obese-without EI reduction
Time (min)
D
Energy intake (kcal)
2000
1500
1000
500
0
0 10 20 30 40 50 60 70 80 90
Trp/LNAA ratio

Figure 2.1
Distributions of energy intake in lean and obese participants (**A**), plasma tryptophan/LNAA ratios in lean and obese participants (**B**) and in individuals who reduced their energy intake compared with those who did not (**C**), and the relationship between energy intake with the plasma tryptophan/LNAA ratio in lean participants (**D**), in response to intragastric administration of 3 g tryptophan. (**A**) Tryptophan reduced energy intake in 8/16 lean (change from control: -311±29 kcal), and 9/16 obese (change from control: -180±52 kcal) participants. (**B**) Tryptophan increased the plasma tryptophan/LNAA ratio in both lean (*P<0.001) and obese participants ($^{\#}$P<0.001) compared with control, and the plasma tryptophan/LNAA ratio was lower in obese, than in lean, participants ($^{\delta}$P<0.05). (**C**) The plasma tryptophan/LNAA ratio was greater in lean participants who reduced their energy intake compared with those who did not (*P<0.05). Moreover, in participants who reduced their energy intake, the plasma tryptophan/LNAA ratio was lower in obese, than in lean, participants ($^{\#}$P<0.05). (**D**) There was a trend for an inverse correlation between energy intake with the plasma tryptophan/LNAA ratio in lean participants (r=-0.4, P=0.08). Data are means±SEM. Data were analysed using general linear model mixed-model ANOVA.

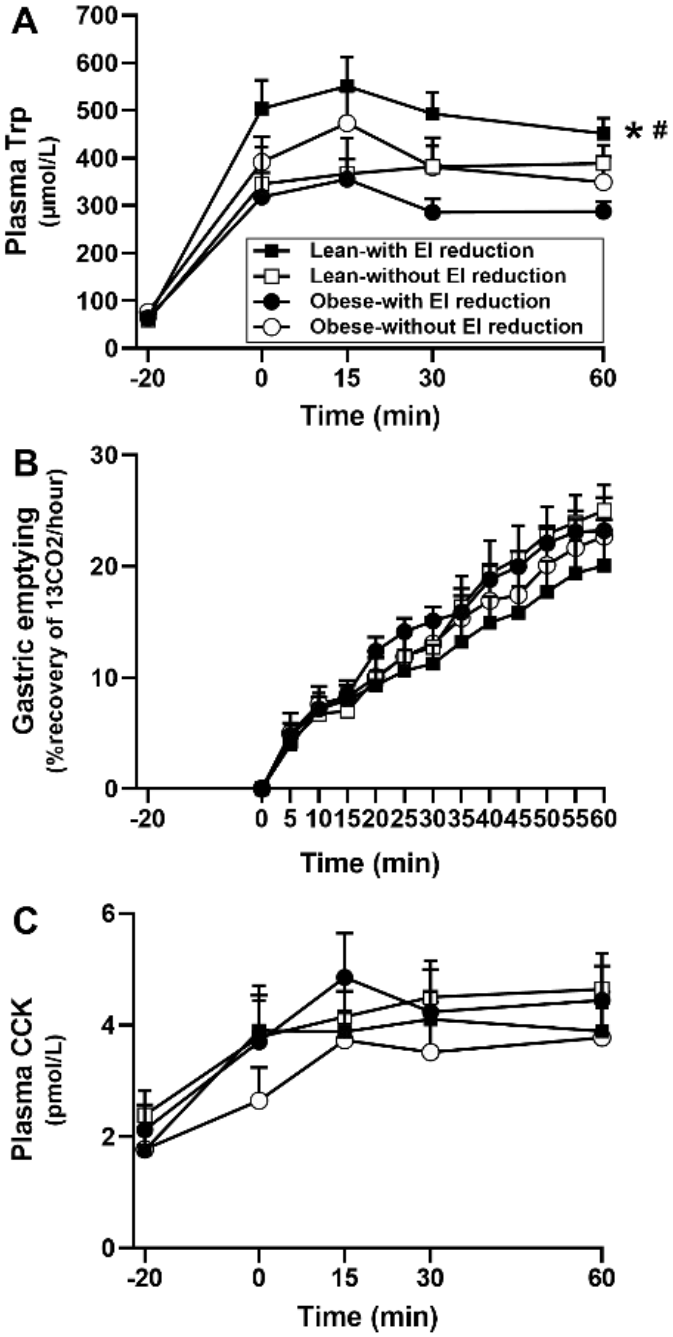

Figure 2.2
Plasma tryptophan concentrations (**A**), gastric emptying (**B**) and plasma CCK concentrations (**C**) following intragastric administration of 3 g tryptophan in participants who reduced their energy intake compared with those who did not. (**A**) Plasma tryptophan was greater in lean participants who reduced their energy intake compared with those who did not (*P<0.05). In participants who reduced their energy intake, plasma tryptophan was lower in obese, compared with lean, participants ($^{\#}$P<0.05). There were no significant differences in gastric emptying (**B**) or plasma CCK (**C**) in response to tryptophan, between participants who reduced their energy intake and those who did not in either lean or obese participants. Data are means±SEM. Data were analysed using general linear model mixed-model ANOVA.

2.5 DISCUSSION

We have shown that the increase in the plasma tryptophan/LNAA ratio after IG administration of 3 g TRP is much greater (by ~65%) in lean participants than in those with obesity, and greater (by ~37%) in those lean participants who reduced their energy intake in response to TRP. The plasma tryptophan/LNAA ratio also tended to be correlated with energy intake in lean, but not in those with obesity, participants. In contrast, gastric emptying and plasma CCK did not differ between the two groups. These findings, accordingly, are consistent with the hypothesis that obesity is associated with a diminished plasma tryptophan/LNAA response and that the latter is potentially a determinant of the suppression of energy intake by TRP in the lean.

The plasma tryptophan/LNAA ratio is regarded as an indicator of brain serotonin synthesis, and there is a close link between serotonin and food intake [329,330]. Both pharmacological and dietary manipulations influence serotonin synthesis and availability [329], and serotoninergic agents, such as fenfluramine and the selective serotonin reuptake inhibitors, fluoxetine and sertraline, reduce food intake in healthy humans and those with obesity [357]. Changes in the macronutrient composition of the diet also affect the plasma tryptophan/LNAA ratio [327]. For example, in humans, carbohydrate-rich meals (containing ~70 g [358], and ~115 g [334] carbohydrate) lead to an insulin-mediated decrease in plasma branched-chain amino acids, but a lesser reduction in tryptophan levels, thus, raising the plasma tryptophan/LNAA ratio. On the other hand, consumption of protein-rich meals (containing ~50 g [358], and ~100 g [334] protein) or a high-protein diet (150 g egg protein per day) for 5 days [359], reduces the postprandial tryptophan/LNAA ratio, because most proteins are rich in LNAAs, which are not catabolised by the liver, while tryptophan is utilised for hepatic protein synthesis. In contrast, α-lactalbumin-enriched whey protein, ingested either as a drink (12 g) [335] or as part of a standard

breakfast (20 g), increases the plasma tryptophan/LNAA ratio, compared with a drink containing casein [332]. Moreover, addition of 300 mg tryptophan to a carbohydrate-rich meal (containing ~60 g carbohydrate) increased the plasma tryptophan/LNAA ratio beyond the effect of that of the carbohydrate load [350]. However, only few studies evaluated energy intake in response to the interventions [334,335]. In response to a 60% increase in the tryptophan/LNAA ratio after a high-carbohydrate meal, self-reported intake was found to be lower, but did not correlate with the tryptophan/LNAA ratio, in food-craving women [334], while a 16% increase in the tryptophan/LNAA ratio after an α-lactalbumin-containing meal did not affect energy intake in lean men [335], and other studies achieving greater rises in the tryptophan/LNAA ratio (up to 75%) did not evaluate energy intake [350,359]. Thus, it is possible that the magnitude of diet-induced changes in the tryptophan/LNAA ratio, and associated brain serotonin synthesis, may have been insufficient to suppress appetite and food intake.

In our study, 3 g TRP, a dose well tolerated and effective in modulating GI functions and energy intake [167,192], 15 min before a mixed-nutrient, led to a substantial 9-fold elevation in the plasma tryptophan/LNAA ratio in lean participants, with fasting ratios comparable with those reported in other studies [332,338,350,359]. The plasma tryptophan/LNAA ratio was unchanged on the control day, thus, the nutrient drink, which contained casein and soy protein isolates, as protein sources, and the carbohydrates, maltodextrin and sucrose, per se had no effect. However, the purpose of the drink in our main study was not to manipulate the plasma tryptophan/LNAA ratio, but to provide a carbohydrate source in order to evaluate postprandial blood glucose in response to TRP consumption. We observed a trend for an inverse correlation between energy intake and the plasma tryptophan/LNAA ratio in lean participants, indicating a role for the plasma tryptophan/LNAA ratio in energy intake regulation. Our data, therefore,

suggest that intragastric tryptophan suppresses acute energy intake, at least in part, via post-absorptive pathways in the brain.

Although both the plasma tryptophan and tryptophan/LNAA ratio were also increased in those with obesity, the responses to TRP were substantially lower than in lean participants, in line with previous findings [338]. While it could be argued that lower tryptophan/LNAA ratios and circulating concentrations of tryptophan may be due to larger body weights and/or larger blood volumes in those with obesity, we took the effect of blood volume into account and adjusted our data for it, and in line with a previous study, the differences remained even after correcting the tryptophan dose for body weight [338], or the plasma tryptophan/LNAA ratio and tryptophan for blood volume in the current study. Lower tryptophan concentrations and tryptophan/LNAA ratios could be due to metabolism-related dysregulations, affecting the plasma appearance rate of tryptophan differentially in obesity, including insulin resistance, inflammatory pathways and pathways of tryptophan metabolites [360,361], as well as different rates of uptake and degradation of tryptophan in the liver [362]. Thus, individuals with obesity may require larger doses of tryptophan in order to reach plasma levels comparable to those in lean controls [338].

The plasma tryptophan/LNAA ratio was highest in lean participants who reduced their energy intake in response to tryptophan, compared with those who did not. Interestingly, there were no significant differences amongst lean participants who did not reduce their energy intake and the group with obesity, although the mean profile was somewhat higher in the lean group. The data may, therefore, suggest that either progressive lowering of the plasma tryptophan/LNAA ratio may occur, e.g. in response to overeating, or, alternatively, a lower ratio may put lean people at risk of becoming obese [334,358,363]. These questions warrant evaluation in prospective studies.

Specific GI motor functions, including gastric emptying and gut hormones, play important roles in the acute regulation of appetite and energy intake [140,195], and we have reported that the magnitude of the stimulation of pyloric pressures (a key determinant of the slowing of gastric emptying) and plasma CCK are independent determinants of energy intake in response to intraduodenal nutrients, particularly lipid [146]. While intragastric tryptophan slows gastric emptying [191,192] and intraduodenal tryptophan stimulates plasma CCK and pyloric pressures [167,169], there was no difference in gastric emptying or CCK between participants who reduced their energy intake compared with those who did not. While our data, therefore, suggest that these GI factors may not play a role in the regulation of energy intake by tryptophan, it is worth noting that mean gastric emptying curves were lowest in lean participants who reduced their energy intake, suggesting somewhat slower gastric emptying, which may then also have affected the release of CCK by tryptophan. It is possible that the lack of statistical significance is due to a type 2 error, due to the small sample size, thus, the role of gastric emptying warrants further evaluation in prospective studies.

Some limitations of our study should be noted. We only included young men (primarily to avoid effects of the menstrual cycle on energy intake [364]), thus, extrapolation of our results to other populations, e.g. women, older individuals or people with type 2 diabetes, should be done with caution. The current analysis was an exploratory secondary analysis and the power calculation based on tryptophan data indicated that the study was powered to detect differences in tryptophan values between treatments, however, the observation of trends, particularly for correlations, may have been due to insufficient statistical power, Moreover, our sample sizes in the sub-groups were small. Hence, this should be addressed in future, prospective studies. It is possible that our study design, providing the mixed-nutrient drink 15 min after tryptophan

and 60 min before the buffet lunch, may have interfered with the effects of tryptophan on energy intake by attenuating the GI effects of tryptophan.

In conclusion, intragastric tryptophan increases the plasma tryptophan/LNAA ratio much more in lean participants than in those with obesity, and the suppression of energy intake in response to tryptophan was related to the plasma tryptophan/LNAA ratio in the lean. Thus, the plasma tryptophan/LNAA ratio appears to play a role in the regulation of acute energy intake in response to tryptophan in lean individuals, however, further studies including larger sample sizes are required to evaluate the role of the plasma tryptophan/LNAA ratio in the regulation of energy intake, as well as potential mechanisms underlying the changes in obesity.

CHAPTER 3: Suppression of energy intake by intragastric L-tryptophan in lean and obese men: relations with appetite perceptions and circulating cholecystokinin and tryptophan

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Name of Principal Author (Candidate)	Maryam Hajishafiee		
Contribution to the Paper	Performed experiment and data analysis, interpreted results, 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. I am the primary author of this paper.		
Signature		Date	17 / 8 / 2021

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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3.1 ABSTRACT

Background and aims: L-tryptophan reduces energy intake in healthy men. The underlying mechanisms, including appetite, plasma cholecystokinin (CCK), tryptophan (TRP), and the ratio of TRP to LNAAs ratio, and whether responses differ in lean individuals and those with obesity, are uncertain. We evaluated the effects of intragastric TRP on energy intake (primary outcome) and their potential mechanisms, pre- and post-meal, in lean men and those with obesity.

Methods: Twelve lean men (mean±SD age: 30±3 y; BMI (in kg/m2): 23±1) and 13 men with obesity (mean±SD age: 31±3 y; BMI: 33±1) received, on 3 separate occasions, in double-blind, randomised order, 3 g ("TRP-3") or 1.5 g ("TRP-1.5") TRP, or control ("C"), intragastrically, 30 min before a buffet-meal. Energy intake from the buffet-meal, hunger, fullness, and plasma CCK and amino acid concentrations were measured in response to TRP alone and for 2 hours post-meal. Data were analysed using maximum likelihood mixed-effects models, with treatment, group, and treatment-by-group interaction as fixed effects.

Results: TRP alone increased plasma CCK, TRP, and the TRP/LNAAs ratio (all P<0.001), with no difference between groups. TRP suppressed energy intake (P<0.001), with no difference between groups (lean, C: 1085±102 kcal, TRP-1.5: 1009±92 kcal, TRP-3: 868±104 kcal; obese, C: 1249±98 kcal, TRP-1.5: 1217±90 kcal, TRP-3: 1012±100 kcal). Post-meal, fullness was greater after TRP-3 than after C and TRP-1.5 (all P<0.05), and in men with obesity than in lean men (P<0.05). Plasma TRP and the TRP/LNAAs ratio were greater after TRP-3 and TRP-1.5 than after C (all P<0.001), and tended to be less in men with obesity than in the lean (P=0.07) (TRP/LNAAs ratio: lean, C: 1.5±0.2, TRP-1.5: 6.9±0.7, TRP-3: 10.7±1.4; obese, C: 1.4±0.1, TRP-1.5: 4.6±0.7, TRP-3: 7.8±1.3). There were inverse correlations of energy intake with plasma TRP and the TRP/LNAAs ratio in both groups (lean, both *r*=-0.50, P<0.01; obese, both *r*=-0.40, P<0.05).

Conclusion: Intragastric TRP has potent energy intake–suppressant effects, in both lean men and those with obesity, apparently related to the TRP/LNAAs ratio.

3.2 INTRODUCTION

High-protein diets have potent effects to reduce body weight [72], at least in part because of their capacity to suppress appetite, in health and obesity [157,235,365]. Amino acids, the digestion products of protein, are key to the satiating effects of proteins, and L-tryptophan appears to be particularly potent. When administered orally, intragastrically or intraduodenally, in doses of 2-3 g, tryptophan (TRP) reduces energy intake, increases premeal fullness and decreases premeal hunger in lean people and those with obesity [167,192,316,317]. These effects are associated with the stimulation of appetite-regulatory hormones, including cholecystokinin (CCK), and pyloric pressures [167,169,191,192], which underlie the slowing of gastric emptying [142]. Beyond gut-related functions, a relation between energy intake with circulating TRP has been reported in response to intraduodenal TRP [167], suggesting that post-absorptive mechanisms, possibly via serotonin in the brain, may contribute to appetite suppression [171,318,326,331,349].

The uptake of TRP into the brain, and its conversion to serotonin, is influenced by the concentrations of LNAAs, including tyrosine, valine, isoleucine, leucine and phenylalanine, which share competitive membrane transporters at the blood-brain barrier [327]. Thus, while the effects of TRP on central serotonin cannot be measured directly in humans, the ratio of plasma TRP to the sum of LNAAs (TRP/LNAAs ratio) represents an indirect measure of brain serotonin [327,328]. There is evidence of a relation between eating behavior and the TRP/LNAAs ratio [333-335]. For example, in the fasting state, hunger has been reported to be inversely related to the TRP/LNAAs ratio in both healthy lean people and those with type 2 diabetes [333]. Post-meal desire to binge-eat was also inversely related to a rise (by ~60%) in the TRP/LNAAs ratio

following a high-carbohydrate (114 g) meal, which stimulates insulin secretion and leads to a decrease in plasma levels of amino acids competing with TRP, thus, raising the TRP/LNAAs ratio [334]. In contrast, energy intake and post-meal appetite ratings were not affected by a modest (~16%) increase in the TRP/LNAAs ratio following an alpha-lactalbumin-enriched meal, which is relatively high in TRP, in healthy men [335].

There is evidence that TRP metabolism may be modified in obesity. For example, the rise in both plasma TRP and the TRP/LNAAs ratio after a high-carbohydrate meal has been reported to be less in individuals with obesity than the lean [338]. Moreover, in a secondary analysis of data from a previous study investigating the effects of acute administration of TRP in doses of 1.5 g and 3 g, we have recently reported a relation between the suppression of energy intake and the TRP/LNAAs ratio, in response to TRP, in lean men, but not those with obesity [366]. However, the sample size was small and the study design suboptimal (i.e. a mixed-nutrient drink was provided before the meal from which energy intake was assessed, which could interfere with the effects of TRP on intake and the TRP/LNAAs ratio), thus, the relations between energy intake with plasma tryptophan concentrations and the TRP/LNAA ratio in response to tryptophan, and potential differences between lean individuals and those with obesity, remain unclear.

