Gastrointestinal Nutrient Sensing in Obesity and Type 2 Diabetes: Role in Appetite Regulation and Glycaemic Control

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BHSc (Honours, First Class)

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"It is paradoxical, yet true, to say, that the more we know, the more ignorant we become in the absolute sense, for it is only through enlightenment that we become conscious of our limitations. Precisely one of the most gratifying results of intellectual evolution is the continuous opening up of new and greater prospects."

- *Nikola Tesla*

Conference Proceedings

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Cvijanovic, N., Isaacs, N.J., Rayner, C.K., Feinle-Bisset, C., Young, R.L., Little, T.J. Pyloric motility and energy intake responses to intraduodenal fat in lean, overweight and obese humans. Oral presentation at the ANZOS Annual Scientific Meeting, 16th-18th October 2014, Sydney, Australia.

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Cvijanovic, N., Feinle-Bisset, C., Young, R.L., and Little, T.J. (2015). Oral and intestinal sweet and fat tasting: impact of receptor polymorphisms and dietary modulation for metabolic disease. Nutrition Reviews, 73(5), 318-334. doi:10.1093/nutrit/nuu026.

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Cvijanovic, N., Bound M, Little T.J., Feinle-Bisset C., Wu T., Rayner C.K., and Young R.L. Altered expression of sweet taste receptors and glucose transporters in poorly controlled type 2 diabetes. Manuscript in preparation.

Abbreviations

β2M; Beta-2 microglobulin

3-OMG; 3-ortho-methylglucose

ANOVA; Analysis of variance

AUC; Area under the curve

BBM; Brush border membrane

BMI; Body mass index

CCK; Cholecystokinin

CD36; Cluster of differentiation-36

CV; Circumvallate

DIO; Diet-induced obese

FFA; Free fatty acid

FFAR; Free fatty acid receptor

FFQ; Food frequency questionnaire

GI; Gastrointestinal

GIP; Glucose-dependent insulinotropic peptide

GLP-1; Glucagon-like peptide-1

GPR; G-protein coupled receptor

GLUT2; Glucose transporter-2

HFD; High-fat diet

iAUC; Incremental area under the curve

ID; Intraduodenal

IV; Intravenous

KO; Knock-out

LCFA; Long-chain fatty acid

mRNA; Messenger RNA

MUFA; Monounsaturated fatty acid

OEA; Oleoylethanolamide

OGTT; Oral glucose tolerance test

OM; Osborne-Mendel

PIP2; Phosphatidylinositol 4,5-bisphosphate

PPARα; Peroxisome-proliferator-activated receptor-α

PUFA; Polyunsaturated fatty acid

PYY; Peptide YY

RNA; Ribonucleic acid

RT-PCR; Reverse transcription polymerase chain reaction

SEM; Standard error of the mean

SGLT-1; Sodium-glucose co-transporter-1

SNP; Single-nucleotide polymorphism

T2D; Type 2 diabetes

T1R2; Taste receptor type 1, member 2

T1R3; Taste receptor type 1, member 3

TAG; Triacylglycerol

TFEQ; Three-Factor Eating Questionnaire

TRPM5; Transient receptor potential cation channel, subfamily M, member 5

WT; Wild-type

Abstract

Background: The sensing of nutrients by the small intestine generates signals, including the secretion of gastrointestinal (GI) hormones, which are important determinants of subsequent energy intake and postprandial glycaemia. Recent studies have identified that specific free fatty acid (FFA) and sweet taste sensors/receptors, localised to enteroendocrine cells and/or absorptive cells, in the small intestine, play a central role in mediating nutrient-induced GI hormone release. Furthermore, studies in knock-out (KO) and diet-induced obese (DIO) animal models have revealed that altered expression of a number of these receptors attenuates GI hormone secretion, and consequently alters food intake and glycaemic control, thereby, providing evidence that intestinal nutrient sensing plays a significant role in the pathophysiology of obesity and type 2 diabetes (T2D). However, our understanding of the relationships between expression of nutrient receptors in the small intestine, nutrient-induced release of GI hormones, appetite regulation, and glycaemic control in human health and metabolic conditions such as obesity and T2D remains limited.

Aims: The studies presented in this thesis aimed to characterise the expression and functional role of duodenal nutrient sensors for fats and carbohydrates in human health, obesity and T2D. Specifically, the aims were to investigate:

1) The effect of acute intraduodenal (ID) nutrient exposure (lipid or glucose) on duodenal nutrient sensor expression.
2) Relationships between the expression of nutrient sensors at baseline (fasted), and after nutrient infusion, with the secretion of GI hormones involved in regulating appetite, energy intake and glycaemia.

3) Relationships between the expression of nutrient sensors at baseline (fasted), and after nutrient infusion, with appetite perceptions, habitual energy and macronutrient intakes.

Methods: For the studies presented in **Chapter 3** and **4**, 57 volunteers classified as lean (n = 20, body mass index (BMI) 18-24 kg.m^2), overweight (n = 18, BMI 25-29 kg.m^2) or obese (n = 19, BMI ≥ 30 kg.m^2) underwent unsedated endoscopy. Duodenal biopsies were collected at baseline (following a 12 hour fast), and 30 min after an ID infusion of 10% Intralipid® (2 kcal/min). Duodenal expression of free fatty acid receptor 1 (FFAR1), FFAR4, G-protein coupled receptor 119 (GPR119), and the cluster-of-differentiation-36 (CD36) was assessed by quantitative reverse-transcription polymerase chain reaction (RT-PCR), relative to expression of the housekeeper gene β-2 microglobulin (β2M). On a separate visit, the effects of a 120 min ID infusion of Intralipid® (2 kcal/min) infusion on blood glucose, and plasma cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), peptide-YY (PYY), insulin and leptin concentrations were evaluated, followed by an *ad libitum* buffet-meal, from which energy and macronutrient intake was quantified. Habitual dietary intake was assessed using food frequency questionnaires (FFQs).

For the study presented in **Chapter 5**, 12 healthy control individuals (HC), 12 patients with well-controlled T2D (WC-T2D; HbA1c 6.3 ± 0.2%), and 9 patients with poorly-controlled T2D (PC-T2D; HbA1c 10.6 ± 0.5%) undertook an oral glucose tolerance test (OGTT) following an overnight fast, as previously described[1]. These participants were then studied during a euglycaemic clamp (5 ± 1 mmol/L), with duodenal biopsies collected at baseline (fasted) and after a 30 min ID glucose infusion (4 kcal/min). Copy numbers of taste receptor type 1, member 2 (T1R2), the sodium-glucose co-transporter 1 (SGLT-1) and glucose-

transporter 2 (GLUT2) transcript were assessed at t = 0, 10 and 30 min by RT-PCR. Plasma concentrations of GIP, GLP-1, and C-peptide were measured at 10 min intervals from baseline (t = 0 min) for 60 min (t = 60 min). Plasma concentrations of 3-ortho-methylglucose (3-OMG) were measured at t = 30 and 60 min, using mass spectrometry, to assess glucose absorption.

Results:

Duodenal fatty acid sensing receptor expression in lean, overweight and obese individuals

During fasting, duodenal expression of FFAR1 and FFAR4 was lower ($P \leq 0.05$), and CD36 higher ($P \leq 0.001$), in obese, compared with lean and overweight, participants. ID lipid increased GPR119 and FFAR1 transcript levels independent of BMI (both $P \leq 0.05$), while levels of CD36 and FFAR4 did not change. The lipid-induced change in FFAR1 was positively associated with the incremental area under the curve (iAUC) of GIP ($r = 0.3$, $P \leq 0.05$). ID lipid induced the secretion of GIP, GLP-1, CCK, PYY and insulin, but there was no relationship between hormone levels with fat sensor expression. There was no relationship between acute energy and macronutrient intake at the buffet-meal and duodenal expression of fat sensors, however, habitual consumption of polyunsaturated fatty acids (PUFAs) was negatively associated with GPR119 in healthy, lean participants ($r = -0.5$, $P \leq 0.05$) (**Chapter 3**, **Chapter 4**).

Duodenal sweet taste receptor (STR) and glucose transporter expression in health, and patients with well- and poorly-controlled type 2 diabetes

Blood glucose concentrations were higher in PC-T2D than WC-T2D and HC groups before and during the OGTT ($P \leq 0.001$). Basal T1R2 transcript levels were similar across groups, while SGLT-1 transcripts were lower in PC-T2D than in the WC-T2D group ($P \leq 0.01$), and GLUT2 transcripts lower in PC-T2D than in both WC-T2D and HC groups ($P \leq 0.01$). Plasma GIP concentrations were higher in WC-T2D than in the HC group at baseline ($P \leq 0.01$), with no group differences in GLP-1 and C-peptide concentrations. ID glucose increased SGLT-1 and decreased GLUT2 transcripts at 10 min (group × time interaction) in both HC and WC-T2D groups (both $P \leq 0.001$, $P \leq 0.05$ respectively), but had no effect on SGLT-1 or GLUT2 transcripts in the PC-T2D group. T1R2 transcripts were lower in PC-T2D at 10 min than in the WC-T2D group ($P \leq 0.05$), while transcript levels of all targets were similar across groups at t = 30 min. ID glucose increased plasma GIP, GLP-1 and C-peptide concentrations (all $P \leq 0.001$), with GIP higher in PC-T2D (iAUC, $P \leq 0.05$) than in the HC group, GLP-1 higher in WC-T2D than the HC group ($P \leq 0.05$), and C-peptide highest in HC compared to both WC-T2D and PC-T2D groups ($P \leq 0.01$, $P \leq 0.001$). T1R2 and GLUT2 transcripts at baseline, and in response to ID glucose, were unrelated to GIP, GLP-1 or C-peptide iAUC. GIP concentrations after 10 min were negatively associated with basal SGLT-1 transcripts ($r = -0.6$, $P \leq 0.05$), and the degree of change in SGLT-1 during ID glucose ($r = -0.5$, $P \leq 0.05$). Serum 3-OMG at 30 min was positively related to the change in T1R2 transcript level at 10 min in HC participants ($r = 0.7$, $P \leq 0.05$) (**Chapter 5**).