The aims of this study were, therefore, to evaluate, in a prospective study, the effects of intragastric administration of tryptophan (to avoid its unpleasant taste) on energy intake from a buffet-meal, pre- and post-meal appetite perceptions, including hunger and fullness, plasma CCK, tryptophan and the TRP/LNAA ratio in both lean men and those with obesity. We included men to avoid any effects of the menstrual cycle on energy intake [364].

3.3 PARTICIPANTS AND METHODS

3.3.1 Participants

Twelve healthy, lean men (mean±SD age: 30±3 y; range: 18–48 y; mean±SD BMI (in kg/m2): 23±1; range: 21–25) and 13 men with obesity (mean±SD age: 31±3 y; range: 19–49 y; mean±SD BMI: 33±1; range: 30–37) were recruited through advertisements on online sites (University of Adelaide and Gumtree) and by flyers placed at the University of Adelaide, University of South Australia, and Royal Adelaide Hospital (**Figure 3.1**). All participants had been weight-stable (i.e., <5% fluctuation) and not practicing any dietary restrictions in the 3 months preceding the study. Participants were excluded if they had significant gastrointestinal disorders or symptoms, cardiovascular or respiratory diseases or surgery, took medications known to affect gastrointestinal function and/or appetite, had low serum ferritin (<30 μg/L) or iron (<8 μmol/L) concentrations (a requirement by the Human Research Ethics Committee), lactose intolerance, consumed protein supplements, or >2 standard drinks (20 g) of alcohol on >5 days a week, or were vegetarians, smokers, high performance athletes, or restrained eaters (score > 12 on the restrained eating component of the 3-factor eating questionnaire) [351]. Diabetes was excluded by measurement of glycated haemoglobin, which was <6% in all cases. After inclusion, each participant was allocated to a treatment order of balanced randomisation (www.randomization.com) by a research officer who was not involved in data analysis. The Human Research Ethics Committee of the Central Adelaide Local Health Network approved the study protocol, and the study was performed in accordance with the Declaration of Helsinki and the National Health and Medical Research Council of Australia Statement on Ethical Conduct in Human Research. All participants provided written informed consent before inclusion.

3.3.2 Study outline

Study design

In both groups, we evaluated the effects of intragastric administration of aqueous solutions containing 1) 3 g ("TRP-3") or 2) 1.5 g ("TRP-1.5") TRP, or 3) control (0.9% saline) ("C"), on energy intake (primary outcome), appetite perceptions, plasma CCK, TRP, and the TRP/LNAAs ratio (secondary outcomes).

Study treatments

TRP solutions were prepared by dissolving 1.5 g or 3 g TRP (PureBulk Inc.), 58 mg CaCl2·H2O, and 1.75 g or 1.65 g NaCl in 200 mL water for irrigation. C solutions consisted of 58 mg CaCl2·H2O and 1.85 g NaCl in 200 mL water. The solutions were approximately iso-osmolar (C: 296 mOsm/kg; 1.5 g: 318 mOsm/kg; 3 g: 335 mOsm/kg) and had a pH of ∼7 and a temperature of ∼23◦C. They were prepared by a research officer, who was not involved in the performance of the studies or data analysis, on the morning of each study and administered via a nasogastric catheter directly into the stomach. Syringes were covered, so that both the study participant and the investigators performing the study were blinded to the nature of the solution. The doses of TRP (1.5 g and 3 g) were chosen based on our previous work [192], in which particularly the 3 g dose slowed gastric emptying and lowered postprandial blood glucose in both lean participants and those with obesity.

Study protocol

Each participant was studied in a randomised, double-blind, crossover fashion on 3 occasions, each separated by 3–7 d. Participants were provided with a standardised meal (Beef lasagne, McCain Food; energy content: 603 kcal), to be consumed between 18:30 and 19:00 on the night before each study. They were asked to maintain their normal eating habits between study days

and instructed to refrain from strenuous exercise and alcohol for 24 hours before each study. They were also asked to abstain from any other food and drink, except water (which was allowed until 07:00), during and after the evening meal until they attended the Clinical Research Facility at the University of Adelaide at 08:30 the next morning. The participant was provided with a standardised light breakfast (1 slice (30 g) wholemeal bread, 11 g peanut butter, and a cup (200 mL) of black tea; ~140 kcal in total). At 11:20, an intravenous cannula was placed into a forearm vein, the arm was kept warm with a heat pad for regular sampling of "arterialised" blood, and a baseline blood sample for measurement of plasma CCK and amino acid concentrations was taken. The participant also completed a visual analog scale (VAS) questionnaire to assess hunger, fullness, nausea, and drowsiness. They were then intubated with a soft-silicon feeding tube (outer diameter: 4 mm; Dentsleeve), which was inserted through an anesthetised nostril into the stomach. Immediately thereafter (t=-31 min, ~11:30), the participant received the study treatment (i.e., 1 of the 2 doses of TRP or C) within 1 min. The tube was then removed. Further blood samples and VAS ratings were collected at t=-20, -10, and -1min. At t=0min, the participant was presented with a standardised, cold, buffet-style meal, and instructed to eat until they were comfortably full, for up to 30 min (t=0–30 min). The meal comprised 4 slices (~120 g) of wholemeal 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 ~2302 kcal (~27 energy% fat, ~52 energy% carbohydrate, and ~21 energy% protein) and weight of ~2924 g [355]. Participants were not informed that the purpose of the buffet-meal was to assess energy intake. Each participant was studied individually, therefore, energy intake from the buffet-meal was evaluated separately in each participant. After the buffet-meal (t=30 min),

blood samples and VAS ratings were taken every 30 min for a further 2 hours (t=30–150 min). At t=150 min, the intravenous cannula was removed and the participant was allowed to leave the laboratory.

3.3.3 Measurements

Energy intake

The amount of food and drink consumed (g) was quantified by weighing each item of the buffet-meal before and after it was presented to the participant. Energy intake (kcal) was calculated using commercially available software (Foodworks 8, Xyris Software).

Perceptions of hunger, fullness, nausea, and drowsiness

Perceptions of hunger and fullness were assessed using paper-based, validated 100-mm VAS questionnaires [367]. Nausea and drowsiness, which can be induced by tryptophan [167,316], were also assessed. VASs consisted of 100-mm horizontal lines, where 0 mm represented "not felt at all" and 100 mm "felt the strongest possible." Participants were asked to place a vertical mark on each horizontal line to rate the strength of each sensation felt at that point in time.

Plasma CCK and amino acid concentrations

Blood samples (10 mL) were collected into ice-chilled EDTA-containing tubes. Plasma was obtained by centrifugation at 1832×g for 15 min at 4◦C within 15 min of collection and stored at −80◦C until subsequent analysis. Plasma CCK-8 concentrations (pmol/L) were analysed by RIA using an adaptation of the method of Santangelo et al. [354]. Intra-assay CV was 10.9% and inter-assay CV was 13.8%. The detection limit was 1 pmol/L. Plasma concentrations of free amino acids (μmol/L) were quantified by ultra-performance LC using the method of Milan et al. [368]. Amino acids were assayed from 20 μL serum with 15 μM l-Norvaline (as internal

standard) extracted with 20 μL 10% sodium tungstate and 160 μL 0.04Msulfuric acid. Amino acid concentrations were calculated from standard curves generated for each amino acid from the standard injections. The internal standard signal in each chromatogram was used for data normalisation for analyte recovery and quantification.

3.3.4 Statistical analysis

The number of participants was determined by power calculations based on our previous study [190]; thus, *n*=12 participants in each group would allow detection of differences between treatments in energy intake of 249 kcal for the lean group, and 463 kcal for the group with obesity, assuming within-subject SDs of 232 kcal and 431 kcal, respectively, and a difference between groups of 471 kcal, assuming a between-group SD of 334 kcal. Overall significance was set at 0.05 and adjusted for multiple treatment comparisons, with power of 80%. Raw data for energy intake and the amount consumed from the buffet-meal, and AUCs for plasma CCK and TRP concentrations, the TRP/LNAAs ratio (determined at each time point by dividing plasma TRP concentration by the sum of the concentrations of the other LNAAs (i.e., tyrosine, valine, isoleucine, leucine, and phenylalanine)), and VAS ratings, were calculated, using the trapezoidal rule, from t=-31 to -1 min and t=30–150 min. To take into account the potential influence of any difference in blood volume between men with obesity and the lean, data for plasma CCK, TRP, and the TRP/LNAAs ratio were corrected for blood volumes using the equation by Nadler et al. [356]. Data were analysed using maximum likelihood mixed-effects models, with treatment, group, and the treatment-by-group interaction as fixed effects, baseline (i.e., fasting) values as a fixed covariate (except for the analysis of energy intake and amount consumed), and an unstructured covariance matrix to account for the repeated visits per participant. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed when significant main effects or interactions were found. Within-subject

correlations [369], using AUCs for all variables, were performed to evaluate relations of energy intake with $AUCs_{-31\ to\ -1min}$ and $AUCs_{30\ to\ 150min}$ of appetite perceptions, plasma CCK, TRP, and the TRP/LNAAs ratio. Statistical analysis was performed using SPSS software version 25 (IBM). Differences were considered statistically significant at $P \leq 0.05$. All data are reported as means±SEMs.

3.4 RESULTS

All participants completed the study and tolerated the study conditions well. All participants were unrestrained eaters (score range: lean: 1–10; obese: 1–11). There were no differences in baseline values of VAS ratings, plasma CCK, TRP, or the TRP/LNAAs ratio within or between groups.

3.4.1 Energy intake

There were effects of treatment, but not group or an interaction, on energy intake ($P < 0.001$) and the amount consumed ($P<0.05$). Energy intake was less after TRP-3 than after C and TRP-1.5 ($P<0.001$ and $P<0.05$, respectively) (**Figure 3.2.A**). Moreover, the amount consumed was less after TRP-3 than after C ($P<0.05$) and tended to be less than after TRP-1.5 ($P=0.06$) (**Figure 3.2.B**).

3.4.2. Hunger

Response to TRP alone: There was no effect of treatment or group, or an interaction, on hunger (**Figure 3.3.A, Table 3.1**).

After the meal: Hunger scores decreased after the meal on each day. There was an effect of treatment on hunger ($P<0.01$), which tended to be less after TRP-3 than after C ($P=0.09$). There

was also a trend for an effect of group on hunger (P=0.08), which tended to be less in participants with obesity than in the lean group (P=0.08).

3.4.3. Fullness

Response to TRP alone: There was no effect of treatment or group, or an interaction, on fullness (**Figure 3.3.B, Table 3.1**).

After the meal: Fullness scores increased after the meal on each day. There was a treatment-by-group interaction for fullness (P<0.01), which was greater after TRP-3 than after C and TRP-1.5 in lean participants (all P<0.05), but not in those with obesity. Fullness was greater after TRP-1.5 in participants with obesity than in the lean group (P<0.05).

3.4.4. Nausea

Response to TRP alone: There was an effect of treatment, but not group or an interaction, on nausea (P=0.05); however, there were no significant effects between treatments (**Figure 3.3.C, Table 3.1**).

After the meal: Nausea scores were low and did not change after the meal on any day. There was no effect of treatment or group, or an interaction, on nausea.

3.4.5. Drowsiness

Response to TRP alone: There was a treatment-by-group interaction for drowsiness (P<0.05), which was greater after TRP-3 than after C in lean men (P<0.05), but not in those with obesity. Drowsiness after TRP-3 was less in men with obesity than in the lean group (P<0.01) (**Figure 3.3.D, Table 3.1**).

After the meal: Drowsiness scores did not change after the meal on any day. There was an effect of treatment, but not group or an interaction, on drowsiness (P<0.01). Drowsiness was greater after TRP-3 than after C and TRP-1.5 (all P<0.05).

3.4.6. Plasma CCK concentrations

Response to TRP alone: There was an effect of treatment, but not group or an interaction, on plasma CCK (P<0.001), which was greater after TRP-3 and TRP-1.5 than after C (P<0.001 and P<0.01, respectively) (**Figure 3.4.A, Table 3.2**).

After the meal: Plasma CCK increased after the meal on each day; however, there was no effect of treatment or group, or an interaction.

3.4.7. Plasma tryptophan concentrations

Response to TRP alone: There was an effect of treatment, but not group or an interaction, on plasma TRP (P<0.001), which was greater after TRP-3 and TRP-1.5 than after C (all P<0.001), and after TRP-3 than after TRP-1.5 (P<0.01) (**Figure 3.4.B, Table 3.2**).

After the meal: There was a progressive fall in plasma TRP on each day, except for C. There was an effect of treatment, but no interaction, on plasma TRP (P<0.001), which was greater after TRP-3 and TRP-1.5 than after C (all P<0.001), and after TRP-3 than after TRP-1.5 (P<0.001). There was a trend for an effect of group on plasma TRP (P=0.07), which tended to be less in participants with obesity than in the lean (P=0.07).

3.4.8. Plasma tryptophan/LNAAs ratio

Response to TRP alone: There was an effect of treatment, but no interaction, on the TRP/LNAAs ratio (P<0.001), which was greater after TRP-3 and TRP-1.5 than after C (all

P<0.001), and after TRP-3 than after TRP-1.5 (P<0.01). There was also a trend for an effect of group on the TRP/LNAAs ratio (P=0.06), which tended to be less in participants with obesity than in the lean (P=0.06) (**Figure 3.4.C, Table 3.2**).

After the meal: There was a progressive fall in the TRP/LNAAs ratio on each day, except for C. There was a treatment-by-group interaction for the TRP/LNAAs ratio (P<0.05), which was greater after TRP-3 and TRP-1.5 than after C (all P<0.001), and after TRP-3 than after TRP-1.5 (P<0.001), in both groups. The TRP/LNAAs ratio was less after TRP-1.5 (P<0.05), and tended to be less after TRP-3 (P=0.07), in participants with obesity than in the lean group.

3.4.9. Correlations of energy intake with plasma TRP, the TRP/LNAAs ratio, CCK, and perceptions

There were significant inverse correlations of energy intake with premeal AUCs for plasma TRP and the TRP/LNAAs ratio in both groups (lean, both *r*=-0.50, P<0.01; obese, both *r*=-0.40, P<0.05). There were no significant correlations between energy intake and premeal AUCs for hunger, fullness, nausea, drowsiness, and plasma CCK. There were significant positive correlations between post-meal AUCs for fullness and post-meal AUCs for TRP and the TRP/LNAAs ratio in lean participants (all *r*=0.50, P<0.01), but not in those with obesity.

Table 3.1
AUCs of VAS ratings in response to intragastric administration of TRP, in doses of 1.5 g ("TRP-1.5") or 3 g ("TRP-3"), or C (t=-31 to -1 min), and after a buffet-meal (t=30-150 min), in lean men and those with obesity[1]

	Treatment						**P values**		
	Lean			**Obese**			**Treatment-by-group interaction**	**Treatment effect**	**Group effect**
	Control	**TRP-1.5**	**TRP-3**	**Control**	**TRP-1.5**	**TRP-3**			
Hunger									
$AUC_{-31\ to\ -1min}$ (mm*min)	1510±188	1570±181	1670±211	1560±182	1370±176	1460±202	0.26	0.56	0.63
$AUC_{30\ to\ 150min}$ (mm*min)	3430±670^{a}	3090±440	2620±365^{b}	2260±644^{a}	1960±430	1720±351^{b}	0.29	<0.01	0.08
Fullness									
$AUC_{-31\ to\ -1min}$ (mm*min)	745±126	838±171	1060±177	808±121	778±167	853±170	0.36	0.16	0.73
$AUC_{30\ to\ 150min}$ (mm*min)	6880±1000^{a}	7600±877^{ab}	8730±981^{c}	9150±966	9740±851^{d}	9080±941	<0.01	<0.05	0.14
Nausea									
$AUC_{-31\ to\ -1min}$ (mm*min)	233±37.7	286±67.4	350±84.5	158±35.5	292±66.0	261±81.1	0.46	0.05	0.46
$AUC_{30\ to\ 150min}$ (mm*min)	947±230	1120±268	1760±469	656±218	859±261	1080±450	0.53	0.11	0.89
Drowsiness									
$AUC_{-31\ to\ -1min}$ (mm*min)	664±108^{a}	809±108	1000±104^{b}	459±103	547±105	393±100^{c}	<0.05	0.10	<0.01
$AUC_{30\ to\ 150min}$ (mm*min)	2870±560^{d}	3210±602^{d}	4890±815^{e}	1960±536^{d}	2150±584^{d}	2760±783^{e}	0.40	<0.01	0.17

[1]*n*=12 lean men, *n*=13 men with obesity. Values are means±SEMs. Data were analysed using maximum likelihood mixed-effects models, with treatment, group, and the treatment-by-group interaction as fixed effects, baseline (i.e., fasting) values as a fixed covariate, and an unstructured covariance matrix to account for the repeated visits per participant. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed when significant main effects or interactions were found. Hunger: b<a, P=0.09. Fullness: c>a, d>b, all P<0.05. Drowsiness: b>a, P<0.05; c<b, P<0.01; e>d, P<0.05. C, control; TRP, tryptophan; VAS, visual analog scale.

Table 3.2
AUCs of plasma CCK, TRP, and the TRP/LNAAs ratio in response to intragastric administration of TRP, in doses of 1.5 g ("TRP-1.5") or 3 g ("TRP-3"), or C (t=-31 to -1 min), and after a buffet-meal (t=30 to 150 min), in lean men and those with obesity[1]

	Treatment						**P values**		
	Lean			**Obese**			**Treatment-by-group interaction**	**Treatment effect**	**Group effect**
	Control	**TRP-1.5**	**TRP-3**	**Control**	**TRP-1.5**	**TRP-3**			
Plasma CCK									
$AUC_{-31\ to\ -1min}$ (pmol/L*min)	17.0±0.90[a]	21.0±1.60[b]	21.3±1.05[b]	17.0±0.90[a]	21.6±1.60[b]	22.0±1.01[b]	0.98	<0.001	0.60
$AUC_{30\ to\ 150min}$ (pmol/L*min)	125±14.6	117±14.2	113±14.4	131±14.1	136±14.0	131±14.0	0.45	0.64	0.10
Plasma tryptophan									
$AUC_{-31\ to\ -1min}$ (µmol/L*min)	225±5.50[a]	836±62.0[b]	1130±115[c]	218±5.03[a]	715±61.0[b]	899±111[c]	0.34	<0.001	0.13
$AUC_{30\ to\ 150min}$ (µmol/L*min)	1040±96.0[d]	4210±406[e]	6750±833[f]	1035±90.0[d]	3450±395[e]	5540±800[f]	0.11	<0.001	0.07
TRP/LNAA ratio									
$AUC_{-31\ to\ -1min}$	0.40±0.01[a]	1.60±0.12[b]	2.10±0.22[c]	0.40±0.01[a]	1.24±0.12[b]	1.60±0.21[c]	0.17	<0.001	0.06
$AUC_{30\ to\ 150min}$	1.52±0.15[d]	6.85±0.70[eg]	10.7±1.35[fi]	1.36±0.14[d]	4.64±0.70[eh]	7.81±1.30[fj]	<0.05	<0.001	<0.05

[1]*n*=12 lean men, *n*=13 men with obesity. Values are means±SEMs. Data were analysed using maximum likelihood mixed-effects models, with treatment, group, and the treatment-by-group interaction as fixed effects, baseline (i.e., fasting) values as a fixed covariate, and an unstructured covariance matrix to account for the repeated visits per participant. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed when significant main effects or interactions were found. Plasma CCK: b>a, P<0.01. Plasma TRP: c>a, b>a, both P<0.001; c>b, P<0.01; f>d, e>d, f>e, all P<0.001. TRP/LNAAs ratio: c>a, b>a, all P<0.001; c>b, P<0.01; f>d, e>d, f>e, all P<0.001; h<g, P<0.05; j<i, P=0.07. C, control; CCK, cholecystokinin; LNAA, large neutral amino acid; TRP, tryptophan.

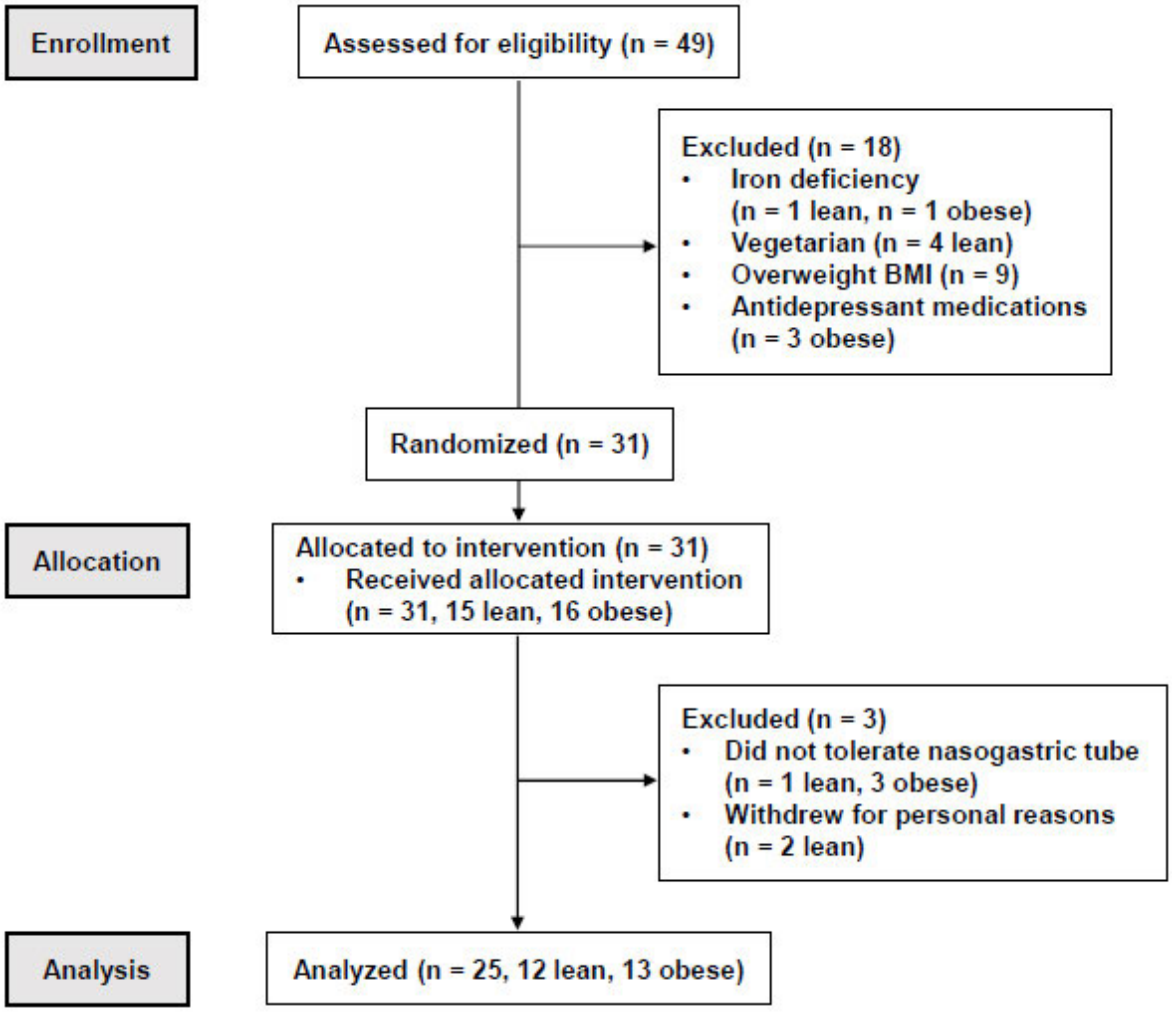

Figure 3.1
CONSORT Flow diagram.

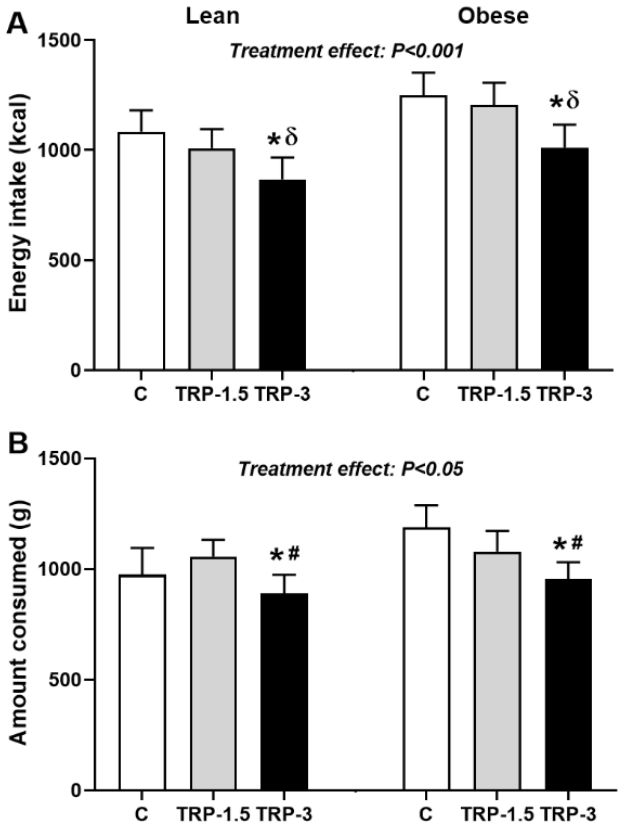

Figure 3.2
Energy intake (**A**) and amount consumed (**B**) after intragastric administration of 3 g (TRP-3) or 1.5 g (TRP-1.5) TRP, or C, in lean men and those with obesity. Data were analysed using maximum likelihood mixed-effects models, with treatment, group, and the treatment-by-group interaction as fixed effects and an unstructured covariance matrix to account for the repeated visits per participant. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed when significant main effects or interactions were found. (**A**) *TRP-3 and C differ, P<0.001. $^{\delta}$TRP-3 and TRP-1.5 differ, P<0.05. (**B**) *TRP-3 and C differ, P<0.05. $^{\#}$Trend for TRP-3 and TRP-1.5 to differ, P=0.06. Data are expressed as means±SEMs; *n*=12 lean men, *n*=13 men with obesity. C, control; TRP, tryptophan.

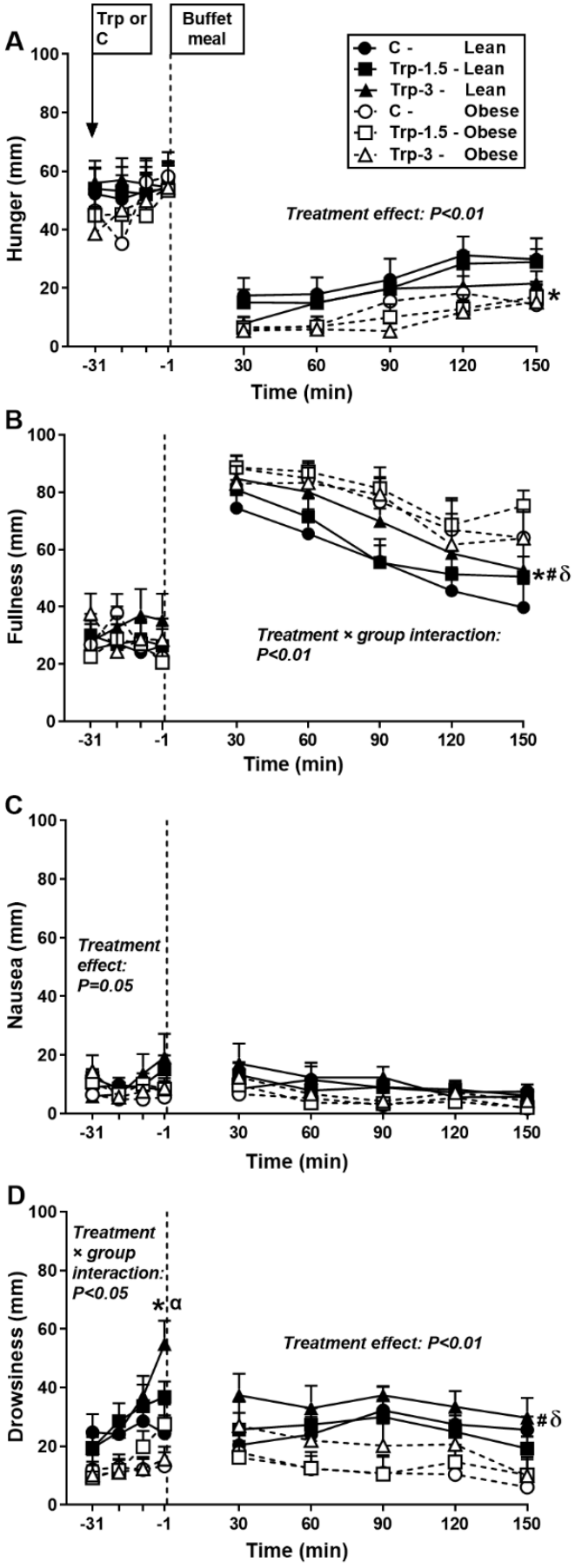
A
Trp or C
Buffet meal
C - Lean
Trp-1.5 - Lean
Trp-3 - Lean
C - Obese
Trp-1.5 - Obese
Trp-3 - Obese
Hunger (mm)
Treatment effect: P<0.01
*
Time (min)
B
Fullness (mm)
*#δ
Treatment × group interaction: P<0.01
Time (min)
C
Nausea (mm)
Treatment effect: P=0.05
Time (min)
D
Drowsiness (mm)
Treatment × group interaction: P<0.05
*α
Treatment effect: P<0.01
#δ
Time (min)

Figure 3.3
Hunger (**A**), fullness (**B**), nausea (**C**), and drowsiness (**D**) ratings after intragastric administration of 3 g (TRP-3) or 1.5 g (TRP-1.5) TRP, or C, in lean men and those with obesity. Data were analysed using maximum likelihood mixed-effects models, with treatment, group, and the treatment-by-group interaction as fixed effects, baseline (i.e., fasting) values as a fixed covariate, and an unstructured covariance matrix to account for the repeated visits per participant. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed when significant main effects or interactions were found. (**A**) Response to the meal: *trend for TRP-3 and C to differ, P=0.09. (**B**) Response to the meal: *TRP-3 and C differ in lean, P<0.05; $^{\#}$TRP-3 and TRP-1.5 differ in lean, P<0.05; $^{\delta}$TRP-1.5 differs in lean and obese, P<0.05. (**D**) TRP alone: *TRP-3 and C differ in lean, P<0.05; $^{\alpha}$TRP-3 differs in lean and obese, P<0.01. Response to the meal: $^{\#}$TRP-3 and C differ, P<0.05; $^{\delta}$TRP-3 and TRP-1.5 differ, P<0.05. Data are expressed as means±SEMs; *n*=12 lean men, *n*=13 men with obesity. C, control; TRP, tryptophan.

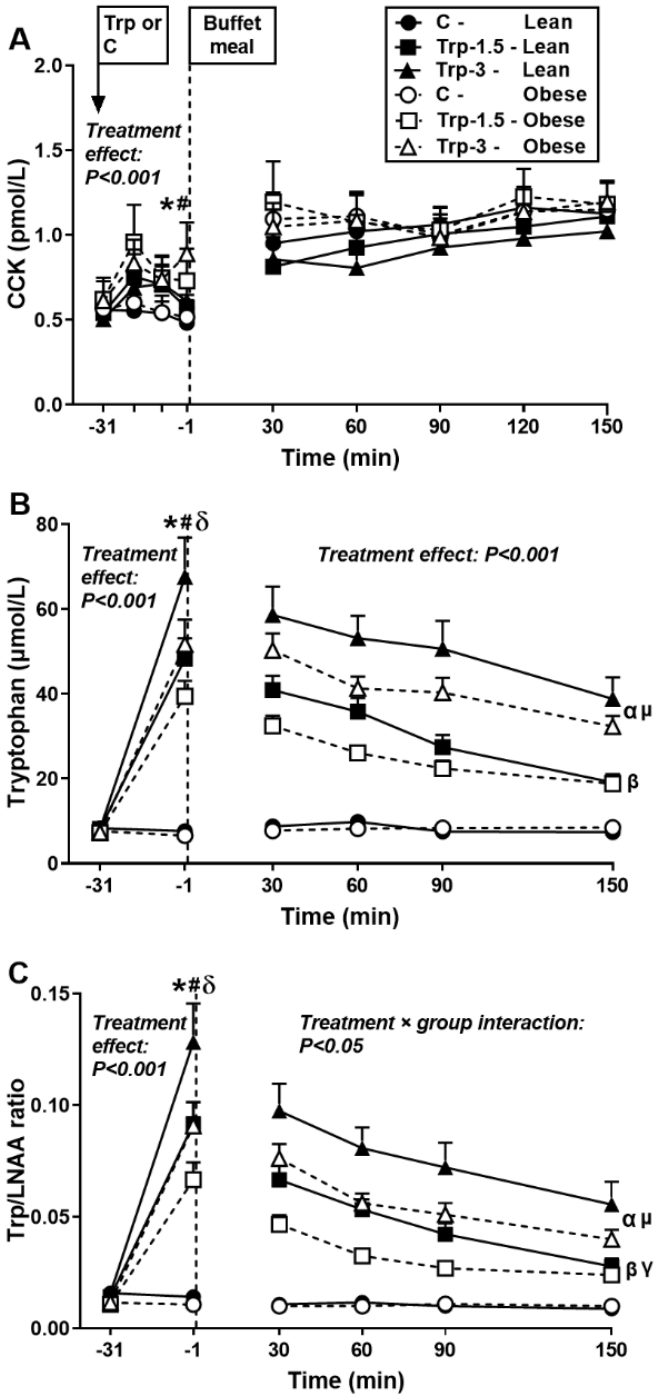

Figure 3.4
Plasma CCK (**A**), TRP (**B**), and the TRP/LNAAs ratio (**C**) after intragastric administration of 3 g (TRP-3) or 1.5 g (TRP-1.5) TRP, or C, in lean men and those with obesity. Data were analysed using maximum likelihood mixed-effects models, with treatment, group, and the treatment-by-group interaction as fixed effects, baseline (i.e., fasting) values as a fixed covariate, and an unstructured covariance matrix to account for the repeated visits per participant. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed when significant main effects or interactions were found. (**A**) TRP alone: *TRP-3 and C differ, P <0.001; #TRP-1.5 and C differ, P<0.01. (**B**) TRP alone: *TRP-3 and C

differ, P<0.001; [#]TRP-1.5 and C differ, P<0.001; [δ]TRP-3 and TRP-1.5 differ, P<0.01. Response to the meal: [α]TRP-3 and C differ, P<0.001; [β]TRP-1.5 and C differ, P<0.001; [μ]TRP-3 and TRP-1.5 differ, P<0.001. (**C**) TRP alone: *TRP-3 and C differ, P<0.001; [#]TRP-1.5 and C differ, P<0.001; [δ]TRP-3 and TRP-1.5 differ, P<0.01. Response to the meal: [α]TRP-3 and C differ, P<0.001; [β]TRP-1.5 and C differ, P<0.001; [μ]TRP-3 and TRP-1.5 differ, P<0.001; [γ]TRP-1.5 differs in lean and obese, P<0.05. Data are expressed as means±SEMs; *n*=12 lean men, *n*=13 men with obesity. C, control; CCK, cholecystokinin; LNAA, large neutral amino acid; TRP, tryptophan.