Conclusions: These studies have identified notable differences in the duodenal expression of the FFA sensors FFAR1, FFAR4 and CD36 in human obesity at baseline. GPR119 was linked to habitual PUFA consumption in health, indicating that dietary fatty acid composition, rather than high-fat diet (HFD) consumption *per se*, may influence fat sensor

expression. Overall, the response of FFA sensors to acute ID lipid remained intact in obesity, with BMI-independent increases in FFAR1 and GPR119, but no association between FFA sensor expression and fat-induced secretion of gut hormones across the cohort. In separate studies we demonstrated that baseline expression of duodenal glucose transporters SGLT-1 and GLUT2 was lower in PC-T2D patients at euglycaemia. Incretin and transcriptional responses to glucose infusion, and 3-OMG absorption, was similar in WC-T2D and HC, however, PC-T2D patients showed a dysregulated T1R2 response, lack of transcriptional change in SGLT-1 and GLUT2 to ID glucose infusion, and exaggerated GIP secretion and 3-OMG absorption. Therefore, impaired glycaemic control in PC-T2D patients may be linked to impairment of luminal sweet sensing and its downstream signals. Further investigations are needed to define the functional connections between altered GI nutrient sensing and the pathophysiology of obesity and T2D.

Chapter 1: Thesis Synopsis

Obesity (BMI $\geq$ 30 kg.m^2) is a prevailing condition in westernised societies, and is associated with a number of co-morbidities, which significantly impact both individual and population health. The global prevalence of obesity has doubled since 1980[2]. Although a complex interplay of factors, including genetics, contribute to the development of obesity, lifestyle factors, including the consumption of energy-dense diets, high in fats and sugars, and increasingly sedentary lifestyles, have undoubtedly contributed to the current epidemic[3,4]. While there is a desperate need to prevent and treat obesity and its serious co-morbidities, in particular T2D, the underlying pathophysiology of these metabolic conditions remains unclear, limiting the long-term efficacy of current therapeutic interventions.

Lifestyle interventions (i.e. calorie restricted diets and exercise) are the first line therapy for obesity, yet weight loss is often modest, and a lack of compliance presents issues with long-term effectiveness. Additionally, metabolic adaptations to weight loss can also occur, making caloric restriction and weight loss difficult to maintain[5]. Pharmacological interventions can result in modest weight reductions, however these are not maintained long-term and are often associated with significant side effects. Currently, Roux-en-Y gastric bypass surgery (RYGB) is the most effective surgical treatment for obesity[6,7], and is commonly associated with rapid remission of T2D[8]. Importantly, the effectiveness of RYGB is associated with changes in the secretion of specific hormones from the GI tract which regulate satiety, energy intake and glycaemia. Therefore, it is of substantial interest to understand the mechanisms governing the secretion of these GI hormones, as they may present novel targets for the development of therapies which could mimic the effectiveness of RYGB.

It has recently been shown that the GI tract is equipped to detect and respond to ingested nutrients via intestinal ‘taste’ sensors on enteroendocrine cells. Changes in the expression or

function of these intestinal taste systems in mammals (e.g. genetic KO models, human polymorphisms) attenuates the effects of fats and carbohydrates on GI hormone secretion, appetite and energy intake, and glycaemia. While the existence of these GI sensors has been established in rodents, there are limited human studies. **Chapter 2** comprises a comprehensive review of the current state of knowledge of oral and intestinal fatty acid and sweet taste sensors in health, obesity, and T2D.

The study presented in **Chapter 3** characterised the expression of duodenal FFA sensors in health, during fasting, and following an acute ID lipid infusion. Examination of relationships between fat sensor expression and GI hormone secretion, appetite, and acute energy and macronutrient intake in response to lipid was also undertaken. Relationships between habitual dietary intake and fat sensor expression were examined to determine whether dietary patterns influenced intestinal fat sensitivity. **Chapter 4** extended this investigation, to determine whether expression of fat sensors was 1) altered in obese individuals, and 2) responded differently to an acute lipid stimulus compared to responses in healthy individuals. Relationships between transcriptional changes of fat sensors with GI hormone secretion, appetite, and energy intake were also assessed to evaluate whether changes in fat sensors influenced these events.

Chapter 5 focussed on the intestinal sweet taste system, and whether changes in the regulation of intestinal glucose sensing and transport had the potential to impact on control of glycaemia in patients with T2D. We previously showed that transcriptional regulation of the intestinal sweet taste system was similar in healthy individuals and patients with diet-controlled T2D at 'normal' glycaemic levels (euglycaemia), but was dysregulated in the latter when blood glucose levels were elevated (hyperglycaemia). In the hyperglycaemic

state, patients with T2D also showed an exaggerated glucose absorption, and worsened postprandial glycaemia. It is unknown, however, whether the sweet taste system is more profoundly altered as T2D progresses, in patients who have prevailing hyperglycaemia due to 'poorly' managed disease. This study, therefore, aimed to characterise transcriptional regulation of duodenal sweet taste receptors (STRs) and glucose transporters SGLT-1 and GLUT2 in healthy individuals, and patients with 'well' or 'poorly' controlled T2D, in response to ID glucose infusion at euglycaemia. As patients with T2D are known to have an increased capacity for glucose absorption, and may show changes in their glucose-dependent release of incretin hormones which govern postprandial glycaemia, these factors were investigated in healthy individuals and patients with different degrees of T2D control in association with transcript changes.

The studies presented in this thesis provide novel information regarding intestinal nutrient sensors for fats and carbohydrates in healthy individuals, and individuals with metabolic diseases (obesity and T2D). The studies explored the relationships between both fasting, and nutrient-stimulated changes in these nutrient sensors, and subsequent GI signalling regulating appetite, energy intake, and glycaemic control.

Chapter 2: Oral and Intestinal Sweet and Fat Tasting: Impact of Receptor Polymorphisms and Dietary Modulation for Metabolic Disease

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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);

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2.1 Abstract

The human body has evolved with a disposition for nutrient storage, allowing for periods of irregular food availability and famine. In contrast, the modern diet is characterised by excessive consumption of fats and sugars, resulting in a surge in the rates of obesity and T2D. Although these metabolic disorders arise from a complex interaction of genetic, social, and environmental factors, evidence now points to fundamental changes in nutrient metabolism at the cellular level contributing to the underlying pathology. Taste receptors detect nutrients in the oral cavity and GI tract and can influence the hormonal response to nutrients; they may also become maladaptive in conditions of excess fat or sugar consumption. Precise links between taste receptor activity, and downstream effects on energy intake and glycaemia are not well defined. This review outlines the candidate taste receptors for carbohydrates and fats in the oral cavity and within the small intestine, highlighting the contributions of underlying genetics (polymorphisms) and sensory challenges (e.g., HFD) to the development of obesity and T2D.

2.2 Introduction

Fats and sugars activate reward pathways in the brain that elicit pleasant taste and hedonistic sensations, enhance palatability, reinforce taste preference, and promote consumption[9-12]. Humans have developed an innate preference to consume fats and sugars in energy-dense foods and then to store energy (lipids, glycogen), limit glucose utilisation, and preserve protein in preparation for periods of irregular food availability[9]. However, in today's environment, energy-dense, high-fat, and high-sugar foods are readily available; in the absence of periods of food scarcity, the innate human preference for these foods promotes excess energy intake and the development of obesity and its comorbidities (cardiovascular disease, hypertension, stroke, osteoarthritis, dyslipidaemia[13]), most notably, T2D[14] (as reviewed by Cordain et al.[4]). It has been proposed that sensory challenges such as a high-fat or high-sugar diet may trigger maladaptation of the homeostatic mechanisms of nutrient detection and energy regulation in the GI tract. Significant evidence now points to fundamental changes in cellular nutrient metabolism as the potential source of maladaptation, although the specific mechanisms involved are poorly defined.

The GI tract generates powerful signals that act pre- and postprandially to regulate energy intake and glycaemia via modulation of food selection, digestion, and absorption[15]. Food selection is driven through a combination of sensory inputs that include sight, smell (olfactory), texture (trigeminal), and taste (gustatory) cues, as well as post oral GI cues. Taste receptors on both the lingual and olfactory epithelium form the front line of sensory input to the GI tract and detect the chemical composition of ingested foods to provide key sensory inputs in order to determine those that are of nutritional benefit (e.g., sugar, fat, protein) and those that present risk, such as toxic compounds (e.g., bitter, sour). A large number of animal and human studies have established that taste receptors, analogous to oral taste receptors, are found throughout the length of the GI tract, where they detect the presence of

monosaccharides, FFAs, and peptides/amino acids and trigger the release of the key hormones that regulate satiety and glycaemia from the gut wall (see review by Depoortere[16]). The present review highlights the emerging evidence for a role of these GI taste receptors for sweet and fat in the regulation of energy intake and glycaemia, as well as the mechanisms underlying their role, while acknowledging that present understanding of the precise intracellular mechanisms that link GI taste receptors to hormone release and downstream effects is rapidly evolving.

Significant research is currently dedicated to uncovering the mechanisms of nutrient detection in health and potential dysregulation or maladaptation in metabolic diseases. In order to expand present knowledge in this area, an understanding of how the form and/or function of taste receptors may be altered in metabolic disease states is of critical importance. The relative contributions of underlying genetic predispositions (e.g., receptor polymorphisms) or environmental influences such as chronic exposure to a HFD or excess consumption of sugars and artificial sweeteners to changes in oral or GI nutrient receptors remains to be established.

This review provides a brief overview of GI signals that regulate energy intake and glycaemia as well as a summary of oral and small intestinal receptors for sweet and fat taste. For each of these taste modalities, functionality in health, obesity, and T2D is compared, as is the contribution of sensory challenges (e.g., high-fat and high-sugar diets or non-caloric sweeteners) and functional maladaptation (e.g., polymorphisms) of these sensor systems to these metabolic conditions. Importantly, how these nutrient sensors may serve as novel targets for therapeutic benefits in the settings of obesity and T2D is highlighted.

2.3 Gastrointestinal signals critically control energy intake and glycaemia

GI signals triggered in response to a meal are critical in the regulation of subsequent food intake, and the efficient coordination of nutrient digestion, absorption, and subsequent utilisation in the body[17]. Satiation signals are initiated at two sites – in the stomach, largely through gastric distension and activation of gastric mechanoreceptors, and in the small intestine, upon nutrient-dependent release of peptides from enteroendocrine cells within the gut wall[17]. Other cell types such as brush cells (tuft cells) may also contribute to luminal chemosensing[18]; however, understanding of the role of these cells is limited, and a comprehensive discussion is beyond the scope of the current review. Signals arising from the small intestine also determine the rate of gastric emptying and are major determinants of the postprandial glycaemic response; it is, therefore, critical to understand how nutrient sensors and their effectors in the small intestine function in a state of health as well as their potential for maladaptation in disease.