3.5 DISCUSSION

The major novel findings of this study were that 1) 3 g, but not 1.5 g, intragastric TRP suppressed energy intake, with no difference between lean men and those with obesity, but did not affect premeal hunger or fullness; 2) at both doses, TRP potently stimulated CCK before the meal, with no difference between groups; 3) TRP predictably increased plasma TRP and the TRP/LNAAs ratio, with the effect of TRP-3 greater than C and TRP-1.5, and a trend for the TRP/LNAAs ratio to be less in men with obesity than in lean participants; 4) energy intake correlated with premeal plasma TRP and the TRP/LNAAs ratio, but not CCK, in both groups; 5) despite substantially lower energy intakes in response to TRP-3, post-meal hunger tended to be less, and fullness greater, than in C; and 6) post-meal plasma TRP and the TRP/LNAAs ratio remained elevated after TRP-3 and TRP-1.5 and tended to be less in men with obesity than in the lean.

Our finding of a substantial suppression of energy intake (by ∼20%) by intragastric TRP in both lean men and those with obesity is consistent with earlier studies reporting that 2 g and 3 g TRP, given in capsules, suppressed energy intake at lunch 45 min later in healthy lean men [316] and 60 min later in participants with obesity [317], and our own study in which energy intake from a buffet-lunch was markedly suppressed after 90-min intraduodenal infusion of TRP (total amount: 3.3 g) in lean men [167]. Interestingly, whereas the 2 earlier studies found an energy intake–suppressant effect of the 2-g dose, a dose of 1.5 g TRP was ineffective in the current study, suggesting that the effective dose lies between 1.5 and 2 g. Whereas the previous 2 studies did not allow direct comparisons between lean participants and those with obesity, our data indicate that there were no significant differences in the energy intake–suppressant effect of TRP between the 2 groups; this is in contrast to the responses to fatty acids, which have been found to be less in obesity [186].

A number of mechanisms may be involved in the appetite suppressant effects of TRP, including the stimulation of CCK [167,192]. Consistent with a previous study in which TRP was administered intraduodenally [167], intragastric TRP stimulated the release of CCK within 10–20 min after administration, although the magnitude of effect in the current study appeared to be less, presumably because intragastric administration was associated with a lesser, or slower, exposure of small intestinal receptors to TRP than intraduodenal infusion. In contrast to the previous study, energy intake was not correlated with plasma CCK in the current study. Although this does not exclude CCK as a mediator of energy intake suppression by TRP, the involvement of endogenous CCK would need to be clarified using a specific CCK-receptor antagonist [370].

In our previous study, in response to intraduodenal TRP, energy intake was strongly correlated with circulating plasma TRP in lean men [167], suggesting that the appetite-suppressant effect of TRP may involve direct effects of TRP and/or act via serotonergic pathways in the brain. Furthermore, in another study, 3 g TRP, 15 min before a mixed-nutrient drink, led to a substantial 9-fold elevation in the TRP/LNAAs ratio in lean participants [192]. In the current study, administration of 3 g TRP was associated with an 8-fold increase in the TRP/LNAAs ratio in the lean, which correlated inversely with energy intake, supporting the important role of the TRP/LNAAs ratio in the suppression of energy intake. We found a trend for a difference in the TRP/LNAAs ratio between men with obesity and lean men, with values somewhat less in those with obesity, in line with our previous study [192] and work by others [338]. However, in contrast to the previous study, energy intake was also correlated with circulating TRP and the TRP/LNAAs ratio in men with obesity. Although we do not have a clear explanation for this apparent discrepancy, it may reflect a difference in study designs. In both previous studies [192,338], in which the TRP/LNAAs ratio was less in people with obesity, the participants also

ingested a carbohydrate-containing meal. Moreover, although individuals with obesity had comparable BMIs in both the previous [192] and current studies, the duration of their obesity may differ, which may affect TRP metabolism [339].

In contrast to some studies [167,316], TRP did not affect premeal appetite perceptions. The duration of 30 min may have been insufficient: longer time intervals, including 45 min and 90 min between TRP administration [167,316], or 150 min between serotonergic drugs, and a subsequent meal [371], were associated with an increase in fullness and a suppression of hunger. Of interest, although 3 g TRP suppressed energy intake substantially, hunger scores remained low, and fullness elevated, for 2 hours after the meal. That is, despite eating less, participants were less hungry and more full. Hunger also tended to be less, and fullness was greater, in men with obesity. There were also relations between post-meal fullness and plasma TRP and the TRP/LNAAs ratio, although only in the lean, suggesting that a sustained increase in circulating TRP and the TRP/LNAAs ratio and, thus, brain serotonin, contributed to sustained appetite suppression for ≥2 hours after the meal. How this translates to energy intake at a subsequent meal warrants further investigation.

It is unlikely that the suppression of energy intake by TRP was related to nausea, because scores were low, and there was no relation between nausea and energy intake. Although it has been shown that TRP increases sleepiness and decreases alertness [372], drowsiness is unlikely to have reduced food intake, because scores were low and there was no link between drowsiness and energy intake. Moreover, serotonin receptor antagonists, such as cyproheptadine, induce drowsiness, but also increase food intake [373].

Some limitations of our study should be noted. The study only evaluated men (both lean and those with obesity). Because previous studies have indicated sex-specific changes in TRP pathways in the brain and greater serotonin synthesis in men than in women [374,375], extrapolation of our results to other populations, e.g., women, older individuals, or people with type 2 diabetes, should be done with caution. We demonstrated that TRP sustained appetite suppression for 2 hours after the meal; however, we did not evaluate energy intake from a subsequent meal, therefore determination of the effects of TRP on energy intake at subsequent meals requires further evaluation. The doses of TRP used were based on previous studies showing a potent effect of TRP at ∼3 g on energy intake [167,192]. Although it could be argued that higher doses of TRP may result in more potent energy intake–suppressant effects, they are also associated with adverse effects, including nausea nausea [167].

In conclusion, intragastric TRP has potent energy intake–suppressant effects, which are maintained in obesity. Suppression of energy intake was related to circulating TRP and the TRP/LNAAs ratio, supporting a role of post-absorptive mechanisms in the observed effects. TRP also sustained appetite suppression for 2 hours after the meal. The findings have important implications for the use of TRP as an appetite-suppressant strategy in obesity. Further studies are warranted to evaluate the effects of TRP on energy intake and appetite perceptions at subsequent meals to investigate whether the effect is sustained for the rest of the day or whether energy compensation occurs later, and the mechanisms underlying the observed effects.

CHAPTER 4: Effects of intragastric administration of L-tryptophan on the glycaemic response to a nutrient drink in men with type 2 diabetes - impacts on gastric emptying, glucoregulatory hormones and glucose absorption

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Contribution to the Paper	Performed experiment and data analysis, interpreted results, 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. 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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4.1 ABSTRACT

Background and aims: The rate of gastric emptying and glucoregulatory hormones are key determinants of postprandial glycaemia. Intragastric administration of L-tryptophan slows gastric emptying and reduces the glycaemic response to a nutrient drink in lean individuals and those with obesity. We investigated whether tryptophan decreases postprandial glycaemia and slows gastric emptying in type 2 diabetes.

Methods: Twelve men with type 2 diabetes (age: 63±2 years, HbA1c: 49.7±2.5 mmol/mol, BMI: 30±1 kg/m^2) received, on 3 separate occasions, 3 g ("TRP-3") or 1.5 g ("TRP-1.5") tryptophan, or control (0.9% saline), intragastrically, in randomised, double-blind fashion, 30 min before a mixed-nutrient drink (500 kcal, 74 g carbohydrates), containing 3 g 3-O-methyl-D-glucose (3-OMG) to assess glucose absorption. Venous blood samples were obtained at baseline, after tryptophan, and for 2 h post-drink for measurements of plasma glucose, C-peptide, glucagon and 3-OMG. Gastric emptying of the drink was quantified using 2-dimensional ultrasound.

Results: Tryptophan alone stimulated C-peptide (P=0.002) and glucagon (P=0.04), but did not affect fasting glucose. In response to the drink, TRP-3 lowered plasma glucose from t=15-30 min and from t=30-45 min compared with control and TRP-1.5, respectively (both P<0.05), with no differences in peak glucose between treatments. Gastric emptying tended to be slower after TRP-3, but not TRP-1.5, than control (P=0.06). Plasma C-peptide, glucagon and 3-OMG increased on all days, with no major differences between treatments.

Conclusion: In people with type 2 diabetes, intragastric administration of 3 g tryptophan modestly slows gastric emptying, associated with a delayed rise, but not an overall lowering of, postprandial glucose.

4.2 INTRODUCTION

Gastric emptying and the release of the glucoregulatory hormones, insulin and glucagon, are important determinants of the postprandial blood glucose response to carbohydrate-containing meals, in health, obesity and type 2 diabetes [255]. For example, gastric emptying, which exhibits a substantial inter-individual variation, accounts for about 35% of the variance in peak postprandial blood glucose [255]. Slowing of gastric emptying, by dietary or pharmacological means, attenuates postprandial glycaemic excursions, particularly during the first 30 - 60 min postprandially [376,377]. Gastric emptying and the release of glucoregulatory hormones can be influenced by nutrients [147,378]. For example, in type 2 diabetes, whey protein, when given as a preload 30 min before a high-carbohydrate meal, slowed gastric emptying and stimulated insulin, leading to a reduction in the glycaemic response [297]. These effects of protein appear to be mediated, at least in part, by their digestion products, amino acids – both circulating amino acids and as a result of the interaction of amino acids with the small intestine [171,323,379,380].

A number of amino acids have been shown to reduce the blood glucose response to glucose or mixed-nutrient drinks [192,303,305,311,381]. The aromatic amino acid, tryptophan, is of particular interest; it potently stimulates pyloric motility and plasma cholecystokinin (CCK) concentrations, which are both central to the slowing of gastric emptying [167,191]. Solutions containing tryptophan, when administered intragastrically or orally, empty from the stomach more slowly than control solutions [169,170]. We reported that intragastric administration of 3 g tryptophan slowed gastric emptying of a mixed-nutrient drink, consumed 15 min later, associated with a reduced glycaemic response in the first 30 min, in both lean individuals and those with obesity who do not have type 2 diabetes [192]. That intragastric tryptophan had a minimal [167], or no [169], effect to stimulate insulin, and may reduce insulin [192], suggests that the attenuation in postprandial blood glucose is unlikely to reflect an insulinotropic effect of

tryptophan. Like other amino acids, tryptophan has been reported to stimulate glucagon [167,192], which may potentially counteract glucose-lowering by stimulating glycogenolysis and gluconeogenesis. Hence, slowing of gastric emptying is likely to be fundamental to a reduction in postprandial glycaemia by tryptophan. The above considerations are of relevance to the management of type 2 diabetes; it is now appreciated that postprandial glycaemic excursions are a major determinant of overall glycaemic control, as assessed by measurement of glycated haemoglobin, and the consequent risk of microvascular complications (i.e. retinopathy, nephropathy and neuropathy). However, there is no information about the effects of tryptophan in type 2 diabetes. The observed reduction in postprandial glucose resulting from slowing of gastric emptying, induced by tryptophan, should intuitively be associated with a reduction in the rate of carbohydrate absorption, as supported by animal studies [382]. Glucose absorption can be measured in humans using plasma concentrations of the non-metabolisable glucose analogue, 3-orthomethyl-glucose (3-OMG) [383].

The aim of the current study was to evaluate the effects of intragastric administration (to avoid potential confounding effects of taste) of tryptophan on the glycaemic response to, as well as glucoregulatory hormones and glucose absorption, and gastric emptying of, a mixed-nutrient drink in people with type 2 diabetes.

4.3 PARTICIPANTS AND METHODS

4.3.1 Participants

Twelve men with type 2 diabetes (mean age: 63±2 years, BMI: 30±1 kg/m^2, HbA1c: 49.7±2.5 mmol/mol (6.7±0.1%), duration of diabetes: 10±2 years) participated in the study. Their diabetes was managed by metformin (500-2000 mg/day) alone (*n*=7), or in combination with a DPP-IV inhibitor (*n*=2) or a sodium-glucose transport inhibitor (*n*=3). Participants were

recruited by flyers placed at the Royal Adelaide Hospital and Diabetes South Australia, and through advertisements on online sites (University of Adelaide and Gumtree). Participants were excluded if they had significant gastrointestinal disorders or symptoms, cardiovascular or respiratory diseases or surgery, used medication known to affect gastrointestinal function and/or appetite, were lactose-intolerant, consumed protein supplements or >2 standard drinks (20 g) alcohol on >5 days a week, were vegetarians or smokers, or had an estimated glomerular filtration rate <45 mL/min, or low serum ferritin (<30 ug/L) or iron (<8 umol/L) levels (a requirement by the Human Research Ethics Committee). In all cases, autonomic nerve function was evaluated using standardised cardiovascular reflex tests, and the presence of autonomic neuropathy (i.e. a score ≥3) represented an exclusion [384]. After inclusion, each participant was allocated to a treatment order of balanced randomisation (www.randomization.com) by a research officer who was not involved in data analysis. The Human Research Ethics Committee of the Central Adelaide Local Health Network approved the study protocol, and the study was performed in accordance with the Declaration of Helsinki and the National Health and Medical Research Council of Australia Statement on Ethical Conduct in Human Research. All participants provided written informed consent before inclusion. The study was registered as a clinical trial with the Australia and New Zealand Clinical Trials Registry (www.anzctr.org.au) (trial number: ACTRN12613000899741).

4.3.2 Study outline

Study design

We investigated the effects of intragastric administration of (i) 3 g ("TRP-3") or (ii) 1.5 g ("TRP-1.5") tryptophan, or (iii) control (0.9% saline) on plasma glucose (the primary outcome), C-peptide (a measure of insulin secretion), and glucagon concentrations and glucose

absorption in response to, and gastric emptying of, a mixed-nutrient drink, consumed 30 min after the intragastric treatments.

Study treatments

Tryptophan treatments were prepared by dissolving 1.5 g or 3 g food-grade tryptophan (PureBulk Inc., Roseburg, Oregon, USA), 58 mg $CaCl_2xH_2O$, and 1.75 g or 1.65 g NaCl, respectively, in 200 mL water for irrigation. Control solutions consisted of 58 mg $CaCl_2xH_2O$ and 1.85 g NaCl in 200 mL water. The solutions were approximately iso-osmolar (control: 296, 1.5 g: 318, 3 g: 335 mOsm/kg) and had a pH of ~7 and a temperature of ~23 °C. Solutions were prepared by a research officer, who was not involved in the performance of the studies or data analysis, on the morning of each study and administered via a nasogastric catheter directly into the stomach. Syringes were covered, so that both study participants and the investigators performing the study were blinded to the nature of the solution. The doses of tryptophan were chosen based on our previous work and were well tolerated [192].

Study protocol

Participants were studied in a randomised, double-blind, cross-over fashion on 3 occasions each separated by 3-7 days. Participants were provided with a standardised meal (Beef lasagne, McCain Food, Wendouree, Victoria, Australia; energy content: 603 kcal), to be consumed between 6.30 p.m. and 7 p.m. on the night prior to each study. All participants were instructed to maintain their normal eating habits between study days and to discontinue their glucose-lowering medication for 48 hours, and refrain from strenuous exercise and alcohol for 24 hours, before each study. Participants were also asked to abstain from any solids or liquids, except water (which was allowed until 7 a.m.), after the evening meal until they attended the Clinical Research Facility at the University of Adelaide at 8.30 a.m. the next morning. An intravenous

cannula was placed into a forearm vein, and the arm was kept warm with a heat pad for regular sampling of "arterialized" blood. A baseline blood sample for measurement of plasma glucose and hormones, and a baseline antral area measurement, using 2-dimensional ultrasound, with the participant seated in an upright position, were taken. Participants also completed a visual analogue scale (VAS) questionnaire to assess gastrointestinal symptoms. Each participant was then intubated with a soft-silicon feeding tube (outer diameter: 4 mm; Dentsleeve, Mississauga, Ontario, Canada), which was inserted through an anaesthetised nostril into the stomach. Immediately thereafter (t=-31 min, ~8.45 a.m.), participants received the 200-mL intragastric bolus of one of the two doses of tryptophan, or control, within 1 min. The tube was then removed. Further blood samples and VAS ratings were collected at t=-20, -10 and -1 min. At t=-1 min, each participant consumed, within 1 min, a mixed-nutrient drink (350 mL, 500 kcal, 74 g carbohydrates, including maltodextrin and sucrose, 18 g protein and 15 g fat) consisting of 325 mL Nestle Resource Plus® vanilla (Nestle Healthcare Nutrition, Tongala, Victoria, Australia) plus 25 mL water to make up the final volume) containing 3 g 3-OMG (Sigma-Aldrich, Milwaukee, Wisconsin, USA). Blood samples and VAS ratings were collected subsequently at 15-min intervals from t=15 to 60 min and at 30-min intervals from t=60 to 120 min. Measurements of antral area were obtained immediately after the drink (t=0 min), at 5-min intervals from t=0 to 60 min, and at 15-min intervals from t=60 to 120 min. At t=120 min, the cannula was removed, and participants were provided with a light lunch, after which they were free to leave the laboratory.

4.3.3 Measurements

Plasma glucose, C-peptide, glucagon and serum 3-OMG concentrations

For glucose and hormones, venous blood samples (7 mL) were collected into ice-chilled ethylenediaminetetraacetic acid-containing tubes. For serum 3-OMG, 3-mL venous samples

were collected into untreated tubes and allowed to clot. Plasma and serum were each separated by centrifugation at 3200 rpm for 15 min at 4 °C within 15 min of collection and stored at -80 °C until subsequent analysis.

Plasma glucose concentrations (mmol/L) were quantified by the glucose oxidase method using a glucose analyser (YSI 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, Ohio, USA). Intra- and inter-assay CVs were ≤2%.

Plasma C-peptide concentrations (pmol/L) were measured by ELISA immunoassay (10-1136-01, Mercodia, Uppsala, Sweden). The minimum detectable limit was 15 pmol/L and the inter- and intra-assay CVs were 8.3% and 2.9%, respectively. C-peptide reflects endogenous insulin secretion, since it is not extracted by the liver and its half-life is longer than that of insulin [385].

Plasma glucagon concentrations (pg/mL) were measured by radioimmunoassay (GL-32K, Millipore, Billerica, Massachusetts, USA). The minimum detectable limit was 15 pg/mL, and inter- and intra-assay CVs were 6.9% and 4.2%, respectively.

Serum 3-OMG concentrations (mmol/L) were measured by liquid chromatography and mass spectrometry, with a sensitivity of 0.0103 mmol/L [386].

Gastric emptying

Gastric emptying was evaluated by measuring antral area using 2-dimensional ultrasound (Aloka SSD-650 CL; ALOKA Co. Ltd., Tokyo, Japan). Imaging was performed with the transducer positioned vertically to obtain a sagittal image of the antrum, with the superior mesenteric vein and the abdominal aorta in a longitudinal section [387]. Images were taken at the

end of inspiration to minimise the effects of the motion of the stomach that occurs with regular breathing [140]. A region-of-interest was drawn around the cross-section of the antrum using in-built software. The intragastric retention of the meal, expressed as a percentage of the meal at t=0 min, at any given time [387], and the gastric half-emptying time (T50), defined as the time taken for 50% of the meal to empty from the stomach [388], were calculated.

GI symptoms

Nausea and bloating were quantified using a VAS questionnaire [367]. The strength of each sensation 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 indicate how they were feeling at each time point by placing a vertical mark on the 100-mm line.

4.3.4 Statistical analysis

The number of participants included was determined by power calculations based on our previous studies [167,192]. We calculated that n=12 participants would allow detection of a 1.0 mmol/L reduction in blood glucose at α=0.05 with a power of 80% [192].

Areas under the curve (AUCs) for plasma glucose, C-peptide, glucagon and VAS ratings were calculated using the trapezoidal rule, from t=-31 to -1 min to assess the effects of tryptophan alone, and from t=-1 to 120 min (t=0 to 120 min for gastric emptying and serum 3-OMG) to assess the responses to the mixed-nutrient drink. AUCs were analysed using general linear model mixed-model analysis, with baseline values as a covariate. C-peptide and glucagon concentrations at t=-1 min (i.e. immediately pre-drink), which we reported previously to correlate with subsequent glycaemic control [192], peak plasma glucose, time to peak glucose and gastric half-emptying time (T50) were analysed using repeated-measures ANOVA. Post-hoc

comparisons, adjusted for multiple comparisons by Bonferroni correction, using parametric tests, were performed when significant ANOVA effects were found. Statistical analysis was performed using SPSS software (version 25, IBM, Chicago, Illinois, USA). Differences were considered statistically significant at $P \leq 0.05$, and $0.05 < P \square 0.1$ as trend. All data are reported as means±SEM.

4.4 RESULTS

All participants completed the study and tolerated the study conditions well. There were no effects of treatment on nausea or bloating, neither in response to tryptophan alone nor after the mixed-nutrient drink (**Table 4.1**). No participant had evidence of autonomic nerve dysfunction (mean total score: 1.1±0.1; range: 0-2).

4.4.1 Plasma glucose concentrations

Effect of tryptophan alone: There was no effect of treatment on fasting plasma glucose.

Effect following the mixed-nutrient drink: There was a substantial rise in plasma glucose after the drink on all days. While there was no effect of treatment on overall plasma glucose $AUC_{-1 \text{ to } 120min}$, there was a treatment*time interaction for plasma glucose (P=0.05). Plasma glucose was lower after TRP-3 from t=15-30 min compared with control, and from t=30-45 min compared with TRP-1.5 ($P<0.05$ for all). There was no effect of treatment on peak glucose concentration or the time to peak concentration (**Figure 4.1**, **Table 4.1**).

4.4.2 Gastric emptying

There was an effect of treatment on gastric retention $AUC_{0 \text{ to } 120min}$ (P=0.04), which tended to be greater after TRP-3 compared with control (P=0.06). While there was also an effect of

treatment on T50 (P=0.05), following post-hoc comparisons the difference between treatments was not significant (**Figure 4.2**, **Table 4.1**).

4.4.3 Plasma C-peptide concentrations

Effect of tryptophan alone: There was an effect, albeit very small, of treatment on fasting C-peptide $AUC_{-31\ to\ -1min}$ (P=0.002), which tended to be greater after TRP-3 (P=0.06), and was greater after TRP-1.5 (P=0.002), compared with control. There was also an effect on C-peptide at t=-1 min (P=0.01), which was greater after both TRP-3 and TRP-1.5 compared with control (P<0.05 for both).

Effect following the mixed-nutrient drink: C-peptide rose substantially after the drink on all days. There was an effect of treatment on C-peptide $AUC_{-1\ to\ 120min}$ (P=0.01), which tended to be greater after TRP-1.5 (P=0.06), but not TRP-3, compared with control. There was no treatment*time interaction (**Figure 4.3.A**, **Table 4.1**).

4.4.4 Plasma glucagon concentrations

Effect of tryptophan alone: There was an effect of treatment on fasting glucagon $AUC_{-31\ to\ -1min}$ (P=0.04), however, this was lost on post-hoc comparisons. There was also an effect on glucagon at t=-1 min (P=0.007), which tended to be greater after TRP-3 compared with control (P=0.07).

Effect following the mixed-nutrient drink: There was a marked rise in plasma glucagon after the drink on all days. However, there was no effect of treatment on glucagon $AUC_{-1\ to\ 120min}$, or a treatment*time interaction (**Figure 4.3.B**, **Table 4.1**).

4.4.5 Serum 3-OMG concentrations

There was no effect of treatment on serum 3-OMG $AUC_{0\ to\ 120min}$, or a treatment*time interaction (**Figure 4.3.C**, **Table 4.1**).

Table 4.1
AUCs of plasma glucose, C-peptide and glucagon concentrations and VAS ratings in response to tryptophan alone (t=-31 to -1 min) and a mixed-nutrient drink (t=-1 to 120 min), and gastric emptying and serum 3-OMG responses to, the mixed-nutrient drink, ingested 30 min after intragastric administration of tryptophan, in doses of 3 g (TRP-3) or 1.5 g (TRP-1.5), or control†

	Treatments			P value
	Control	**TRP-1.5**	**TRP-3**	**(ANOVA)**
Plasma glucose				
$AUC_{-31\ to\ -1min}$ (mmol/L*min)	234±1	233±2	231±1	NS‡
$AUC_{-1\ to\ 120min}$ (mmol/L*min)	1390±52	1412±44	1341±42	NS
Peak (mmol/L)	13±1	13±1	13±1	NS
Time to peak (min)	81±8	82±5	90±6	NS
Gastric emptying				
$AUC_{0\ to\ 120min}$ (% retention*min)	5172±637	5650±532	6026±662*	0.04
T50 (min)	34±8	43±8	49±7	0.05
Plasma C-peptide				
$AUC_{-31\ to\ -1min}$ (pmol/L*min)	26140±335	28986±427**	28651±839*	0.002
$AUC_{-1\ to\ 120min}$ (pmol/L*min)	244611±21630	269441±24255*	237282±25079	0.01
Plasma glucagon				
$AUC_{-31\ to\ -1min}$ (pg/mL*min)	1259±14	1386±50	1378±47	0.04
$AUC_{-1\ to\ 120min}$ (pg/mL*min)	7513±366	7967±400	8457±328	NS
Serum 3-OMG				
$AUC_{0\ to\ 120min}$ (mmol/L*min)	22±2	23±2	21±2	NS
Nausea				
$AUC_{-31\ to\ -1min}$ (mm*min)	191±36	135±16	186±67	NS
$AUC_{-1\ to\ 120min}$ (mm*min)	836±619	846±640	996±662	NS
Bloating				
$AUC_{-31\ to\ -1min}$ (mm*min)	156±53	157±57	182±74	NS
$AUC_{-1\ to\ 120min}$ (mm*min)	1147±604	1467±737	1780±675	NS

Data are means±SEM, *n*=12. AUCs of plasma glucose, gastric emptying, plasma C-peptide, glucagon, serum 3-OMG and VAS ratings, and T50 were analysed using general linear model mixed-model analysis, including baseline as a covariate, with adjustments for multiple comparisons made using Bonferroni correction.

† AUC: area under the cure, VAS: visual analogue scale, T50: gastric half-emptying time, 3-OMG: 3-O-methyl-D-glucose

‡ NS: not significant

* Trend for difference from control (P=0.06)

** Significantly different from control (P=0.002)

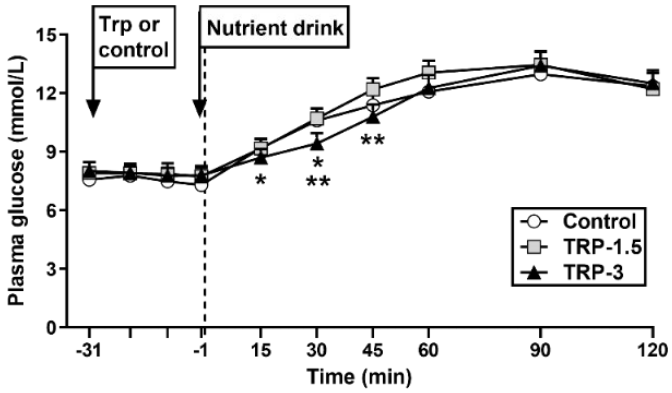

Figure 4.1
Plasma glucose concentrations following intragastric administration of 3 g (TRP-3) or 1.5 g (TRP-1.5) tryptophan, or control, in participants with type 2 diabetes. After tryptophan alone (e.g. t=-31 to -1 min), there was no effect on fasting plasma glucose. Following the mixed-nutrient drink (e.g. t=0 to 120 min), there was no effect on overall plasma glucose $AUC_{-1\ to\ 120min}$. However, there was a significant treatment*time interaction for plasma glucose (P=0.05); Plasma glucose after TRP-3 was lower from t=15 to 30 min compared with control (*P<0.05), and from t=30 to 45 min compared with TRP-1.5 (**P<0.05). Data are expressed as means±SEM; *n*=12. Data were analysed using general linear model mixed-model analysis, with baseline as covariate, and repeated-measures ANOVA, and adjustments for multiple comparisons were made using Bonferroni correction.

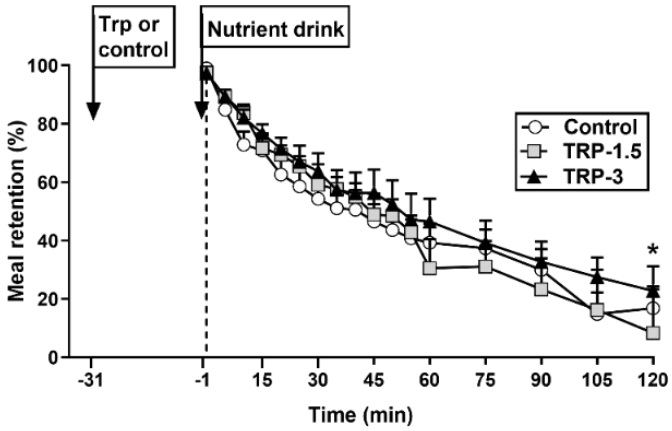

Figure 4.2
Gastric emptying, expressed as % drink retention, following intragastric administration of 3 g (TRP-3) or 1.5 g (TRP-1.5) tryptophan, or control, in participants with type 2 diabetes. There was an effect of treatment on gastric emptying $AUC_{0\ to\ 120min}$ (P=0.04), which tended to be greater after TRP-3 compared with control (*P=0.06). There was also an effect of treatment on T50 (P=0.05), although this was lost on post-hoc comparisons. Data are expressed as means±SEM; *n*=12. Data were analysed using general linear model mixed-model analysis, with baseline as covariate, and adjustments for multiple comparisons made using Bonferroni correction.

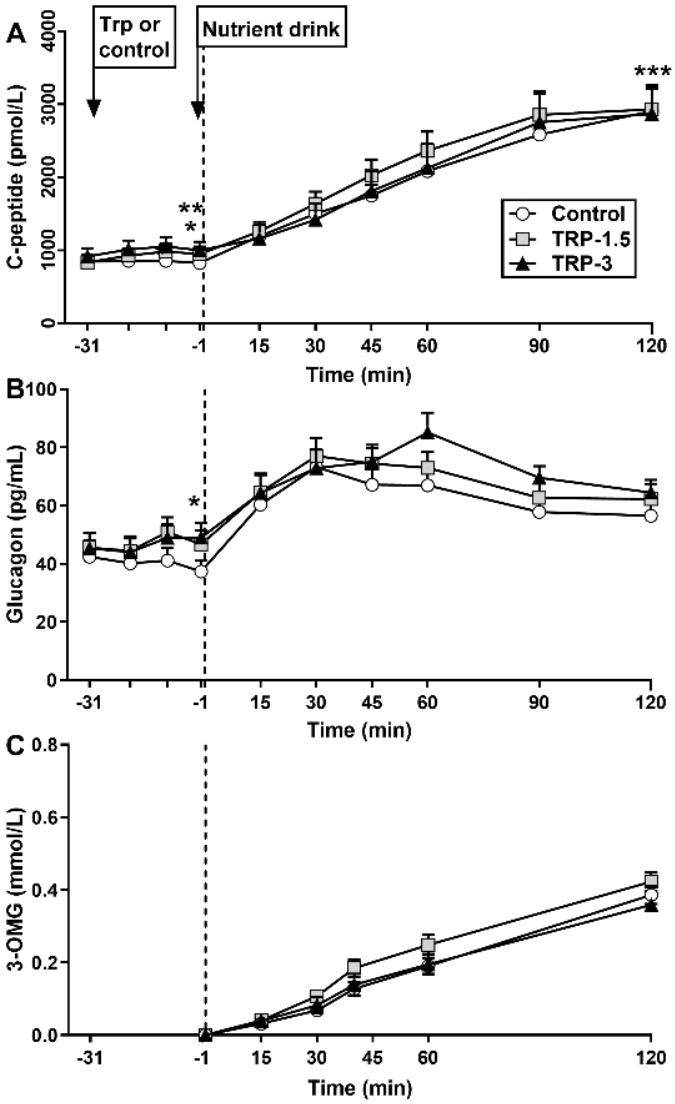

Figure 4.3

Plasma C-peptide (**A**), glucagon (**B**) and serum 3-OMG (**C**) concentrations following intragastric administration of 3 g (TRP-3) or 1.5 g (TRP-1.5) tryptophan, or control, in participants with type 2 diabetes. (**A**) After tryptophan alone (e.g. t=-31 to -1 min), there was an effect of treatment on C-peptide $AUC_{-31\ to\ -1min}$ (P=0.002), which tended to be greater after TRP-3 (*P=0.06), and was greater after TRP-1.5 (**P=0.002), compared with control. There was also an effect on C-peptide at t=-1 min (P=0.01), which was greater after both TRP-3 and TRP-1.5 compared with control (*P<0.05 for both). Following the mixed-nutrient drink (e.g. t=0 to 120 min), there was an effect of treatment on C-peptide $AUC_{-1\ to\ 120min}$ (P=0.01), which tended to be greater after TRP-1.5, but not TRP-3 (***P=0.06), (**B**) After tryptophan alone, there was an effect of treatment on fasting glucagon $AUC_{-31\ to\ -1min}$ (P=0.04), however, this was lost on post-hoc comparisons. There was also an effect on glucagon at t=-1 min (P=0.007), which tended to be greater after TRP-3 compared with control (*P=0.07). Following the mixed-nutrient drink, plasma glucagon rose on all days. However, there was no effect of treatment on glucagon $AUC_{-1\ to\ 120min}$, or a treatment*time interaction, (**C**) There was no effect of treatment on serum 3-OMG $AUC_{0\ to\ 120min}$, or a treatment*time interaction. Data are expressed as means±SEM; *n*=12. Data were analysed using general linear model mixed-model analysis,

with baseline as covariate, and repeated-measures ANOVA, adjusted for multiple comparisons using Bonferroni correction.

4.5 DISCUSSION

Our study indicates that in type 2 diabetes, intragastric administration of tryptophan, in a dose of 3 g, but not 1.5 g, delays the rise in, but does not result in an overall lowering of, the plasma glucose response to a carbohydrate-containing mixed-nutrient drink, contrasting our previous observation in lean individuals or those with obesity [192]. Tryptophan slowed gastric emptying modestly, in line with our recent findings in lean people and normoglycaemic people with obesity [192], which was likely responsible for delaying the rise in postprandial plasma glucose. Tryptophan also stimulated C-peptide, reflecting insulin secretion, although this effect was very small, as well as glucagon; the latter, therefore, most likely counteracted any glucose-lowering effect of insulin.

Protein "preloads" containing whey [297] and oral administration of specific amino acids [389] have been shown to diminish postprandial glycaemic excursions in type 2 diabetes and, accordingly, have the potential to be used in the management of type 2 diabetes. We have reported that TRP-3, but not TRP-1.5, modestly lowered blood glucose in response to a mixed-nutrient drink in lean people and those with obesity [192]. We, therefore, hypothesised that, in people with type 2 diabetes, in whom postprandial blood glucose is characteristically elevated, the blood glucose-lowering effect of tryptophan would be enhanced. Contrary to our hypothesis, tryptophan did not diminish the overall postprandial blood glucose, at either dose. However, TRP-3 did reduce the initial glycaemic response to the drink, but modestly, by delaying the rise in blood glucose.

Gastric emptying is a major determinant of the glycaemic response to carbohydrate-containing meals in health, obesity and type 2 diabetes [255], and our previous study showed that TRP-3,

administered intragastrically, slowed gastric emptying in both lean people and those with obesity [192]. Accordingly, the finding that tryptophan, in the same dose, slowed gastric emptying in people with type 2 diabetes is consistent with these observations and is likely to account for the delayed rise in blood glucose. It needs to be recognised, though, that the magnitude of the slowing of gastric emptying was modest, which may explain the lack of effect on blood glucose lowering. The slowing of gastric emptying after TRP-3 may explain the apparent lack of effect of tryptophan on glucose absorption, as measured by serum 3-OMG. That is, any effect of TRP-3 on glucose absorption may have been masked by its effect to slow gastric emptying. Because our study did not include healthy participants as controls, the effect of tryptophan on glucose absorption in health, and whether any effect of tryptophan on glucose absorption may be altered in type 2 diabetes, remains unclear.