2.4 Effects of gastrointestinal hormones on satiation and glycaemia

More than 20 different hormones are released by specific populations of small intestinal enteroendocrine cells in response to ingested nutrients (as reviewed by Rindi et al.[19]). These hormones modulate a variety of GI functions, including pancreatic secretion, motility, glycaemia, and, importantly, energy intake and satiety[18]. Key gut hormones involved in the satiety response include CCK derived from I-cells[20], and GLP-1 and PYY from L-cells[21,22]. Traditionally, each GI hormone was viewed as being secreted from a specific cell type; however, there is now evidence of considerable overlap in hormone expression between I-, K, and L-cells, with enteroendocrine cells co-expressing numerous functionally related peptides[23]. This suggests that a single cell type may be tuned to secrete specific hormones depending on the cells' location along the axis of the gut and their exposure to specific

dietary components. Further studies in this area will be critical to determine the factors that control the phenotypes of enteroendocrine cells along the axis of the intestine. CCK, GLP-1, and PYY work to modulate GI motility, appetite, and energy intake, and they slow the rate of gastric emptying. GLP-1 in conjunction with K-cell-derived GIP acts as an incretin hormone to substantially augment insulin secretion when glucose is given orally, compared to as an isoglycaemic intravenous (IV) infusion[24]. By contrast, ghrelin, the best known orexigenic hormone, is produced in enteroendocrine cells of the gastric mucosa; it powerfully stimulates food intake in humans[25], increases motility, and decreases insulin secretion[26].

It is now well established that intestinal taste receptors can trigger the release of CCK, PYY, GLP-1, and GIP[27-30], which supports a link between taste receptor expression and/or functionality and the control of energy intake and/or glycaemia. To date, there is limited knowledge regarding a link between nutrient-induced ghrelin suppression and taste receptor activation, with available functional evidence highlighting α-gustducin activity linked to bitter receptor activation in ghrelin- releasing cells in the stomach[31]. The focus of the current review is on the localisation and characteristics of sweet and fat taste receptors in the tongue and small intestine, as well as their links with GI hormone release from open enteroendocrine cells. The review also documents recent evidence of how these sweet and fat taste receptors may be dysregulated in metabolic disease states, such as in obesity and T2D (**Table 2.1**)[1,3, 27-29, 32-65].

Table 2.1: Candidate free fatty acid (FFA) and sweet taste receptors (STRs) in the gastrointestinal (GI) tract: their location, GI mediators and physiological responses to metabolic challenge and genetic knockout (KO).

Gene and reference	Location	Example of ligands	Gastrointestinal mediator	Physiological response to HFD/obesity/T2D	Physiological change in response to genetic KO
FFAR4 Matsumura et al. (2007)[32]; Martin et al. (2012)[33]; Cartoni et al. (2010)[34]; Hirasawa et al. (2005)[28]; Ichimura et al. (2012)[35]; Duca et al. (2013)[36]; Kawai et al. (2003)[37]; Tanaka et al. (2008)[27]	Oral: CV, foliate, fungiform papillae[32-34] Small intestine: enteroendocrine L cells[28]	Unsaturated LCFA[28, 33]	GLP-1[28], PYY[28],CCK[27]	HFD: ↓protein expression[36]	↓Fat taste preference[37]; ↓GLP-1[28, 35]; ↓glucose tolerance[35]; ↑insulin resistance[35], ↑obesity[35]
FFAR1 Cartoni et al. (2010)[34]; Itoh et al. (2003)[38]; Edfalk et al. (2008)[39]; Liou et al. (2011)[40]; Lan et al. (2008)[41]; Steneberg et al. (2005)[42]; Latour et al. (2007)[43]; Briscoe et al. (2003)[44]; Kebede et al. (2008)[45]	Oral: CV, foliate, rarely fungiform[34] Small intestine: enteroendocrine L and K cells[39] Pancreas: pancreatic β cells[38]	Saturated and unsaturated medium- and LCFA[34]	GLP-1[39], GIP[39], CCK[40]	KO on HFD[42]: ↓insulin secretion; ↓insulin resistance; ↔glucose tolerance KO on HFD[41, 42]: ↑insulin resistance; ↑liver steatosis, ↑obesity	↓**Fat taste preference**[34]; ↓GLP-1[39]; ↓CCK[40]; ↔weight[43]; ↔insulinemia[43]; ↔plasma FFA[43]; ↔fasting blood glucose[43]
GPR119 Lauffer et al. (2009)[46]; Lan et al. (2009)[47]; Overton et al. (2006)[48]; Hansen et al. (2012)[49]; Chu et al. (2008)[50]	Small intestine: enteroendocrine L cells[46] Pancreas: pancreatic β cells[47]	OEA[48], 2-oleyl glycerol, 2-monoacylglycerols[49]	GLP-1[47, 50], GIP[50]		↓GLP-1[46]
CD36 Laugerette et al. (2005)[3]; Simons et al. (2011)[51]; Drover et al. (2005)[52]; Zhang et al. (2011)[53]; Poirier et al. (1996)[54]; Lobo et al. (2001)[55]; Schwartz et al. (2008)[56]	Oral: CV, foliate[3, 51] Small intestine: brush border of enterocytes[54, 55]	Saturated and unsaturated LCFA (C≥ 16)[3], OEA[56]	OEA[56]	HFD: ↓CV transcript expression[53]	↓Fat taste preference[3]; hypertriglyceridaemia[52]; ↓OEA mobilisation in response to fat[56]
T1R2/T1R3 Nelson et al. (2001)[57]; Margolskee et al. (2007)[58]; Jang et al. (2007)[59]; Dyer et al. (2005)[60]; Young et al. (2009)[61]; Young et al. (2013)[1]; Daly et al. (2012)[66]	Oral: CV[57] Small intestine: enteroendocrine I and K cells[1, 59-61]	Sugars, d-amino acids, sweet proteins, non-caloric sweeteners (e.g., saccharin[58], acesulfame-k[58])[57]	GLP-1[58, 59], GIP[58], GLP-2[66]	T2D vs healthy[1]: ↔*T1R2* transcript at baseline; ↑*T1R2* by luminal glucose at euglycaemia, ↓at hyperglycaemia (healthy); ↑by luminal glucose in T2D irrespective of glycaemia (T2D) ↑glucose absorption at hyperglycaemia	T1R3/α-gustducin KO[58]: ↔*SGLT-1* transcript on low or high-carbohydrate diet; ↔*SGLT-1* transcript or protein in response to dietary non-caloric sweeteners
SGLT-1 Yoshida et al. (1995)[62]; Gorboulev et al. (2012)[63]; Dyer et al. (2002)[64]; Margolskee et al. (2007)[58]; Stearns et al. (2010)[65]; Moran et al. (2010)[29]	Small intestine: brush border membrane of enterocytes[62, 64]	Substrates: D-glucose[29, 65], D-galactose Inducing ligands: D-fructose[58], saccharin[29, 58, 65], sucralose[58]	GLP-1[58], GIP[67]	T2D: ↑transcript and protein ↑brush border membrane vesicle transport[64]	Glucose-galactose malabsorption syndrome[63]; ↓glucose-mediated GIP and GLP-1 secretion[63]

Abbreviations: circumvallate (CV); long-chain fatty acid (LCFA); glucagon-like peptide-1 (GLP-1); glucagon-like peptide-2 (GLP-2); peptide tyrosine tyrosine (PYY); cholecystokinin (CCK); high-fat diet (HFD); glucose-dependent insulinotropic peptide (GIP); knockout (KO); oleoylethanolamide (OEA); type 2 diabetes (T2D); sodium-glucose co-tranporter-1 (SGLT-1)

2.5 Gastrointestinal taste receptors

Well-developed knowledge of oral taste receptors has enabled rapid advances in understanding the intestinal nutrient-sensing mechanisms following the identification of so-called GI taste receptors in the intestine. The recognition of morphological and phenotypic similarities between lingual taste cells and intestinal enteroendocrine cells has refocused research efforts towards understanding the mechanisms by which intestinal enteroendocrine cells sense nutrients and initiate signalling to regulate food intake and glycaemia. Both cell types are polarised and possess apical microvilli exposed to the lumen that are equipped with specific G protein-coupled receptors (GPRs) tuned to individual taste modalities. These receptors detect sweet, sour, salty, bitter, and umami tastants, as well as fatty acids, and, in turn, activate intracellular signalling pathways comprised of ion channels, ligand-gated channels, and enzymes, leading to the release of specific mediators at their basolateral membrane[68]. These mediators then act in an autocrine, paracrine, or endocrine fashion, and can activate cognate receptors on adjacent sensory nerve endings to initiate signalling to brain centres[68]. Activation of oral taste receptors and lingual afferents elicits a conscious perception of taste, and, via higher brain centres, generates immediate and critical response mechanisms driving food selection and avoidance[69]. Activation of intestinal taste receptors does not elicit a conscious perception of taste; however, the capacity of these receptors to trigger hormone release and activate gut-brain signalling pathways via the vagus nerve significantly contributes to the generation of meal-related sensations[70].

2.6 Sweet sensing in the gastrointestinal tract

2.6.1 Sweet taste receptors in the oral epithelium

Sweet tastants are detected by a heterodimer of the GPR subtypes T1R2 and T1R3, which acts as a broadly tuned receptor for sugars, D-amino acids, sweet proteins, and non-caloric

sweeteners[1,57-61,66]. Studies focused on the oral sweet taste receptor heterodimer have demonstrated multiple binding sites for non-caloric sweeteners, supporting broad tuning of STRs to structurally diverse stimuli[71]. Interaction of sweet tastants with oral sweet taste receptors leads to activation of the taste-specific G protein, α-gustducin[60], and in turn, to a rise in intracellular calcium and the gating of a taste-specific transient receptor potential ion channel, transient receptor potential cation channel subfamily M member 5 (TRPM5)[72]. Gating of this channel facilitates cell depolarisation, the basolateral release of mediators, including noradrenaline, acetylcholine, serotonin and glutamate, and activation of inputs from the lingual afferent nerves to the nucleus of the solitary tract in the brainstem to generate the perception of sweet taste[68,73].