TRP-3 tended to stimulate, and TRP-1.5 had a small effect to increase, plasma C-peptide immediately before the nutrient drink. We have reported that intraduodenal tryptophan, at a load of 0.15 kcal/min, providing ~3.3 g over 90 min, had a small effect to stimulate insulin in health [167]. This stimulation of insulin may reflect a direct effect of circulating amino acids [389] or be secondary to the secretion of the incretin hormones, GLP-1) or GIP, which both have glucose-dependent insulinotropic effects [266,276]. The insulin-stimulatory effect of GIP is known to be markedly attenuated in type 2 diabetes [390]. In healthy participants, the small insulinotropic effect of tryptophan is likely to be independent of GLP-1 and GIP [167], as insulin stimulation occurred at blood glucose levels of <8 mmol/L [391]. After the drink, TRP-1.5, but not TRP-3, tended to increase C-peptide compared with control; this may reflect the lack of effect of TRP-1.5 to slow gastric emptying. Nevertheless, overall the insulinotropic capacity of tryptophan in the doses examined is modest. Therefore, the absence of overall glucose-lowering by tryptophan is most likely due to only small insulin stimulation and modest slowing of gastric

emptying, combined with relatively more potent stimulation of glucagon, possibly as a direct response to circulating amino acids [389]. A progressive rise in glucagon for ~30 min, followed by a sustained elevation, was evident on all three days, consistent with the well-established failure of postprandial glucagon suppression characteristic of type 2 diabetes [392].

The potential mechanism(s) underlying the differences in effects of tryptophan observed in this study, compared with those in healthy people [192], deserve some consideration. Tryptophan may modulate GI motility, and, thus, gastric emptying, locally via its metabolite, serotonin [393], or via CCK [167,294]. Tryptophan-induced stimulation of CCK most likely involves Ca^{+2}-sensing receptors (CaSR) on enteroendocrine L cells [394]. Whether tryptophan-induced stimulation of CCK is altered in type 2 diabetes warrants evaluation in future studies. The effect of tryptophan to stimulate insulin may be directly at the pancreatic β-cell via activation of G protein-coupled receptor 142 (GPR142) and/or CaSR [395-397], and may also involve serotonin [398]. Since people with type 2 diabetes have β-cell dysfunction, it is possible that the effects of tryptophan on one or more of these mechanisms may be attenuated, or the expression of these receptors altered, in type 2 diabetes [399]. This warrants further investigation.

Some potential limitations of our study should be appreciated. We deliberately studied patients with well-controlled type 2 diabetes (i.e. glycated haemoglobin is more dependent on postprandial glucose) without autonomic neuropathy and managed on oral hypoglycaemic drugs, but not insulin, as we considered that a glucose-lowering effect of tryptophan would be most evident in this group. It would be anticipated that in the cohort studied, the rate of gastric emptying would be normal, or slightly accelerated [255]. The two doses of tryptophan were based on our previous studies; while it is tempting to speculate that larger doses may have more potent effects, our previous studies have indicated that these may be associated with adverse

effects [167]. Our study utilised intragastric administration of tryptophan, however, administration in capsules is also possible [338], providing for a more practical application outside the laboratory setting. We only included men to avoid any effects of the menstrual cycle on gastric emptying [364], thus, extrapolation of our observations to females should, of necessity, be circumspect.

In conclusion, our study suggests that in type 2 diabetes, administration of tryptophan in a dose of 3 g before a carbohydrate-containing drink delays the rise in plasma glucose, probably as a result of slowing of gastric emptying, but does not affect the overall blood glucose response.

CHAPTER 5: Effects of intraduodenal infusion of lauric acid and L-tryptophan, alone and combined, on glucoregulatory hormones, gastric emptying and postprandial glycaemia in healthy men

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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. I am the primary author of this paper.		
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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 and aims: In healthy men, intraduodenal administration of the fatty acid, lauric acid ("C12") and the amino acid, L-tryptophan ("TRP"), at loads that individually do not affect energy intake, reduce energy intake substantially when combined, associated with greater stimulation of CCK. Cholecystokinin potently slows gastric emptying, a key determinant of postprandial blood glucose. Accordingly, the combination has the potential for greater postprandial blood glucose lowering than either nutrient alone.

Methods: Twelve healthy, lean men (age: 28±2 years) received, on 4 separate occasions, 45-min intraduodenal infusions of C12 (0.3 kcal/min), TRP (0.1 kcal/min), C12+TRP (0.4 kcal/min), or 0.9% saline (control), in randomised, double-blind fashion. 30 min after commencement of the infusion a mixed-nutrient drink, containing 100 mg ^{13}C-acetate, was consumed and gastric emptying measured (^{13}C breath-test) for 3 hours. Blood samples were obtained at baseline, in response to treatments alone, and for 2 h post-drink for measurements of plasma glucose, C-peptide and GLP-1. "Early" (first 30 min) and "overall" glycaemic and hormone responses were evaluated.

Results: C12+TRP and C12 delayed the early rise ($P<0.05$), but did not affect overall postprandial plasma glucose, compared with control and TRP. C12+TRP slowed gastric emptying compared with control ($P<0.01$) and TRP ($P<0.05$), and C12 tended to slow gastric emptying compared with control ($P=0.09$). C12+TRP tended to delay ($P=0.07$), and C12 delayed ($P<0.05$), the early rise in C-peptide compared with control; moreover, C12+TRP delayed ($P<0.05$), and C12 tended to delay ($P=0.06$), the early rise in C-peptide compared with TRP. Only C12+TRP stimulated early and overall GLP-1 compared with control (all $P<0.05$).

Conclusion: Both C12+TRP and C12 delay the rise in postprandial glucose, probably primarily by slowing of gastric emptying, while TRP was ineffective. Therefore, in health, C12+TRP and C12, in the loads administered, appear to have comparable blood glucose-lowering effects.

5.2 INTRODUCTION

The functions of the upper GI tract, including the release of gut hormones, particularly CCK and GLP-1, and the rate of gastric emptying, are fundamental to the regulation of postprandial glycaemic excursions and energy intake [126,130,379]. Dietary nutrients, particularly fat and protein, when infused into the duodenum, potently stimulate gut hormones and pyloric pressures (a major mechanism underlying the slowing of gastric emptying) [144,160,256] and suppress subsequent energy intake [147]. Moreover, nutrient "preloads", e.g. 30 mL olive oil or 55 g whey protein, when consumed ~30 min before a carbohydrate meal, slow gastric emptying, stimulate GLP-1 and attenuate the postprandial rise in glucose [297,400]. These effects, however, necessitate administration of relatively large caloric loads (~220-270 kcal), probably because of the time required for digestion of fat and protein to release fatty acids [148,149] and amino acids [162,163], required to elicit the effects.

Amongst the products of fat and protein digestion, the fatty acid, lauric acid ("C12"), and the amino acid, L-tryptophan ("TRP"), appear to have particularly potent effects [167,293]. For example, a 90-min intraduodenal infusion of C12, at a load of 0.4 kcal/min (~4 g), stimulated pyloric pressures and plasma CCK and GLP-1 concentrations, and suppressed *ad libitum* energy intake in healthy men [293]. Moreover, in people with type 2 diabetes, pellets containing 2.35 g C12, as well as other nutrients, stimulated GLP-1 release and attenuated postprandial glucose [295]. Intraduodenal TRP, at a load of 0.15 kcal/min for 90 min (~3.3 g), also stimulated pyloric pressures and plasma CCK and suppressed energy intake [167]. Moreover, in men with obesity intragastric administration of 3 g TRP 15 min before a mixed-nutrient drink reduced the glycaemic response and slowed gastric emptying in both lean men and those with obesity [192].

To maximise effects of gut stimuli to suppress appetite and energy intake, and minimise the potential for adverse effects, there has been interest in combining different stimuli, including nutrients. For example, in healthy men the combination of gastric distension, achieved by gradually filling a bag with air, and intraduodenal lipid infusion increased the perception of fullness much more than either stimulus alone [124]. Moreover, the combination of gastric distension with a water-filled balloon and intravenous infusion of CCK-8, at levels that individually had no effect, reduced subsequent food intake [139]. Most recently, we demonstrated that 90-min intraduodenal infusions of C12 and TRP, at loads of 0.3 kcal/min and 0.1 kcal/min, respectively, that individually had no effect on energy intake, reduced energy intake substantially when combined, associated with much greater stimulation of plasma CCK and suppression of ghrelin [346]. In contrast, both C12 and the combination of C12 and TRP, but not TRP, stimulated plasma GLP-1 comparably [346]. Since CCK potently slows gastric emptying [401], the combination of C12 and TRP has the potential to lead to greater postprandial blood glucose lowering than either nutrient alone. Alternatively, if the observed effect on GLP-1 is more reflective of the outcome, then blood glucose lowering may be greater in response to C12 and C12+TRP.

The aim of the current study was to assess the effects of intraduodenal infusion of C12 and TRP, alone and combined, on the glycaemic and glucoregulatory hormone responses to, and gastric emptying of, a mixed-nutrient drink in healthy lean men.

5.3 PARTICIPANTS AND METHODS

5.3.1 Participants

Twelve healthy, lean men (mean age: 28±2 (range: 18-41) years, BMI: 23.8±0.6 (range: 20-25) kg/m^2) participated in the study (**Figure 5.1**). Men only were included, primarily to avoid the confounding effects of the menstrual cycle on gastric emptying [364]. Participants were recruited through advertisements on online sites (University of Adelaide and Gumtree) and by flyers placed at the Universities of Adelaide and South Australia. All participants had been weight-stable (i.e. <5% fluctuation) in the 3 months preceding the study. They were also unrestrained eaters (score ≤12 on the restrained eating component of the 3-factor eating questionnaire [351]). None had a history of GI disorders or symptoms, cardiovascular or respiratory diseases or surgery, low serum ferritin (<30 ug/L) or iron (<8 umol/L) levels (a requirement by the Human Research Ethics Committee), used supplements or medications affecting GI functions and/or appetite, smoked, consumed >20 g/d of alcohol on >5 days a week, were lactose intolerant or vegetarian. Once a participant was enrolled in the study, they were allocated to a treatment order of balanced randomisation (www.randomization.com) by a research officer who was not involved in data analysis. The Human Research Ethics Committee of the Central Adelaide Local Health Network approved the study protocol, and the study was performed in accordance with the Declaration of Helsinki and the NHMRC Statement on Ethical Conduct in Human Research. All participants provided written informed consent before their inclusion. The trial was registered with the Australian New Zealand Clinical Trial Registry (www.anzctr.org.au; trial number: ACTRN12619001666112).

5.3.2 Study outline

Study design

We investigated the effects of 45-min intraduodenal infusions of 1) lauric acid ("C12") at 0.3 kcal/min (total: 13.5 kcal), 2) L-tryptophan ("TRP") at 0.1 kcal/min (total: 4.5 kcal), 3) a combination of 2) and 3) ("C12+TRP") at 0.4 kcal/min (total: 18 kcal), or 4) isotonic 0.9% saline ("control") on plasma glucose, C-peptide (a measure of insulin secretion), and GLP-1 concentrations in response to, and gastric emptying of, a mixed-nutrient drink, consumed 30 min after the commencement of intraduodenal infusion of treatments (**Figure 5.2**). The nutrient loads and an infusion duration of 90 min were initially selected based on our previous study [346]. However, in preliminary studies we found that a 90-min infusion was poorly tolerated in some volunteers being associated with vomiting ~15-30 min after the nutrient drink. Thus, we reduced the duration of the infusion to 45 min.

Study treatments

The C12 solution contained 5.55 g of food-grade lauric acid (C12:0; Sigma-Aldrich, Milwaukee, Winconsin, USA), which was dissolved with 4.5 g NaCl and 0.65 g NaOH in distilled water. The TRP solution was prepared by dissolving 4.07 g crystalline, food-grade L-tryptophan (PureBulk Inc., Rosenburg, Oregon, USA), 4.1 g NaCl and 0.1 g NaOH in distilled water. The C12+TRP solution contained 5.55 g lauric acid, 4.07g crystalline L-tryptophan, 3.8 g NaCl and 0.08 g NaOH, all dissolved in distilled water. Finally, the control solution consisted of 4.9 g NaCl and 0.08 mL NaOH solution (prepared by dissolving 1.75 g NaOH in 250 mL water) in distilled water. Solutions were isotonic (300 mOsm), had a pH of 7.8-8.1, were prepared to a final volume of 500 mL and infused at a rate of 3 mL/min, so the total volume infused in 45 min was 135 mL. Solutions were prepared on the morning of the study by a research officer, who was not involved in the data analysis, and the infusion pump was covered,

so that neither the investigators performing the study nor the participant were aware of the nature of the infusions.

Study protocol

Participants were studied in a randomised, double-blind, cross-over fashion on 4 occasions, each separated by 3-7 days. Participants were provided with a standardised meal (Beef lasagne, McCain Food, Wendouree, Victoria, Australia; energy content: 603 kcal), to be consumed between 6.30 p.m. and 7 p.m. on the night prior to each study. All participants were instructed to maintain their normal eating habits between study days, refrain from strenuous exercise and alcohol for 24 h before each study, and to abstain from any other food and drinks, except water (which was allowed until 7 a.m.), during and after the evening meal and until they attended the Clinical Research Facility at the University of Adelaide at 8 a.m. the next morning. Participants were intubated with a 17-channel manometric catheter (outer diameter: 3.5 mm, total length: 100 cm; Dentsleeve International, Mui Scientific, Ontario, Canada) [291], which was inserted into the stomach through an anaesthetised nostril and allowed to pass into the duodenum by peristalsis [147]. 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. An additional side-hole was located ~14.5 cm distal to the sleeve and used for intraduodenal infusion of nutrient or control solutions. 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 [402]. Once the catheter was positioned correctly (within ~53±3 min (means±SEM), across study days and participants, at ~9 a.m.), an intravenous cannula was placed into a forearm vein, and the arm was kept warm with a heat pad for regular sampling of "arterialized" blood, for subsequent analysis of plasma glucose, C-peptide and GLP-1

concentrations. Immediately following the completion of phase III of the fasting motility pattern (i.e. during phase I, a period of motor quiescence), at t=-30 min, a fasting blood sample (10 mL) was taken, a visual analogue scale (VAS) questionnaire for the assessment of nausea and drowsiness completed, and intraduodenal infusion of one of the four solutions commenced and continued for 45 min (to t=15 min) (**Figure 5.2**). At t=-1 min, participants consumed, within 1 min, 350 mL of a mixed-nutrient drink (Resource Plus® vanilla (Nestle Healthcare Nutrition, Tongala, Victoria, Australia, 325 mL, 500 kcal, 74 g carbohydrates, including maltodextrin and sucrose, 18 g protein, 15 g fat), plus 25 mL water to make up the final volume) labelled with 100 mg ^{13}C-acetate for measurement of gastric emptying by breath test [352]. Blood samples, VAS ratings and breath samples were obtained at regular intervals throughout the study (**Figure 5.2**). Following the drink, blood samples and VAS ratings were collected for 120 min and breath samples for 180 min. At t=180 min, after a final breath sample, each participant was provided with a light lunch, after which they were allowed to leave the laboratory.

5.3.3 Measurements

Plasma glucose, C-peptide and GLP-1 concentrations

Blood samples (10 mL) were collected into ice-chilled ethylenediaminetetraacetic acid-containing tubes. Plasma was separated by centrifugation at 3200 rpm for 15 min at 4 °C within 15 min of collection and stored at -80 °C until analysed.

Plasma glucose concentrations (mmol/L) were quantified by the glucose oxidase method using a glucose analyser (YSI 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, Ohio, USA). Intra- and inter-assay coefficients of variation (CVs) were ≤2%.

Plasma C-peptide concentrations (pmol/L) were measured by ELISA immunoassay (10-1136-01, Mercodia, Uppsala, Sweden). The sensitivity of the assay was 15 pmol/L and intra- and inter-assay CVs were 6.8% and 3.0%, respectively. C-peptide reflects endogenous insulin secretion, since it is not extracted by the liver [385].

Plasma total GLP-1 concentrations (pmol/L) were measured by RIA (GLPIT-36HK, Millipore, Billerica, Massachusetts, USA). The intra- and inter-assay CVs were 5.8% and 6.6%, respectively. The minimum detectable concentration was 3 pmol/L.

Gastric emptying

Gastric emptying was assessed using a ^{13}C-acetate breath test, measuring $^{13}CO_2$ ($^{1\text{-}13}$C99%, Cambridge Isotope Laboratories, Inc., Andover, Massachusetts, USA) concentrations in exhaled air of end-expiratory breath samples, which were analysed using infrared spectroscopy (FANci2 breath test analyser, Fischer Analysen Instrumente GmbH, Leipzig, Germany). Breath sample data were expressed as percentage recovery of $^{13}CO_2$ in the breath per hour [352,403]. The gastric half-emptying time (T50), defined as the time taken for 50% of the meal to empty from the stomach [404], was also calculated.

Nausea and drowsiness

C12 at loads of >0.4 kcal/min and TRP at loads of >0.15 kcal/min can induce nausea or drowsiness in some individuals [167,291], thus, these were assessed using a VAS questionnaire. This consisted of 100-mm horizontal lines where 0 mm represented “not felt at all” and 100 mm “felt the strongest possible”. Participants were asked to place a vertical mark on each horizontal line to rate the strength of each sensation felt at that point in time.

5.3.4 Statistical analysis

The number of participants studied was determined by power calculations based on our previous work [192]. We calculated that *n*=12 participants would allow detection of a 1.0 mmol/L reduction in blood glucose assuming a within-subjects standard deviation of 1.1 mmol/L, at α=0.05 and a power of 80%.