2.6.2 Oral sweet taste receptors and sweet taste preference

Preference for sweet substances is thought to be inherent rather than learnt. Newborns demonstrate the ability to differentiate varying degrees of sweetness and consume greater volumes of solutions that taste sweeter[74]. Many factors influence food preferences, and even flavours from the maternal diet transmitted through breast milk can influence early food preference[75]. Although the positive hedonic responses to sweet taste are a universal trait, substantial inter-individual variation in the perceived intensity of, and preference for, sweetness has generated significant interest in the field of sweet taste sensing. Although links between sweet taste preference and BMI are not consistently observed[76], there is evidence to support a heightened preference for sweet substances in obesity, which may drive the consumption of excess calories[77]. Interestingly, obese individuals who have undergone RYGB have an enhanced oral perception of sweetness and reduced desire to consume

high-carbohydrate (and high-fat) foods[78,79]. However, the extent to which these changes involve modifications in intestinal STR expression or function requires further investigation.

2.6.3 Sweet taste receptor polymorphisms and sweet taste perception

A number of STR polymorphisms have been identified in humans[80,81] (see **Table 2.2**)[35, 80-88]. For example, two single-nucleotide polymorphisms (SNPs) located upstream of the promoter region of T1R3 are strongly associated with reduced oral sucrose sensitivity in humans[81]. These SNPs resulted in reduced levels of T1R3 transcripts in vitro; however, care must be exercised in interpretation of this finding, as the study used bile-duct derived cells, which may have different proteins interacting at this promoter region[89]. Further evidence of the effects of polymorphisms on sweet taste perception comes from a worldwide survey of human polymorphisms in the T1R receptor family (T1R1, T1R2, T1R3), which revealed that T1R2 is highly diverse compared to other human genes; this corresponds with the variability seen in sweet taste thresholds amongst humans[90]. Importantly, the majority of amino acid variants occur in the first extracellular domain of T1R receptors, the domain that contains the ligand-binding site for carbohydrates. Further investigation is needed to determine the role of STR polymorphisms in driving preference for sweet foods and whether polymorphisms in gut STRs have functional implications for food selection and metabolic signalling in humans[81-88].

Table 2.2: Characteristics of commonly described polymorphisms of fatty acid and sweet sensors in the human GI tract

Gene and reference	Polymorphism	Region	Population	Physiological effects
FFAR1				↔Insulin response (OGTT)
Hamid et al. (2005)[82]	Arg211His (rs2301151)	Coding region	Danish Caucasians (T2D and healthy)	↔Allele frequency between T2D and glucose-tolerant controls
Ogawa et al. (2005)[83]			Japanese males (healthy)	His/His>Arg/Arg: ↑serum insulin levels, ↑insulin resistance, ↔plasma glucose, ↔serum lipids
Vettor et al. (2008)[84]	Gly180Ser	Coding region	Sicilian (healthy and obese)	↑Allele frequency with ↑BMI; transfection of variant in HeLa cells alters FFAR1 function resulting in ↓ability to sense lipids, impaired Ca^{2+} release and β-cell secretion in a model of insulin secretion
FFAR4				↓Receptor expression in human intestinal NCI-H716 cells with ↓GLP-1 release, ↑risk of obesity and insulin resistance
Ichimura et al. (2012)[35]	R270H	Coding region	European (healthy and obese)	
CD36				
Pepino et al. (2012)[85]	rs1761667	Promoter region	African-American, Caucasian (obese)[85]	Oral threshold for oleic acid and trinolein: GG>AG>AA[85]
Keller et al. (2012)[86]	Genotypes: AA, AG, GG		African-American (healthy and obese)[86]	↔Food consumption, fat preference[85], ↑preference for fat (AA genotype) [86]
Bokor et al. (2010)[87]	rs3211867, rs3211883, rs3211908, rs1527483	Various	European adolescents (healthy and obese)	↑risk of obesity, ↑adiposity
Heni et al. (2011)[88]	rs9784998, rs3211883, rs3211908, rs3211956	Various	White European (non-diabetics at risk for T2D)	↑BMI, ↑Waist circumference (rs3211883, rs3211908), ↔insulin sensitivity, ↔hepatic lipid accumulation
T1R2				
Eny et al. (2010)[80]	Ser9Cys (rs9701796)	Single-peptide region	White, South Asian, East Asian, other (healthy and T2D)	↔Carbohydrate intake
Eny et al. (2010)[80]	Ile191Val (rs35874116)	Large extracellular domain and ligand binding site	White, South Asian, East Asian, other (healthy and T2D)	↑Carbohydrate consumption (effects seen in BMI ≥ 25) in healthy and T2D
T1R3	rs307355, rs35744813	Upstream of *T1R3* coding sequence	European, Asian, African	↓Sensitivity to sucrose
Fushan et al. (2009)[81]				

Abbreviations: BMI, body mass index; GLP-1, glucagon-like peptide-1; OGTT, oral glucose tolerance test; T2D, type 2 diabetes

2.6.4 Sweet taste receptors in the intestinal epithelium

2.6.4.1 Functional evidence for a role for sweet taste receptors in incretin hormone release

STRs have been localised to subpopulations of enteroendocrine cells within the proximal small intestine of mice and humans, which is a key region of nutrient detection[1,59-61,91-93]. Co-localisation of α-gustducin and GLP-1 within the L-cells of the mouse duodenum provides evidence for a functional relationship between activation of the STR-gustducin pathway and subsequent incretin release[94]. Importantly, animals that lack either α-gustducin, or the T1R3 subunit, demonstrate major defects in glucose-mediated GLP-1 secretion and disrupted glucose homeostasis[59,94]. This provides the strongest functional evidence for a role of the STR-gustducin-coupled pathway as a significant contributor to overall glycaemic control.

The human L-cell line NCI-H716 expresses all components of the sweet taste pathway. Administration of glucose and the non-caloric sweetener sucralose in one study induced GLP-1 secretion, an effect that was inhibited by both RNA interference of α-gustducin, and the sweet taste receptor inhibitor lactisole[59]. In a seminal study of 35 healthy volunteers, Gerspach et al.[30] also demonstrated that glucose-dependent GLP-1 release was attenuated in the presence of lactisole, providing direct evidence of a functional role of intestinal sweet taste receptors in the regulation of incretin hormone release. In this study, lactisole reduced the GLP-1 and PYY response to intragastrically and intraduodenally administered glucose and mixed-nutrient loads, but the effect was greater following intragastric administration. The prominent effect of lactisole from the gastric compartment was unexpected, particularly given that the highest STR expression in humans occurs within the duodenum and since gastric emptying was not altered by lactisole. STR expression was not directly measured in

this study; however, an important point to note is that STR transcript expression is rapidly downregulated in mice receiving jejunal glucose infusion[61]. Accordingly, reduced STR availability to luminal stimuli in the study of Gerspach et al.[30] may have affected the subsequent release of GLP-1. As it is not known whether lactisole antagonism affects the regulation of STR expression, this possibility must be considered. The effect of lactisole on GLP-1 release may also occur via an indirect mechanism that utilises hormones released from the stomach. T1R3 has been localised to gastric brush cells[95], and may trigger hormonal or neural pathways to stimulate satiation peptide release in the intestine. The potential interaction between gastric and intestinal signalling mechanisms is an area that requires further investigation.

2.6.4.2 Effects of intestinal sweet taste receptor activation on glucose transporter availability

Landmark studies in animals have provided strong evidence for a link between intestinal STR activation and an increase in levels of the primary intestinal glucose transporter, SGLT-1, as well as luminal substrate transport capacity[29,58,65]. SGLT-1 is located in the brush border membrane (BBM) of enterocytes in the proximal intestine, and in addition to an absorptive role, the apical transport of sugars by SGLT-1 is also an important determinant of intestinal "incretin" release in both rodents and humans. Rodents administered intraluminal phloridzin (a competitive inhibitor of SGLT-1) show attenuated incretin responses to sweet stimuli[96], while mice deficient in SGLT-1 do not have an effective first phase GLP-1 response, which involves glucose-mediated insulin secretion and inhibition of hepatic glucose production[63]. Intracellular glucose diffuses into the bloodstream via the facilitative transporter GLUT2 at the basolateral membrane of enterocytes[97]. While early rodent studies indicated that GLUT2 was translocated to the apical surface in the presence

of luminal sugars[93], recent studies have indicated that GLUT2 translocation does not occur following a glucose load in healthy humans[63,98]. Further work is needed to understand the potential role of GLUT2 in taste signal transduction.

Indeed, SGLT-1 expression and function is directed by the presence of luminal sweet tastants, and SGLT-1 is upregulated in the presence of a broader range of sweet ligands than its substrate specificity, including by non-caloric sweeteners, highlighting the upstream role of a broadly-tuned sensor[29,58,62,65-98] (see **Table 2.1**). The upregulation of SGLT-1 in enterocytes is known to occur via a cAMP-dependent pathway that involves early post-transcriptional stabilisation of SGLT-1 transcripts[99]. Importantly, intake of dietary sugars and non-caloric sweeteners have been shown to increase intestinal SGLT-1 transcript, protein and function in wild-type (WT) mice, an effect that is absent in mice lacking the sweet taste molecule T1R3 or α-gustducin[58]. These findings indicate that SGLT-1 levels are linked to the broadly-tuned STR, and that intestinal STRs are engaged in the regulation of glucose uptake in animals. In this manner, activation of STRs is likely to trigger the release of autocrine and/or paracrine signals from taste cells, which may act upon adjacent enterocytes, to regulate SGLT-1 expression and function[58]. Candidate signal mediators released include GIP[58], GLP-1[58,59], and GLP-2[100-103], which are co-expressed in many of the enteroendocrine cells that contain T1R2[58]. Accordingly, STRs have been proposed as the intestinal sensor for dietary sweet tastants, with SGLT-1 acting as an important effector[1]. The implications of such a relationship include the potential for therapeutic targets that can specifically work to enhance, or reduce, carbohydrate transport; this is an issue of critical relevance to metabolic diseases, which are characterised by maladaptive carbohydrate absorption.

2.6.5 Disordered sweet taste receptor expression in type 2 diabetes

Patients with T2D often show disordered GI responses to nutrients, with frequently delayed gastric emptying and a high prevalence of GI symptoms[104-106]. There are a number of abnormalities that may impact intestinal glucose sensing in T2D; secretion of GLP-1 and GIP have been inconsistently reported to be diminished in these patients[107,108], while intestinal levels of SGLT-1 may be increased, thus increasing the capacity for glucose absorption[64]. It was recently demonstrated that the intestinal STR system is reciprocally regulated by luminal glucose exposure and blood glucose concentrations in humans. Under euglycaemic conditions, ID glucose infusion increased intestinal expression of T1R2, while the same infusion under hyperglycaemic conditions decreased T1R2 levels; this regulation may serve to limit SGLT-1 recruitment during hyperglycaemia. However, in patients with T2D, T1R2 levels increased upon glucose infusion, irrespective of the prevailing glycaemia, while glucose absorption was increased at hyperglycaemia, as seen by an elevation in levels of the glucose-absorption marker 3-OMG, a non-metabolisable substrate of SGLT-1[1]. Taken together, these results indicate that STR signals persist during hyperglycaemia in T2D patients, and, as a consequence, may sustain the availability of SGLT-1 transport and augment postprandial hyperglycaemia. The proposed pathways for STR signalling in health, and changes that occur in T2D conditions are outlined in **Figure 2.1**. Therapeutics that block STRs, such as lactisole, may therefore improve glycaemic control in the setting of T2D, but this hypothesis needs to be directly tested.