Raw data for plasma glucose, C-peptide and GLP-1 concentrations and VAS ratings were used to calculate total areas under the curve (AUCs), using the trapezoidal rule, from t=-30 to -1 min (to assess the effects of treatment alone), and t=-1 to 120 min (to assess the response to the mixed-nutrient drink), and gastric emptying from t=0 to 180 min. All AUC data and raw data for peak glucose, time to peak glucose and gastric half-emptying time (T50) were analysed using general linear mixed models, including treatment as a fixed factor and baseline as a covariate, and an unstructured covariance structure to account for repeated visits per participant, to evaluate effects of treatments on these outcomes. Post-hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed when significant treatment effects were found. Within-subject correlations [369], using AUCs for all variables, were performed to evaluate relationships between plasma glucose, C-peptide and GLP-1 $AUCs_{\text{-1 to 120min}}$ and $AUCs_{\text{-1 to 30 min}}$ with gastric emptying $AUCs_{\text{0 to 180min}}$ and $AUCs_{\text{0 to 30min}}$, and between plasma glucose $AUCs_{\text{-1 to 120min}}$ and $AUCs_{\text{-1 to 30 min}}$ with C-peptide and GLP-1 $AUCs_{\text{-1 to 120min}}$ and $AUCs_{\text{-1 to 30 min}}$. Statistical analysis was performed using SPSS software (version 25, IBM, Chicago, Illinois, USA). Differences were considered statistically significant at $P \leq 0.05$. All data are reported as means±SEM.

5.4 RESULTS

All participants completed the study and tolerated it well. Three participants reported one episode of loose stools, in both cases within 1-2 hours of the completion of the infusion, one after C12 and two after C12+TRP. These symptoms resolved promptly. Due to technical issues, gastric emptying could only be measured in 10 of the 12 participants. There were no differences in baseline plasma glucose or hormone concentrations, or VAS scores, between study days.

5.4.1 Plasma glucose concentrations

Response to nutrient treatments alone: There was no effect of treatment on plasma glucose $AUC_{-30\ to\ -1min}$ (**Table 5.1**, **Figure 5.3**).

Response to the mixed-nutrient drink: There was a rise in glucose on all days. There was no effect of treatment on plasma glucose $AUC_{-1\ to\ 120min}$. However, there was an effect of treatment on plasma glucose $AUC_{-1\ to\ 30min}$ ($P<0.001$), reflecting the early glucose response, which was less after C12+TRP and C12 compared with control and TRP (all $P<0.05$).

There was no effect of treatment on peak glucose. However, there was an effect of treatment on time to peak glucose ($P<0.01$), which was greater after C12+TRP and C12 compared with control and TRP (all $P<0.05$).

5.4.2 Gastric emptying

There was no effect of treatment on gastric emptying $AUC_{0\ to\ 180min}$ of the mixed-nutrient drink. However, there was an effect of treatment on gastric emptying $AUC_{0\ to\ 30min}$ ($P<0.01$), which was less after C12+TRP compared with control ($P<0.01$) and TRP ($P<0.05$), and tended to be

less after C12 compared with control (P=0.09). There was no effect of treatment on the T50 (P=0.3) (**Table 5.1**, **Figure 5.4**).

5.4.3 Plasma C-peptide concentrations

Response to nutrient treatments alone: There was no effect of treatment on plasma C-peptide $AUC_{-31\ to\ -1min}$ (**Table 5.1**, **Figure 5.5.A**).

Response to the mixed-nutrient drink: There was a rise in C-peptide on all days. There was an effect of treatment on plasma C-peptide $AUC_{-1\ to\ 120min}$ (P<0.01), which tended to be less after C12+TRP compared with TRP (P=0.07). There was also an effect of treatment on plasma C-peptide $AUC_{-1\ to\ 30min}$ (P<0.05), which tended to be less after C12+TRP (P=0.07), and was less after C12, compared with control (P<0.05), and was also less after C12+TRP (P<0.05), and tended to be less after C12, compared with TRP (P=0.06).

5.4.4 Plasma GLP-1 concentrations

Response to nutrient treatments alone: There was a trend for an effect of treatment on plasma GLP-1 $AUC_{-31\ to\ -1min}$ (P=0.08) (**Table 5.1**, **Figure 5.5.B**).

Response to the mixed-nutrient drink: There was a rise in GLP-1 on all days. There was an effect of treatment on plasma GLP-1 $AUC_{-1\ to\ 120min}$ (P<0.05), which was greater after C12+TRP compared with control (P<0.05), and tended to be greater after C12+TRP compared with C12 (P=0.06), but not TRP (P=0.3). There was an effect of treatment on plasma GLP-1 $AUC_{-1\ to\ 30min}$ (P<0.05), which was greater after C12+TRP compared with control (P<0.05), and tended to be greater after C12+TRP compared with TRP (P=0.08), but not C12 (P=0.4).

5.4.5 Nausea and drowsiness

There were no effects of treatment on nausea or drowsiness, in response to nutrients or the drink.

5.4.6 Relationships among plasma glucose, glucoregulatory hormones and gastric emptying

There were positive correlations between early plasma glucose ($AUC_{-1\ to\ 30min}$) ($r=0.56$, $P<0.01$), early C-peptide ($AUC_{-1\ to\ 30min}$) ($r=0.55$, $P<0.01$), and a negative correlation between early GLP-1 ($AUC_{-1\ to\ 30min}$) ($r=-0.52$, $P<0.01$), with early gastric emptying ($AUC_{0\ to\ 30min}$). There was a negative correlation between early plasma glucose ($AUC_{-1\ to\ 30min}$) with early GLP-1 ($AUC_{-1\ to\ 30min}$) ($r=-0.40$, $P<0.01$), and a positive correlation between overall plasma glucose ($AUC_{-1\ to\ 120min}$) with overall C-peptide ($AUC_{-1\ to\ 120min}$) ($r=0.47$, $P<0.01$).

Table 5.1
Plasma glucose, C-peptide and GLP-1 concentrations in response to treatment alone (t=-30 to -1 min) and a mixed-nutrient drink (t=-1 to 120 min; "early" response t=-1 to 30 min), and gastric emptying of the drink (t=0 to 180 min; "early" response t=0 to 30 min), consumed 30 min after the commencement of intraduodenal infusion of control, lauric acid ("C12") at 0.3 kcal/min, tryptophan ("TRP") at 0.1 kcal/min, or C12+TRP at 0.4 kcal/min in healthy men[1].

	Treatment				**P value (main effect)**
	Control	**C12**	**TRP**	**C12+TRP**	
Plasma glucose					
$AUC_{-30\ to\ -1min}$ (mmol/L*min)	137±1	139±2	138±2	136±1	NS
$AUC_{-1\ to\ 120min}$ (mmol/L*min)	652±16	662±21	675±13	641±16	NS
$AUC_{-1\ to\ 30min}$ (mmol/L*min)	162±3	144±4*#	167±4	139±4*#	<0.001
Peak (mmol/L)	6.6±0.2	6.7±0.2	6.7±0.3	6.4±0.2	NS
Time to peak (min)	33±4	49±4*#	30±3	54±6*#	<0.01
Gastric emptying					
$AUC_{0\ to\ 180min}$ (%recovery of $^{13}CO_2$/hour*min)	3219±242	3316±108	3389±77	2825±212	NS
$AUC_{0\ to\ 30min}$ (%recovery of $^{13}CO_2$/hour*min)	233±20	144±24	215±21	95±22α#	<0.01
T50 (min)	170±20	192±11	174±18	226±53	NS
Plasma C-peptide					
$AUC_{-30\ to\ -1min}$ (pmol/L*min)	9532±225	14088±974	13054±554	13925±886	NS
$AUC_{-1\ to\ 120min}$ (pmol/L*min)	179496±12382	181461±19334	195777±21385	160213±23645	<0.01
$AUC_{-1\ to\ 30min}$ (pmol/L*min)	28694±2362	21248±1771*	31704±3755	20769±3665#	<0.05
Plasma GLP-1					
$AUC_{-30\ to\ -1min}$ (pmol/L*min)	412±17	498±37	456±18	532±37	0.08
$AUC_{-1\ to\ 120min}$ (pmol/L*min)	2577±89	2876±210	2840±155	3276±240*	<0.05
$AUC_{-1\ to\ 30min}$ (pmol/L*min)	598±31	747±68	682±55	857±88*	<0.05

[1]Data are means±SEM, *n*=12 (except gastric emptying, *n*=10). AUCs of plasma glucose, gastric emptying, plasma GLP-1 and C-peptide and raw data for peak glucose, time to peak glucose and T50 were analysed using general linear mixed models, including treatment as a fixed factor and baseline as a covariate, and an unstructured covariance structure to account for repeated visits per participant, with adjustments for multiple comparisons made using Bonferroni correction. AUC, area under the curve; GLP-1, glucagon-like peptide-1; T50, gastric half-

emptying time; NS, not significant. * different from control, P<0.05, # different from TRP, P<0.05, α different from control, P<0.01, β different from TRP, P<0.01.

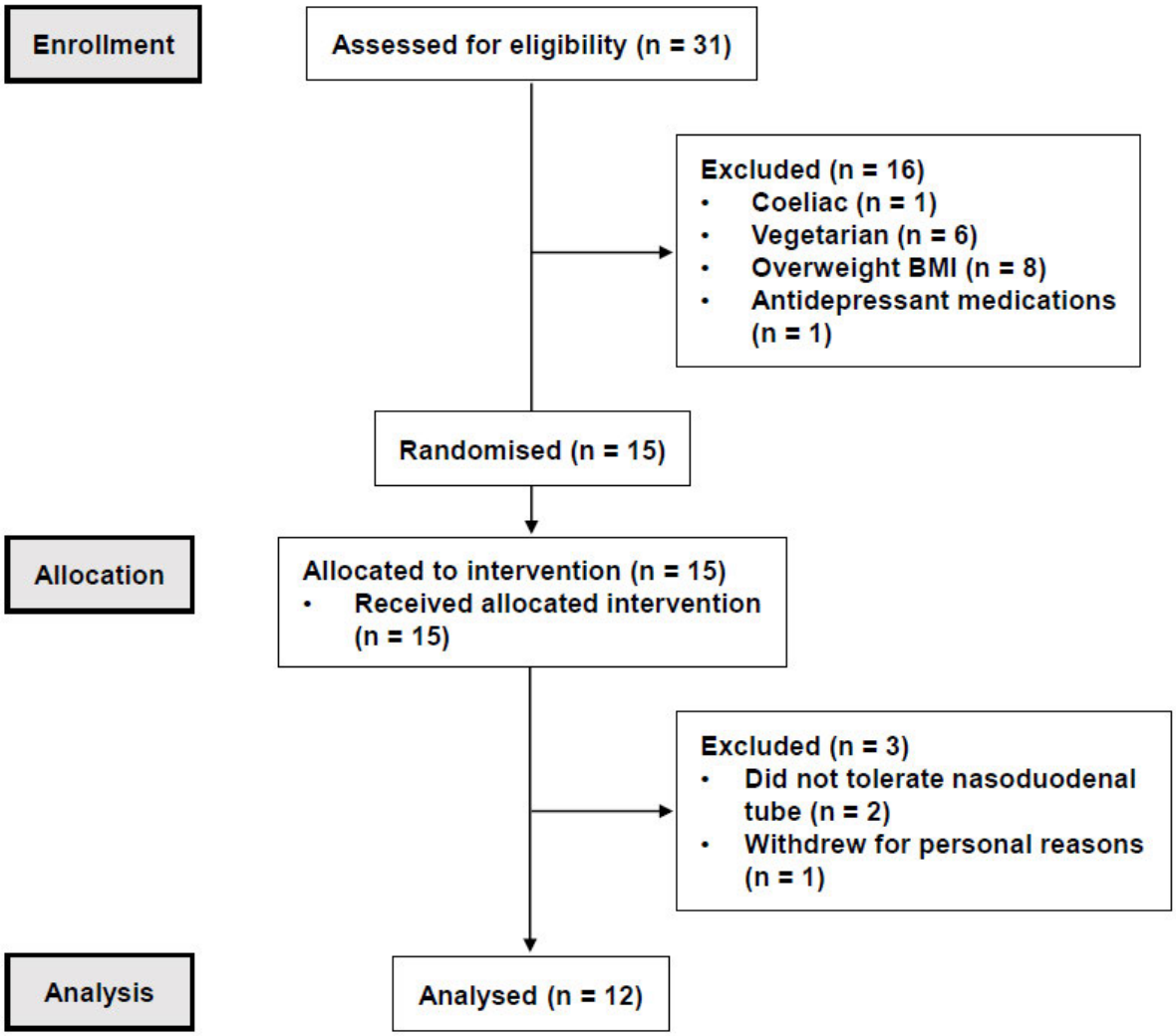

Figure 5.1
CONSORT Flow diagram.

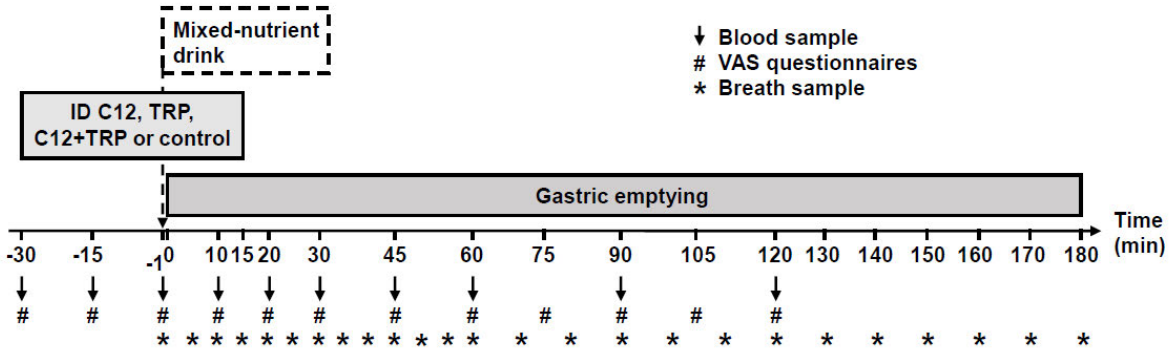

Figure 5.2
Schematic of the study design. On study days, intraduodenal infusion of one of the three treatment solutions (i.e. lauric acid ("C12") at 0.3 kcal/min (total: 13.5 kcal), L-tryptophan ("TRP") at 0.1 kcal/min (total: 4.5 kcal) or a combination of lauric acid and tryptophan ("C12+TRP") at 0.4 kcal/min (total: 18 kcal)) or control (isotonic 0.9% saline) commenced at t=-30 min and continued for 45 min (to t=15 min). At t=-1 min, participants consumed, within 1 min, a mixed-nutrient drink (350 mL; 500 kcal) labelled with 100 mg ^{13}C-acetate for measurement of gastric emptying by breath test. Blood and breath samples and visual analogue scale (VAS) ratings were obtained at baseline and at regular intervals throughout the study. At t=180 min, after a final breath sample, each participant was provided with a light lunch, following which they were allowed to leave the laboratory.

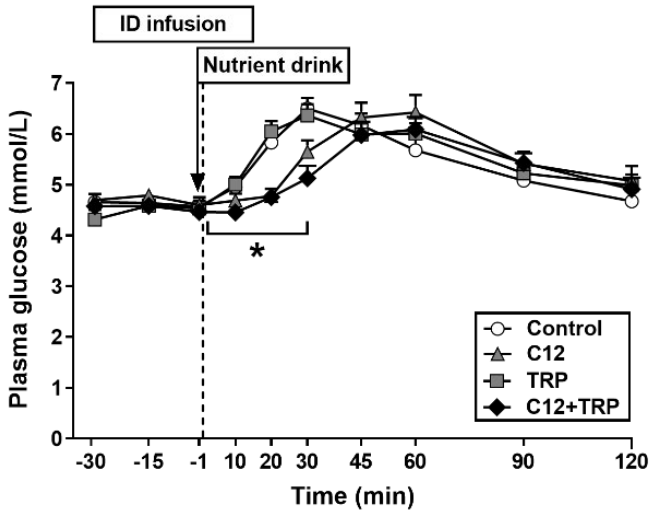

Figure 5.3
Plasma glucose concentrations following intraduodenal infusion of control, lauric acid ("C12") at 0.3 kcal/min, tryptophan ("TRP") at 0.1 kcal/min or C12+TRP at 0.4 kcal/min in healthy men. In response to treatment alone, there was no effect of treatment on plasma glucose $AUC_{-30\ to\ -1min}$. In response to the mixed-nutrient drink, there was no effect of treatment on plasma glucose $AUC_{-1\ to\ 120min}$; however, there was an effect on plasma glucose $AUC_{-1\ to\ 30min}$ ($P<0.001$), which was less after C12+TRP and C12 compared with control and TRP (all *$P<0.05$). Data are expressed as means±SEM, *n*=12. Data were analysed using general linear mixed models, including treatment as a fixed factor and baseline as a covariate, and an unstructured covariance structure to account for repeated visits per participant, and adjustments for multiple comparisons were made using Bonferroni correction.

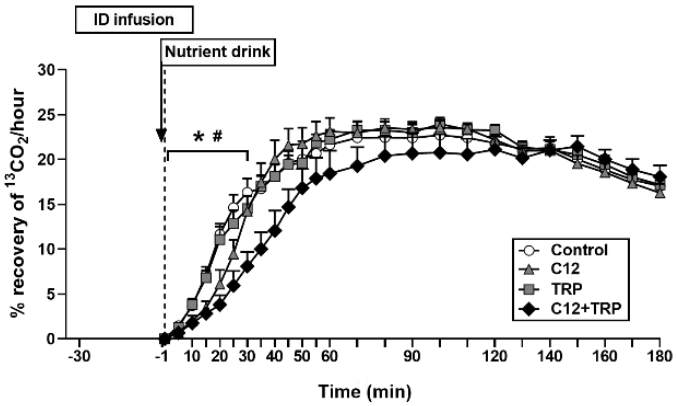

Figure 5.4
Gastric emptying, expressed as %recovery of $^{13}CO_2$/hour, following intraduodenal infusion of control, lauric acid ("C12") at 0.3 kcal/min, tryptophan ("TRP") at 0.1 kcal/min or C12+TRP at 0.4 kcal/min in healthy men. There was no effect of treatment on gastric emptying $AUC_{0\ to\ 180min}$ of the drink; however, there was an effect on $AUC_{0\ to\ 30min}$ ($P<0.01$), which was less after C12+TRP compared with control ($^{*}P<0.01$) and TRP ($^{\#}P<0.05$) and tended to be less after C12 compared with control ($P=0.09$). Data are expressed as means±SEM, *n*=10. Data were analysed using general linear mixed models, including treatment as a fixed factor and baseline as a covariate, and an unstructured covariance structure to account for repeated visits per participant, and adjustments for multiple comparisons were made using Bonferroni correction.