Importantly, to date, no studies have directly investigated whether lactisole blockade of STRs is capable of modifying glucose absorption in human T2D. Given the presence of impaired incretin secretion in T2D, it is of interest to investigate further how STR expression and function is altered in this state. Such knowledge is critical in order to determine the potential therapeutic benefit of lactisole in the clinical setting.

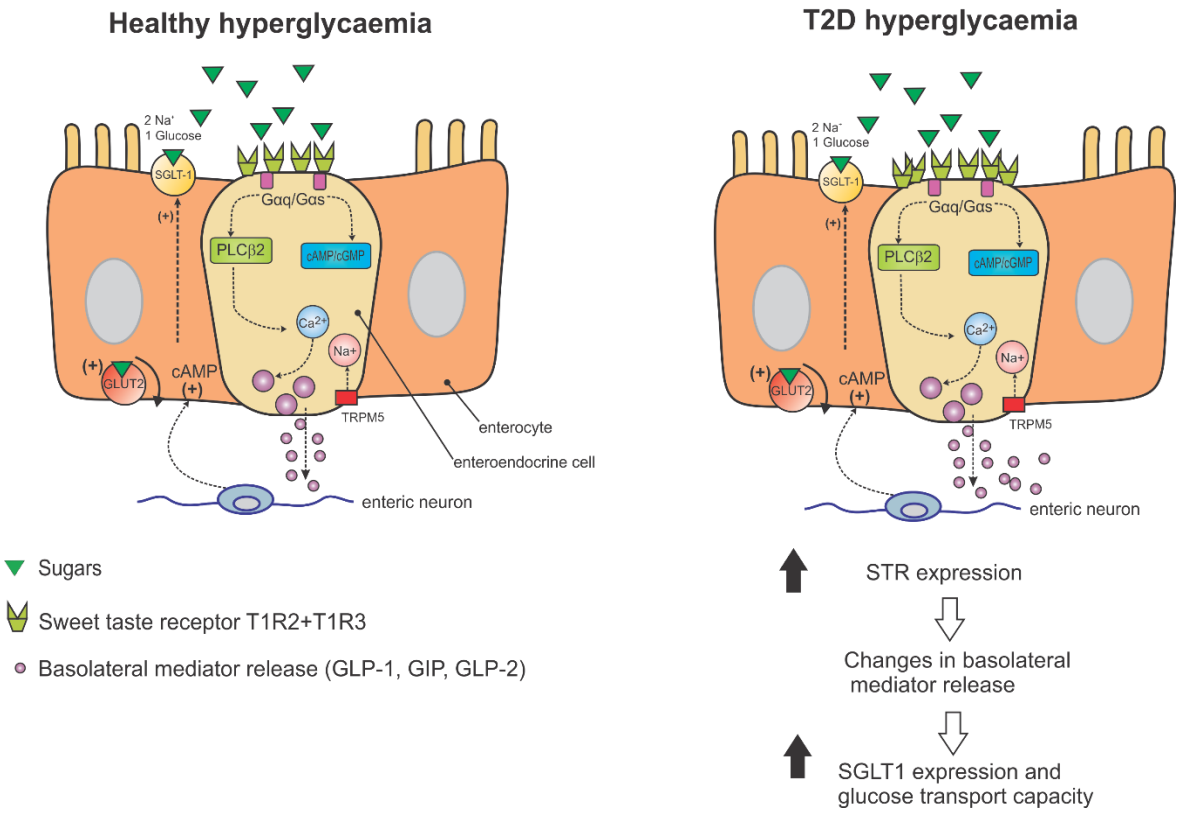

Figure 2.1: Generalised model of intestinal sweet taste sensing in healthy individuals and those with T2D under hyperglycaemic conditions. Heterodimeric STRs comprising the GPR subunits T1R2 + T1R3 detect a wide range of luminal stimuli, such as sugars, D-amino acids, and non-caloric sweeteners. Tastants bind to the receptor and activate the taste-specific G-protein α-gustducin via subunits Gαq to activate PLC βII or Gαs to stimulate cAMP/cGMP-dependent pathway. Upon activation of secondary messengers, calcium is released from inositol triphosphate–sensitive intracellular stores, leading to the gating of TRPM5. The subsequent influx of sodium and cell depolarisation trigger basolateral mediator release of the incretin hormones GLP-1 and GIP or GLP-2, which enter the blood and function as hormones or paracrine signals at adjacent enterocytes to upregulate the primary intestinal glucose transporter, SGLT-1. Upregulation of SGLT-1 increases apical glucose transport capacity, with intracellular glucose entering the bloodstream via the basolateral facilitative GLUT2 transporter. In healthy humans, intestinal STRs are reciprocally regulated by luminal glucose according to prevailing glycaemia, i.e., increased during euglycaemia, decreased during hyperglycaemia. This may limit SGLT-1 function to control postprandial glycaemic excursions. In contrast, in T2D, STR transcript levels increase irrespective of prevailing glycaemia, and glucose absorption is increased during hyperglycaemia.

2.6.6 Activation of sweet taste receptors by non-caloric sweeteners and implications for metabolic disease

Increased consumption of dietary sugars has been linked to the rising incidence of T2D in Western populations[109]. Non-caloric sweeteners have become a topic of much debate. Typically considered to be metabolically inert and consumed as a calorie-free sugar substitute, non-caloric sweeteners have been marketed as a healthy alternative to nutritive sugars as part of a weight loss regimen or for individuals with T2D[110], with initial findings suggesting benefits relating to body weight and decreased energy intake[111]. However, recent epidemiological data have shown that heavy consumers of beverages sweetened with non-caloric sweeteners have an increased risk of developing T2D[112]. Although further research is required, this finding implies that non-caloric sweeteners may not be functionally inert and may negatively affect glycaemic control, thereby offsetting any gains due to reduced energy intake.

Although evidence is convincing in animal models, human studies have not yet established a direct functional role of non-caloric sweeteners. Sucralose, aspartame, and acesulfame-K are known to act on intestinal STRs to increase SGLT-1 expression and function in pigs[29], rats[65], and mice[58]. In studies performed in healthy humans, diet soda containing sucralose and acesulfame-K has been shown to significantly increase GLP-1 release and, consequently, decrease peak glucose levels when ingested prior to an oral glucose load[113,114], but the same effects were not observed in T2D patients[114]. However, these studies did not control for other compounds in the soda that may have affected GLP-1 responses. When these same doses of sucralose and acesulfame-K were infused together into the duodenum of healthy humans, there was no acute effect on gastric emptying, GLP-1 release, or glycaemic response following an oral glucose load[115]. Similarly, other studies have found

that non-caloric sweeteners, such as sucralose[116-118], saccharin[117], aspartame[118], acesulfame-K[118], and stevia[117], when administered alone, are not sufficient to modify acute *in vivo* gut hormone secretion[119]. It has been postulated that the observed increase in GLP-1 in healthy volunteers potentially requires an adjunct caloric, metabolisable sugar, in addition to the non-caloric sweetener, to elicit GLP-1 changes[114]. It is also notable that circulating levels of gut hormones may be a blunt marker for local release based on evidence in rodents, whereby non-caloric sweetener triggered gut hormone levels were several orders higher in intestinal lymph than in circulation[120]. Clearly, additional studies are required to elucidate the effects of non-caloric sweeteners on GI and metabolic functions, particularly in chronic intake settings.

The predominance of non-caloric sweeteners in the modern food supply highlights the importance of understanding the specific intracellular pathways activated by individual non-caloric sweeteners and their downstream effects[110]. For example, the non-caloric sweetener acesulfame-K can induce glucose uptake in rat intestinal cell lines (Caco-2, RIE-1, and IEC-6) at high glucose concentrations by triggering the translocation of GLUT-2 from the basolateral to the apical membrane[121]. The use of a phospholipase βII (PLC βII) inhibitor (U-73122) abolished this effect, indicating the importance of PLC βII in mediating enhanced glucose uptake via increased STR signalling[121]. Research in this area is active, yet no definitive conclusions have been formed.

It is well established that intestinal STRs are engaged in sweet sensing, and may influence glycaemic control; in this, their GI function extends beyond simple nutrient detection in the periphery. However, further research is needed to determine the direct effects of STR

modification on metabolic disease in response to chronic ingestion, and whether actively targeting STRs can be of potential therapeutic benefit for conditions such as T2D.

2.7 Fat sensing in the gastrointestinal tract

2.7.1 Evidence for oral fat taste in humans

Recent evidence for the existence of fat taste has profound implications, particularly in the setting of the current obesity epidemic. The oral perception of dietary fat has long been considered to be based only on texture, odour, and postingestive cues[3,122]. However, this view was challenged by the discovery that triacylglycerols (TAGs), the principal form of fat in food, are digested by lingual lipases to release FFAs, which can act as effective stimuli for oral fatty acid taste receptors in both rodents and humans[37,123]. In humans, lipase activity is sufficient to liberate FFAs at a detectable range of 0.02–6.4 mM[123], and in rats, the addition of the potent lipase inhibitor, orlistat, results in a lower preference for dietary TAG intake, but not FFAs[37], indicating that FFAs are the key stimulus required for oral perception of fat "taste."

2.7.2 Oral sensitivity to fatty acids and fat preference

Fats are an energy-dense source of nutrition possessing hedonistic qualities through the activation of central reward pathways in the brain, enhancing palatability, and reinforcing taste preferences[10-12]. Indeed, a preference for fatty foods is a common trait amongst mammals, and a relationship exists between oral sensitivity to fatty acids and fat preference[124]. Rodents spontaneously prefer fatty foods in a free-choice situation, with dietary fat preferences inversely correlated with oral FFA sensitivity. For example, when placed on a 3-choice macronutrient selection paradigm (protein, carbohydrate, and fat), DIO-prone Osborne-Mendel (OM) rats prefer a HFD, and rapidly become obese, compared

with DIO-resistant (DIO-R) S5B/PL rats, which are naturally inclined to ingest relatively little fat and reduce their total caloric intake when exposed to a HFD in order to stay lean[125]. In humans, studies have shown that when fed a low-fat diet for 4 weeks, both lean and overweight/obese individuals have an increased taste sensitivity (i.e., decreased taste threshold) to oleic acid (C18:1). Conversely, when consuming a HFD, lean individuals have reduced taste sensitivity to C18:1, whereas no change in sensitivity is observed in overweight/obese individuals[126]. Failure of the HFD to alter taste thresholds in the obese may be due to a pre-existing adaptation to a HFD, resulting in a greater preference for, and increased consumption of, high-fat foods[123,126]. Indeed, obese subjects have increased fat detection thresholds relative to lean individuals[127]. However, further investigation is needed to provide empirical evidence of such an adaptation. As such, understanding the mechanisms underlying differences in fat sensitivity, as well as their functional responses, is the next step towards determining whether maladaptation of fat sensors may predispose individuals to obesity.

2.7.3 Fat taste in the small intestine

As with STRs, FFA receptors have been localised to the intestine, where they can trigger gut hormone release, influencing satiety signals and energy intake[27,28,39,46,47]. When TAGs are infused directly into the duodenum of humans, food intake is significantly reduced, an effect that is abolished through addition of the lipase inhibitor tetrahydrolipstatin, highlighting that fat digestion (i.e., the release of FFAs) is also an essential requirement for intestinal fat sensing[21,128]. Importantly, in the small intestine, it is long-chain fatty acids (LCFAs) with a chain length of 12 carbon atoms that induce the most potent, fat-induced hormone secretion, slowing of gastric emptying, and suppression of energy intake[129-133]. Evidence from rodent and human studies supports a relationship between impaired oral receptor-mediated fat-sensing, particularly of LCFA, and obesity, potentially driving energy

overconsumption[123,134]. In the same manner, the sensitivity of FFA receptors within the small intestine may be impaired in obesity[127], leading to overconsumption due to reduced satiety signalling. The potential contribution of FFA receptors to satiety signalling is discussed further below, and the importance of further investigations into these orosensory processes is worth emphasising.

2.7.4 Candidate fatty acid receptors

Multiple receptors for oral and intestinal FFAs have now been identified. This review is focused on the FFAs responsible for the detection of LCFAs, since LCFAs have the most potent suppressive effects on GI function and play a critical role in fat-induced satiety signalling. The lead GPR candidates include FFAR4 (previously GPR120)[27,28,32-36,124], FFAR1 (previously GPR40)[34,38-45], GPR119[46-50], and the multi-functional protein CD36[3,51-56, 86] (**Table 2.1**). Evidence for the effects of HFD/obesity on the availability and function of these receptors is also discussed, with links between receptors and hormonal signalling from the small intestine highlighted for their potential influence on subsequent energy intake (**Figure 2.2**).

2.7.5 Fatty acid receptor FFAR4

2.7.5.1 Oral FFAR4

FFAR4 is expressed in the circumvallate (CV), foliate, and fungiform taste buds of rats[32] and mice[33,34], as well as the lingual taste cells of humans[122]. FFAR4 KO mice demonstrate diminished preference for linoleic and oleic acid, and attenuated lingual nerve responses to several fatty acids, with normal responses to other tastants (e.g., sour, salty, umami)[34].

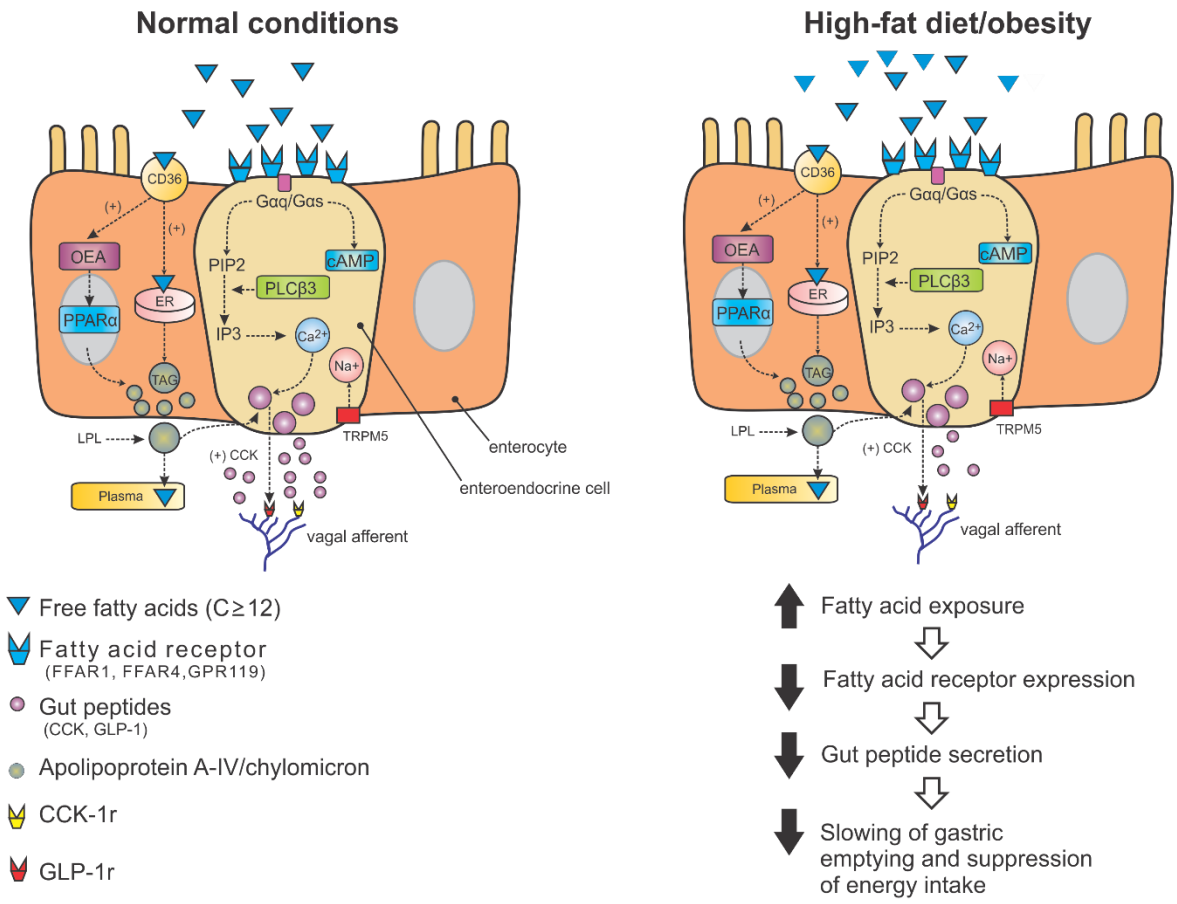

Figure 2.2: Generalised model of intestinal fat taste sensing in normal and in high-fat diet/obese conditions. FFAs, the breakdown product of dietary TAGs, activate GPRs located on the apical surface of small intestinal enteroendocrine cells. LCFAs, potent stimulators of GI function, bind to FFA receptors (FFAR4, FFAR1) and activate a taste-specific G-protein α-gustducin (Gαq) and a secondary messenger cascade via phosphatidylinositol 4, 5-bisphosphate (PIP2) or cAMP (Gαs) in the case of GPR119. PIP2 is converted to inositol triphosphate via activation of PLC βII, triggering the release of calcium from intracellular endoplasmic reticulum stores, gating of cation channel TRPM5, and the influx of sodium and cell depolarisation. Basolateral mediators (e.g., CCK, GLP-1) are subsequently released and act upon vagal afferents within the gut wall, signalling to the brainstem and higher brain centres to regulate satiety via the slowing of gastric emptying and subsequent suppression of energy intake. The multifunctional glycoprotein CD36 is responsible for uptake of FFAs, and is also critical for the production and/or mobilisation of the oleic acid derivative, oleoylethanolamide (OEA), which reduces meal frequency via activation of the peroxisome-proliferator-activated receptor-α (PPAR-α) pathway. PPAR-α also contributes to the formation of TAG-rich chylomicrons within the endoplasmic reticulum and, in particular, the component apolipoprotein A-IV, which potentially mediates CCK-induced satiety signalling. The quantity and quality of chylomicron production may affect blood clearance, potentially affecting atherogenicity of the chylomicrons produced and, in CD36 KO mice, the chylomicrons produced contain less TAG and are much smaller, leading to TAG retention in enterocytes, particularly in HFD conditions[135]. In HFD and obesity conditions, it is hypothesised that the increased luminal exposure to FFAs downregulates FFAR expression, resulting in attenuated secretion of GI signalling peptides and, consequently, impaired fat-induced suppression of gastric emptying and energy intake.

FFAR4 co-localises with GLP-1 within a subset of cells that co-express CD36 in the mouse CV papillae, and may modulate oral sensitivity thresholds for sucrose and unsaturated LCFA[33]. The addition of oleic or α-linoleic acid to isolated mouse CV papillae triggers a rise in active GLP-1 levels, an effect mimicked by the potent FFAR4 agonist, GSK137657A, providing evidence of oral FFAR4-mediated GLP-1 release in the signalling of LCFA[33]. The potential involvement of GLP-1 release in the oral detection of LCFA is further suggested by the observation that GLP-1 receptor KO mice display a reduced preference for oral LCFA, and are unable to detect low concentrations of oil (0.02–0.5% w/v)[33].

2.7.5.2 Effects of a high-fat diet/obesity on intestinal FFAR4: links to the regulation of energy intake

FFAR4 is expressed on L-cells that release GLP-1 and PYY in the mouse small intestine[28]. While a role for FFAR4 in energy regulation has been demonstrated, the precise mechanisms involved remain unclear. For example, rats with HFD-induced obesity have increased levels of FFAR4 transcript and protein in the proximal small intestine (duodenum and jejunum), but decreased CCK, PYY, and GLP-1 protein expression relative to DIO-R rats[36]. In contrast, in mouse STC-1 cells, which model small intestinal enteroendocrine cells, fatty acid-induced GLP-1 release is abolished following silencing of FFAR4 by RNA interference[27,28]. Correspondingly, mice that completely lack FFAR4 receptors demonstrate attenuated GLP-1 secretion in response to fatty acid exposure[28,35], and on a HFD they develop more profound obesity, glucose intolerance, and insulin resistance compared to their WT counterparts[35]. These outcomes indicate a loss of satiety signalling generated through FFAR4 activation, and a subsequent attenuation of the suppressive effects of fat on appetite and energy intake in the development of obesity and its metabolic comorbidities.