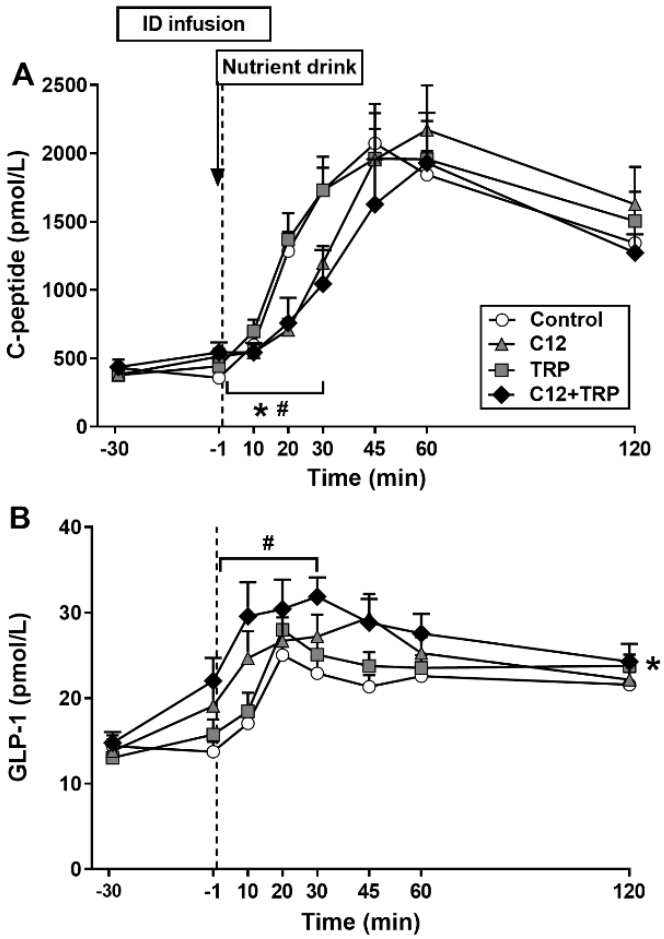

Figure 5.5
Plasma C-peptide (**A**) and GLP-1 (**B**) concentrations following intraduodenal infusion of control, lauric acid ("C12") at 0.3 kcal/min, tryptophan ("TRP") at 0.1 kcal/min, or C12+TRP at 0.4 kcal/min in healthy men. (**A**) In response to treatment alone, there was no effect of treatment on plasma C-peptide $AUC_{-31\ to\ -1min}$. In response to the mixed-nutrient drink, there was an effect of treatment on plasma C-peptide $AUC_{-1\ to\ 120min}$ ($P<0.01$), but no significant post-hoc comparisons. There was also an effect of treatment on plasma C-peptide $AUC_{-1\ to\ 30min}$ ($P<0.05$), which was less after C12 compared with control ($^{*}P<0.05$), and after C12+TRP compared with TRP ($^{\#}P<0.05$). (**B**) In response to treatment alone, there was a trend for an effect of treatment on plasma GLP-1 $AUC_{-31\ to\ -1min}$ ($P=0.08$). In response to the mixed-nutrient drink, there was an effect of treatment on plasma GLP-1 $AUC_{-1\ to\ 120min}$ ($P<0.05$), which was greater after C12+TRP compared with control ($^{*}P<0.05$). There was also an effect of treatment on plasma GLP-1 $AUC_{-1\ to\ 30min}$ ($P<0.05$), which was greater after C12+TRP compared with control ($^{\#}P<0.05$). Data are expressed as means±SEM, *n*=12. Data were analysed using general linear mixed models, including treatment as a fixed factor and baseline as a covariate, and an unstructured covariance structure to account for repeated visits per participant, and adjustments for multiple comparisons were made using Bonferroni correction.

5.5 DISCUSSION

This study evaluated whether intraduodenal infusion of a combination of C12 and TRP has a greater effect to slow gastric emptying, stimulate C-peptide and GLP-1 and suppress postprandial blood glucose than either nutrient alone. Key findings were that 1) C12+TRP delayed the early rise in postprandial glucose, related to slowing of gastric emptying and stimulation of GLP-1, but did not lower the overall glucose response, 2) C12 also delayed the early rise in postprandial glucose and tended to slow gastric emptying, but did not stimulate GLP-1, 3) the plasma C-peptide response was delayed by both C12+TRP and C12 – probably due to the slowing of gastric emptying, thus, insulin is unlikely to have contributed to the observed blood glucose lowering, and 4) TRP did not have any effect to lower glucose, slow gastric emptying or stimulate glucoregulatory hormones. Taken together, it appears that C12+TRP, in the doses used, delayed the rise in postprandial blood glucose, reflecting slowing of gastric emptying and GLP-1 stimulation, and the effect to lower postprandial blood glucose did not exceed that of C12 alone.

Our hypothesis that C12+TRP, when combined, would lead to greater lowering of postprandial plasma glucose than C12 and TRP alone, was based on the outcomes of our previous study, in which C12 and TRP, each administered in doses that had no effect on energy intake, suppressed energy intake substantially when combined, associated with markedly augmented release of CCK [346]. Since CCK is a key regulator of gastric emptying, and slowing of gastric emptying is a major determinant of postprandial blood glucose lowering, we reasoned that the combination of C12 and TRP would reduce postprandial glycaemia more than C12 and TRP alone. While C12+TRP was shown to delay the early rise in postprandial glucose in response to the carbohydrate-containing drink, the overall blood glucose response was not reduced. Interestingly, and contrary to our hypothesis, the effect of C12 to reduce glucose was

comparable to that of C12+TRP, while TRP alone was, indeed, ineffective. We studied healthy individuals in whom postprandial blood glucose is tightly controlled, and the drink, therefore, only caused a modest increase in blood glucose on the control day. Accordingly, evaluation of the effects of these nutrients in people with type 2 diabetes, who exhibit greater rises in postprandial glucose and, hence, may respond to the nutrients with greater blood glucose lowering, is warranted. Our findings also indicate that the effects of C12 and TRP alone, and in combination, on blood glucose differ from those on energy intake, suggesting that the mechanisms involved are likely to differ.

The key GI mechanisms regulating postprandial glycaemic control include the slowing of gastric emptying and the stimulation of glucoregulatory hormones, including insulin and the incretin hormones, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) [255]. C12+TRP substantially slowed gastric emptying, which reduced the delivery of carbohydrate/glucose to the small intestine, accounting for the delayed rise in postprandial glucose. The effect of C12+TRP to slow gastric emptying may reflect the combination of C12 and TRP, given that, in higher doses, each nutrient slows gastric emptying [192,293], and this is associated with the observed reduction in plasma C-peptide concentrations. Thus, early glucose lowering by C12+TRP was likely to primarily reflect the slowing of gastric emptying, but did not involve insulin or the incretin hormones, GLP-1 or GIP, whose insulinotropic properties are glucose dependant [263]. C12+TRP also stimulated GLP-1, as well as CCK [346], although the latter was not measured in the current study; both are likely to have contributed to the slowing of gastric emptying [275]. Unlike GLP-1, GIP, which may be the dominant incretin in health, does not appear to affect gastric emptying [263]. We did not measure plasma glucagon in the current study, however, in our previous study this was unaffected by C12, TRP or C12+TRP in the same doses [405]. Taken together, it appears that the slowing of gastric emptying and the

stimulation of GLP-1, independent of insulin secretion, are central to the effects of C12+TRP to delay the early rise in postprandial blood glucose.

C12 alone also delayed the early rise in blood glucose, and, to our surprise, this effect did not differ from that of C12+TRP. This effect of C12 was associated with a modest effect to slow gastric emptying during the first ~30 min, although the latter was much smaller than that of C12+TRP. However, we have demonstrated that even small changes in the rate of small intestinal glucose delivery can have a substantial impact on blood glucose concentration [258]. Mean levels of GLP-1 were greater, albeit not statistically significantly, after C12. C12 also delayed the rise in C-peptide. Accordingly, it appears that, like C12+TRP, C12 delayed the early rise in blood glucose by a modest slowing of early gastric emptying, possibly due to modest stimulation of GLP-1. We cannot discount the possibility that postabsorptive mechanisms contribute to glucose-lowering by C12. For example, in rats with high-fat diet-induced type 2 diabetes, oral administration of C12 for three weeks decreased fasting blood glucose by activating adenosine monophosphate-activated protein kinase pathways in the liver and increasing GLUT4 translocation with increased glucose uptake [406].

In contrast to C12 and C12+TRP, TRP alone, at a load of 0.1 kcal/min, had no effect to lower blood glucose and, consistent with previous studies [192,346], gastric emptying and C-peptide and GLP-1 release were not affected. Interestingly, in our previous study, using the same dose, TRP had a small, but significant, effect to stimulate CCK. However, it appears that in the absence of other factors to slow gastric emptying, e.g. GLP-1, as was the case with C12, this was insufficient to slow gastric emptying. Hence, TRP alone, unlike C12 alone, may have been unable to affect postprandial glucose.

The limitations of our study should be appreciated. We only evaluated acute effects of the nutrients, and only one dose of each nutrient. The study only evaluated lean men, primarily to avoid the effects of the menstrual cycle on gastric emptying [364]. Thus, extrapolation of our results to other populations, i.e. women, older individuals and people with type 2 diabetes should be circumspect. Our study utilised intraduodenal infusion to avoid interindividual variations in gastric emptying, therefore, the effect of intragastric or oral administration of these nutrients (i.e. in a more physiological setting) requires investigation. We only evaluated acute effects of the nutrients, and only one dose of each nutrient. We did not control for the energy content of the nutrients, and thus, cannot exclude the possibility that the observed effect of C12+TRP on blood glucose lowering was due to its energy content, however, we believe that this is unlikely given the differences in energy content were very small (4.5 and 13.5 kcal). Finally, the effects on the "early", but not "overall", responses may, in part, reflect the experimental paradigm, i.e. nutrients were infused intraduodenally for only 15 min after the drink, therefore, a relatively prompt "offset" in the response would be anticipated.

In conclusion, our findings suggest that in healthy men C12+TRP slowed gastric emptying substantially more than C12 alone, while TRP was ineffective. Similarly, C12+TRP induced a greater stimulation of GLP-1. In contrast, C-peptide stimulation was delayed by both C12+TRP and C12, while TRP alone had no effect. Despite these effects on C-peptide, C12+TRP and C12 delayed the early rise in blood glucose, suggesting that the effect on blood glucose was probably attributable to slowing of gastric emptying, brought about by the stimulation of gut hormones, including GLP-1 and CCK. In light of the observed glucose-lowering effect, studies in patients with type 2 diabetes are now warranted, as, due to their more elevated postprandial glucose concentrations, greater glucose lowering by these nutrients may be anticipated in this

population. These nutrients have the potential to offer a novel, nutrient-based treatment option for the management of type 2 diabetes.

CHAPTER 6: CONCLUSIONS

The studies reported in this thesis provide novel insights into the acute effects of dietary nutrients, specifically, the amino acid, L-tryptophan, and the fatty acid, lauric acid, on GI and pancreatic hormones, gastric emptying, plasma glucose and amino acids concentrations, and the subsequent energy intake in normal-weight participants and those with obesity and type 2 diabetes. The studies have examined (1) the relation between energy intake and the plasma tryptophan/LNAA ratio in lean men and those with obesity, and whether the plasma tryptophan/LNAA ratio differs between individuals who reduce their energy intake in response to intragastric administration of tryptophan compared with those who did not, (2) the effects of intragastric administration of tryptophan on energy intake from a buffet-meal, appetite perceptions, including hunger and fullness, plasma CCK, tryptophan and the tryptophan/LNAA ratio, and whether responses differ in lean men and those with obesity, (3) the effects of intragastric administration of tryptophan on postprandial glycaemia, glucoregulatory hormones, glucose absorption, and gastric emptying of a mixed-nutrient drink in people with type 2 diabetes, (4) the effects of intraduodenal infusion of lauric acid and tryptophan, alone and combined, on glycaemia and glucoregulatory hormone responses to and gastric emptying of a mixed-nutrient drink in healthy lean men.

As discussed in the Introduction, obesity and type 2 diabetes are increasingly prevalent worldwide and current approaches to management are suboptimal. Thus, the development of effective, low cost, and adverse effect-free, strategies for the management and prevention of these disorders is urgently needed. The work presented in this thesis broadly investigated significant gaps in literature in relation to the effects of tryptophan on energy intake and blood glucose and, in combination with lauric acid, on glycaemic control in health, obesity and type 2 diabetes.

The first study, presented in **Chapter 2**, represented a secondary analysis of a previous study with the primary goal to compare the effects of intragastric administration of tryptophan (intragastric administration was used due to the unpleasant taste of the amino acid) on the plasma tryptophan/LNAA ratio in lean individuals and those with obesity, and the associations of the tryptophan/LNAA ratio, gastric emptying and CCK concentrations with energy intake. This study established that intragastric tryptophan, in a dose of 3 g, increased the plasma tryptophan/LNAA ratio much more in the lean than those with obesity, and that the suppression of energy intake in response to tryptophan was related to the plasma tryptophan/LNAA ratio in the lean.

The subsequent study, presented in **Chapter 3**, addressed some of the limitations in study 1, including insufficient power calculation to detect differences in tryptophan concentration between treatments, small sample sizes in sub-groups, and providing a mixed-nutrient drink 15 min after tryptophan and 60 min before a buffet lunch, which may have interfered with the effects of tryptophan on energy intake. Hence, this study administered intragastric tryptophan, in doses of 1.5 g and 3 g, 30 min before a standardised buffet-meal and showed that tryptophan, in the dose of 3 g, suppressed energy intake from that meal, with no difference in the response between lean men and those with obesity, but did not affect premeal hunger or fullness. In both doses, tryptophan stimulated CCK potently before the meal, with no difference between groups. Tryptophan predictably increased both the plasma tryptophan and the tryptophan/LNAA ratio, with the effect of 3 g tryptophan greater than control and 1.5 g tryptophan, and a trend for the tryptophan/LNAA ratio to be less in men with obesity than lean men. Energy intake also correlated with pre-meal plasma tryptophan and the tryptophan/LNAA ratio, but not CCK, in both groups. Despite substantially lower energy intakes in response to 3 g tryptophan, post-meal hunger tended to be less, and fullness greater, compared with control,

and post-meal plasma tryptophan and the tryptophan/LNAA ratio remained elevated after 3 g and 1.5 g tryptophan and tended to be less in men with obesity than the lean. Therefore, intragastric tryptophan has potent energy intake-suppressant effects, which are maintained in obesity. That the suppression of energy intake was also related to circulating tryptophan and the tryptophan/LNAA ratio supports a role of post-absorptive mechanisms in the observed effects.

The study presented in **Chapter 4** aimed to examine whether tryptophan had effects to lower postprandial glycaemia by slowing gastric emptying in type 2 diabetes, given that a glucose-lowering effect of tryptophan had been demonstrated in both lean men and those with obesity. Intragastric administration of tryptophan, in a dose of 3 g, delayed the rise in, but did not result in an overall lowering of, the plasma glucose response to a carbohydrate-containing drink, in contrasts to previous observations in lean individuals and those with obesity. Tryptophan also slowed gastric emptying modestly, which was likely responsible for the delayed rise in plasma glucose. Tryptophan also stimulated C-peptide, reflecting insulin secretion, and glucagon, although the effects were small. The elevation in glucagon may well have counteracted any glucose-lowering effect of insulin.

The study presented in **Chapter 5** aimed to examine whether intraduodenal infusion of a combination of lauric acid, at a load of 3 kcal/min, and tryptophan, at a load of 0.1 kcal/min, for 45 min, would have a greater effect to lower postprandial glycaemia, than either stimulus alone, in lean men. The hypothesis was based on previous reported observations, in which lauric acid and tryptophan, in loads that individually did not affect energy intake, stimulated CCK and reduced energy intake when combined. Since CCK potently slows gastric emptying, and slowing of gastric emptying plays a key role in postprandial blood glucose, the

combination of tryptophan and lauric acid may result in greater blood glucose lowering than each nutrient alone. The study showed that the combination delayed the early rise in postprandial glucose, related to slowing of gastric emptying and, possibly, stimulation of GLP-1, but did not lower the overall glucose response. Lauric acid alone also delayed the early rise in glucose and tended to slow gastric emptying, but did not stimulate GLP-1, while tryptophan did not lower glucose, slow gastric emptying or stimulate glucoregulatory hormones. These findings suggest that, in the doses used, the combination delayed the rise in postprandial blood glucose, probably reflecting slowing of gastric emptying and perhaps GLP-1 stimulation, and the effect to lower postprandial blood glucose did not exceed that of lauric acid alone.

Collectively, the main outcomes of the studies presented in this thesis are that while tryptophan potently suppresses energy intake in lean people and those with obesity, its effect on postprandial blood glucose appears to vary between health, obesity and type 2 diabetes. GI factors, including gastric emptying and gut and pancreatic hormones, contribute to those effects, but to deferring extents, and other extra-intestinal factors (e.g. plasma amino acids) also play a role. Finally, the combination of lauric acid and tryptophan, in individual doses that are ineffective in reducing postprandial glycaemia, has potent effects to delay the early rise in postprandial glucose, an effect most likely driven by lauric acid – induced slowing of gastric emptying.

In conclusion, given evidence to support effects of amino acids, particularly tryptophan on energy intake via post-absorptive mechanisms, the effects of standardised changes in the plasma tryptophan/LNAA ratio across a wide range by manipulating doses of tryptophan and/or large neutral amino acids, on GI functions, appetite and energy intake, warrant further investigation. Moreover, evaluation of the effect of the combination of lauric acid and

tryptophan, given the greater glucose lowering effect warrants assessment in people with type 2 diabetes who, of course, have higher postprandial blood glucose responses than healthy people. Since studies not only in this thesis, but also in most reports available in the literature have evaluated acute exposure to these nutrients, longer-term studies are required to establish whether the effectiveness of these treatments is sustained over a prolonged time period or decreases due to downregulation of receptors, transporters or other compensatory mechanisms.

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