The modulation of FFAR4 in obesity is complex and may involve an initial acute response to increase FFAR4 to compensate for attenuated satiety signalling from L-cells during a HFD. Thereafter, modifications of intracellular and downstream pathways, such as post-translational modifications, may occur, leading to a disconnect between FFAR4 signalling and peptide secretion[36].

Current evidence supports a role for FFAR4 receptors in the detection of LCFAs and release of GLP-1 in both the oral and intestinal epithelium. Moreover, the potential loss of peripheral satiety signalling in FFAR4 KO and DIO rodent models provides strong support for a role of FFAR4 in energy regulation. In humans, key information on FFAR4 regulation is awaited, with studies to date focused on changes in human cell-lines, which may not necessarily reflect the *in vivo* setting. In order to extrapolate FFAR4 dysregulation to the tendency to overeat in obesity, changes at the mucosal level will need to be investigated to determine the luminal availability and subsequent function of the receptor.

2.7.5.3 Effect of FFAR4 polymorphisms on receptor function and body weight

Numerous FFAR4 polymorphisms have been identified in humans; with evidence that these genetic variations are linked to BMI[35] (see **Table 2.2**). A loss-of-function variant of FFAR4 (R270H) has been associated with an increased risk of obesity and insulin resistance in European populations[35] but to a lesser extent in other populations, such as the Japanese[136]. For example, variant R270H is rare in Japan (1 in 1,585 subjects was a heterozygous carrier) but is polymorphic in European populations[35]; this may explain discrepancies in population-based analysis. Few studies have evaluated the functional implications of FFAR4 variations; however, Ichimura et al.[35] reported that LCFA-induced GLP-1 release from human intestinal NCI-H716 cells is attenuated when transfected with the R270H variant. Further research is

needed to fully determine the functional implications of FFAR4 polymorphisms, but studies such as these are generating improved knowledge of how genotypic variation in nutrient receptors manifests in phenotypes of eating behaviour that may increase the risk of obesity.

2.7.6 Fatty acid receptor FFAR1

2.7.6.1 Oral FFAR1

FFAR1 is present in lingual taste cells in mice[34], yet it has been inconsistently reported in rat and human lingual epithelium[32,122,124] and its presence in the human oral epithelium remains controversial. FFAR1 is activated by LCFA, and mice that lack FFAR1 possess diminished taste nerve responses to, and preference for, LCFA (i.e., linoleic acid), while responses to other taste stimuli (e.g., bitter, sweet, salty, and umami) remain intact[34]. Interestingly, six non-fatty acid agonists of FFAR1 (Rosiglitazone, Medica 16, Compound 9.2, Compound 5, Compound 20, and Compound 10.14) are detected in sip-and-spit tests in humans and trigger a fat taste similar to linoleic acid; however, in 2-bottle preference tests in mice, these agonists were not sufficient to modify taste preference[137]. This indicates that FFAR1 activation generates a taste response, but may not be sufficient, alone, to modify taste preference; further human investigations are required to support this notion.

2.7.6.2 Intestinal FFAR1

FFAR1 is highly expressed in pancreatic β-cells, and is also found in L- and K-cells of the proximal intestine in mice, where it co-localises with GLP-1 and GIP, respectively[39]. As such, FFAR1 can enhance glucose-stimulated insulin secretion (GSIS) via direct actions on the pancreas[38], and indirectly via regulation of incretin hormone release from enteroendocrine cells[39]. Due to its tissue distribution and documented effects on fat-mediated insulin release, FFAR1 is an attractive target for potential treatment of obesity

and T2D, with early reports suggesting that FFAR1 KO mice were resistant to numerous HFD-induced effects, including hyperinsulinaemia, hyperglycaemia, hypertriglyceridaemia, and hepatic steatosis[42], and as such, that FFAR1 antagonists have the potential to prevent and treat obesity and T2D. Furthermore, linoleic acid-induced CCK secretion is abolished in pure populations of I-cells isolated from FFAR1 KO mice[40], which is an effect not previously seen in studies using STC-1 cell lines[27] and demonstrates that FFAR1 mediates LCFA-induced CCK secretion in response to dietary fat. However, later studies using the same HFD failed to show any protective effects in FFAR1 KO mice, and HFD exposure caused obesity, insulin resistance, and fatty liver[41]. Moreover, chronic over-expression of FFAR1 in β-cells of transgenic mice led to lipotoxicity and diabetes due to pancreatic dysfunction[42].

Further research is required to determine the extent to which FFAR1 mediates fat-induced effects on satiety and glycaemic control. For example, DIO and DIO-R rats subjected to a 10-week HFD demonstrated upregulated FFAR1 transcript and protein expression upon intragastric nutrient exposure, concurrent with decreased CCK, GLP-1, and PYY peptide expression[36]. The reasons behind this differential response to fatty acids at the GPR and gut peptide level during obesity remain unclear, and the debate continues as to whether FFAR1 agonists or antagonists would constitute the most appropriate therapeutic strategy.

2.7.6.3 FFAR1 polymorphisms

While mutations in FFAR1 have been identified, and include an Arg211His polymorphism, or the rare mutation variant Asp175Asn, there is little agreement on their functional implications[82-84] (see **Table 2.2**). One study found no significant relationship between Arg211His polymorphisms and insulin secretion or T2D risk[82], while another

reported that this polymorphism explained variations in serum insulin levels in Japanese men[83]. A study into a newly identified polymorphism, Gly180Ser, identified impaired intracellular calcium increase as the basis for inadequate β-cell sensing of dietary lipids as an insulin secretory stimulus[84]. While further research is needed to uncover the contribution of genetic differences in FFAR1 to energy intake and glycaemic control, it remains a therapeutic target of interest, due to its potential to control the incretin axis in patients with T2D.

2.7.7 Fatty acid receptor GPR119

The receptor GPR119 was originally localised to pancreatic islets, where it is highly expressed and modulates GSIS[47]. Importantly, however, GPR119 is also localised to L-cells of the small intestine, where it mediates glucose-independent GLP-1 secretion[138]. The restricted localisation of GPR119 makes it an attractive therapeutic target for both obesity and T2D, due to its potential to promote both euglycaemia, and satiety. For example, ileal perfusion of OEA, a naturally occurring fatty acid amide and an endogenous ligand for GPR119[48], induces GLP-1 secretion[46]. GPR119 is also expressed in GLUTag cells, human NCI-H716, and rat primary intestinal cell lines; in the last, OEA has been shown to increase GLP-1 secretion via GPR119 activation[46].

However, OEA-dependent suppression of food intake is intact in mice that lack GPR119, indicating that other pathways are involved in mediating the hypophagic effects of OEA[47]. Oral administration of the GPR119 agonist AR231453 in mice stimulates GLP-1 release and improves glucose tolerance, an effect abolished by blockade of GLP-1 receptor signalling[50]. Combined administration of AR231453 plus sitagliptin, an inhibitor of the GLP-1 breakdown enzyme dipeptidyl peptidase-4, enhanced the effect of AR231453,

whereas the presence of sitagliptin had no such effect in GPR119 KO mice[50]. Despite these findings in mice, the extent to which GPR119 agonists modulate GLP-1 release in humans, and their subsequent effects on energy intake and glycaemia, is unclear, and the few completed Phase II trials have not provided grounds for optimism (see review by Kang[139]).

2.7.8 Fatty acid sensor CD36

2.7.8.1 Oral CD36

CD36 is a receptor-like glycoprotein that binds saturated and unsaturated LCFAs (carbon chain ≥ 16) in foliate and CV papillae in the rodent and human oral epithelium. CD36 is absent from non-gustatory oral tissue, highlighting its taste-specific function[3,51]. CD36 also mediates fat preference; this was demonstrated through a loss of preference for fatty acids (linoleic acid) in CD36 KO mice compared to their WT counterparts, while sensitivity to other taste modalities remained functional[3].

2.7.8.2 Intestinal CD36 and fatty acid transport

CD36 is present in the BBM of enterocytes of the proximal intestine, the primary site of fat absorption, suggesting a role for CD36 in fatty acid uptake[55]. Functional evidence for such a role comes from KO mice, and studies of polymorphisms in rodents and humans. CD36 KO mice fed a HFD for 6 weeks, and equipped with lymph duct fistulae, had impaired lipid secretion into the mesenteric lymph in the proximal small intestine, reflecting an impaired ability to uptake fatty acid into enterocytes, synthesise TAG, and form lipoproteins in the absence of CD36[52].

Research into the intracellular signalling mechanisms linking fat ingestion to satiety have shown that activation of CD36 by LCFA induces the production and/or mobilisation of OEA, an effect that is abolished in CD36 and PPAR-α KO mice[56]. CD36 may also influence the CCK-mediated effects on satiety, with CD36 KO mice also demonstrating a significant reduction in CCK secretion in response to intragastric lipid infusion compared to WT mice[140]. A similar reduction in CCK secretion is also seen in response to fatty acids in STC-1 cells generated to stably express a mutated form of human CD36 (CD36K/A), which impairs CD36-mediated signalling to intracellular calcium but has normal FFA uptake ability[140]. In CD36-deficient humans, abnormal plasma lipid profiles are also observed, including higher postprandial hypertriglyceridaemia, which may also imply a defective clearance of dietary TAGs[135]. However, more research is required into the specific mechanisms involved. Therefore, in addition to a transporter role, CD36 may also influence the satiety effects of fat ingestion, a pathway potentially mediated by OEA.

2.7.8.3 CD36 polymorphisms

The SNP rs1761667 is a common variant in the CD36 gene leading to reduced CD36 expression, and is associated with human obesity. Obese subjects homozygous for the A-allele, which is associated with lower CD36 expression, have lower sensitivity (i.e., higher detection thresholds) to oleic acid and triolein emulsions[85]. Several SNPs of CD36 are also associated with measures of whole-body adiposity, including BMI and waist circumference, in European populations[87,88] (**Table 2.2**). Importantly, many of the numerous genetic variants of CD36 do not confer changes to the protein. While it is important to clarify which polymorphisms affect protein availability and/or function, identifying relationships between the non-functional variants in the gene with phenotypes of eating behaviour will be of value in investigating markers of chronic disease risk[141].

2.7.8.4 CD36 and high-fat diet exposure

Studies in rodents have established links between CD36 expression and susceptibility to obesity. For example, data in mice show that CV papillae protein levels of CD36 reduce significantly 1 hour after re-feeding, and are followed by a progressive return to levels observed during fasting, with an inverse correlation between CD36 protein and food intake[142]. Conversely, exposure to a 3-day HFD upregulates CD36 expression in rats[54] and may explain the increased capacity to absorb fat in the small intestine in obesity[143].

Importantly, there are differences in the responsiveness of CD36 receptors to fat exposure that align with reported differences in CD36 expression among rat strains[53,144]. These findings add support to a role of intrinsic variation in CD36 in response to fatty acid exposure, leading to overconsumption in the obese. For example, CD36 expression on the tongue and duodenum was assessed at 2 time points to assess acute response (3-day HFD) and adaptive response (14-day HFD) in rat strains either resistant to (S5B) or prone to obesity (OM). Basal levels of oral CD36 were not significantly different between strains, however consumption of a HFD induced significant strain-specific changes in CD36[144]. Oral CD36 expression was significantly increased by HFD consumption in OM rats only, with a greater consumption of HFD compared to S5B at both time points, linking increased CD36 with hyperphagia in these rats. Duodenal expression was higher at baseline in S5B rats; however, duodenal CD36 expression only increased following the 14-day HFD, whereas OM rats increased duodenal CD36 expression and persistently overconsumed fat at both time points, indicating an increased absorptive capacity for FFAs but a deficiency in satiety signalling in this strain[144]. This may indicate that S5B rats are more resistant to HFD challenges, with CD36 receptors able to generate sufficient responses to FFAs to promote adequate feelings of satiety. Therefore, increased expression and availability of CD36 may not necessarily confer differences in function/sensitivity. Studies such as these highlight that CD36

signalling can be modulated by chronic fatty acid exposure, with implications for orosensory perception of dietary fats, satiety signalling, and activation of reward pathways, factors which may promote overconsumption, particularly in obesity.

2.7.8.5 CD36 and the gut microbiota

Observations of distinct differences in the gut microbiome in genetically obese rodents and humans have piqued the interest of researchers[145]. Very little is known about the interaction and significance of the microbiome to nutrient sensing, although it has recently been shown that germ-free mice, lacking microbiota, are significantly leaner on a standard-chow diet compared to normal animals, despite having a higher energy intake[146]. Moreover, germ-free mice are completely resistant to HFD-induced obesity[147]. Interestingly, although germ-free mice demonstrate an increased preference for caloric intake from fats relative to normal mice, this is associated with increased lingual levels of CD36, yet a marked decrease in intestinal expression of both FFAR1 and FFAR4, as well as CCK, PYY, and GLP-1[148]. The potential role of gut microbiota in metabolism, energy intake, and body weight, highlights the complexity of investigations into taste receptor function in health and disease. The significance of an altered microbiome in metabolic diseases represents a novel area of gut research, although more investigation is required to ascertain how microbiota communicates with the gut to regulate intestinal nutrient sensing.

2.8 Conclusion

The gut plays a critical role in the control of appetite, energy intake, and glycaemia. The exact mechanisms that mediate gut hormone responses to nutrient intake are not established, but sufficient evidence now indicates that oral and intestinal taste receptors can determine the sensitivity to, preference for, and, ultimately, the metabolic response to ingested

nutrients. Studies of genetic modifications within these sensing pathways (e.g., human polymorphisms, genetically prone obese animal models) are now revealing their functional role in both health and metabolic diseases. The role of STRs in sweet taste sensing is well established in rodents and emerging in humans, with links to glucose absorption highlighting the importance of this luminal sensor in the control of postprandial glycaemia. This is observed through the augmentation of glucose absorption by intestinal STRs during hyperglycaemia in T2D patients, as well as evidence that STR blockade dose-dependently reduces glucose-stimulated incretin hormone release. Whether or not direct blockade of STRs is effective in controlling glycaemia in human T2D remains an exciting new area of investigation with the capacity for widespread benefits in the clinical setting.

Given the high fat intake associated with modern diets, it is now important to increase understanding of how the function of FFA receptors may change in response to dietary cues. Candidate receptors such as GPR119, while initially a promising target to modulate glycaemia through pancreatic and intestinal incretin secretion, has proven to be less successful in the clinical setting. Similarly, there is controversy in the literature regarding the potential for therapeutic development targeting FFAR1, since it is unclear whether agonism or antagonism of this receptor would be more beneficial. Finally, FFAR4 has emerged as a leading receptor target with consistent functional impact, which is particularly evident in KO models via effects on GLP-1 secretion. The critical next step in taste receptor research is to translate the findings that link receptor activation, hormone release, and altered GI function to modifications in eating behaviour and glycaemic control in animals to positive outcomes in patients with obesity and T2D. If this is achieved, these receptors will provide important new targets for the treatment of these human diseases.

Chapter 3: Duodenal Fatty Acid Sensor and Transporter Expression Following Acute Fat Exposure in Healthy Lean Humans

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Overall percentage (%)	75		
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	27/03/2017

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
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3.1 Abstract

Background and aims: FFAs and their derivatives are detected by GPRs on enteroendocrine cells, with specific transporters on enterocytes. It is unknown whether acute fat exposure affects FFA sensors/transporters, and whether this relates to hormone secretion and habitual fat intake.

Methods: We studied 20 healthy participants (10M, 10F; BMI: 22 ± 1 kg/m^2; age: 28 ± 2 years), after an overnight fast, on 2 separate days. On the first day, duodenal biopsies were collected endoscopically before, and after, a 30-min ID infusion of 10% Intralipid®, and relative transcript expression of FFAR1, FFAR4, GPR119 and the FFA transporter CD36 was quantified from biopsies. On the second day, ID Intralipid® was infused for 120-min, and plasma concentrations of CCK and GLP-1 evaluated. Habitual dietary intake was assessed using FFQs.

Results: ID Intralipid® increased expression of GPR119, but not FFAR1, FFAR4 and CD36, and stimulated CCK and GLP-1 secretion. Habitual PUFA consumption was negatively associated with basal GPR119 expression.

Conclusions: GPR119 is an early transcriptional responder to duodenal lipid in lean humans, although this response appeared reduced in individuals with high PUFA intake. These observations may have implications for downstream regulation of gut hormone secretion and appetite. This study was registered as a clinical trial with the Australia and New Zealand Clinical Trial Registry (Trial number: ACTRN12612000376842).

3.2 Introduction

Dietary TAGs stimulate upper GI motor activity and hormone secretion, and suppress appetite and energy intake in humans[149]. The liberation of FFAs is critical for the sensing of fat in the GI tract, since fat-induced responses are attenuated when TAG digestion is blocked by lipase inhibitors[150]. FFAs with a carbon chain length ≥ 12, in particular, are potent stimuli of GI activity and interact with FFAR4 and FFAR1 on enteroendocrine cells within the small intestine[28,40]. Importantly, these fat sensors play a role in mediating GI hormone secretion in rodents, but their expression and function is poorly characterised in humans.

FFAR4 and FFAR1 are present on enteroendocrine cells that release the gut hormones, CCK (from small intestinal I-cells) and GLP-1 (from small intestinal L-cells). Attenuated fat-induced hormone release from these cells has been demonstrated using RNA interference[28] or genetic KO[40] of these receptors. GPR119 is also present on L-cells, but, in contrast to FFARs, is activated by 2-monoacylglycerols and fatty acid derivatives, including the lipid messenger, OEA[46,151]. A functional role for GPR119 in fat-induced hormone signalling is evidenced by RNA inhibition of GPR119 in mouse GLUTag cells, which leads to reduced OEA-induced GLP-1 secretion[46]. The absorption of dietary LCFAs by enterocytes is mediated by the brush border transporter, CD36[152]. This transporter is a critical determinant of OEA mobilisation, which is markedly attenuated in CD36 KO mice[56].

Human obesity appears to be associated with reduced oral and GI sensitivity to fat, reflected in reduced fat-induced suppression of energy intake and GI hormone release[127]. Positive associations between duodenal expression of FFAR4 and CD36 with BMI have been reported, along with reduced numbers of enteroendocrine I- and L-cells[153]. Importantly, these fat sensors in the small intestine represent a unique system which may be dysregulated in obesity. However, whether these receptors respond to an acute fat stimulus, and whether

this is associated with subsequent gut hormone secretion, has not been assessed in humans. Investigating the influence of habitual fat intake on the expression of fat sensors in health is an important first step to determining their role in settings of high-fat consumption and obesity.

The aims of this study were, therefore, to investigate whether acute ID lipid infusion would alter expression of FFAR1, FFAR4, GPR119 and CD36 in the duodenum of lean, healthy participants, and whether this expression would be associated with fat-stimulated CCK and GLP-1 secretion, and related to habitual fat intake. We hypothesised that 1) acute ID lipid infusion would alter expression of these fat sensors, 2) these changes would be associated with fat-induced CCK and GLP-1 release, and 3) habitual fat intake would relate to basal FFAR1, FFAR4, GPR119 and CD36 expression.

3.3 Materials and methods

3.3.1 Participants

Twenty healthy volunteers (10M, 10F; 28 ± 2 years; BMI: 22 ± 1 kg.m^2) participated in the study. Participants were recruited through an existing departmental database, newspaper advertisement, and flyers at local universities and hospitals. All participants were of stable weight for at least 3 months prior to inclusion in the study and were unrestrained eaters (score ≤ 12 in the eating restraint section (Factor 1) of the Three-Factor Eating Questionnaire (TFEQ)[154]). Participants reported no GI symptoms, had no prior GI surgery, did not take medications or supplements known to affect GI motility or appetite, consumed ≤ 20 g of alcohol per week, were non-smokers, and did not regularly consume fish oil supplements. The study protocol was approved by the Royal Adelaide Hospital Research Ethics

Committee and carried out in accordance with the Declaration of Helsinki as revised in 2000. Written informed consent was obtained from each participant prior to inclusion in the study.

3.3.2 Study design and protocols

3.3.2.1 Part A: Endoscopy and collection of duodenal mucosal biopsies

Participants attended the Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, at 0830 h following a standardised evening meal (400g beef lasagne, energy content: 2470 kJ; fat, 20g; protein, 20g; carbohydrate 80g; McCain's Foods, Australia) and an overnight fast of 12 h from solids and 10 h from liquids. Anaesthetic spray (Co-Phenylcaine Forte Nasal Spray, ENT Technologies, Australia) was then administered into the nasal cavity and pharynx, and a small diameter video endoscope (external diameter: 5.3 mm, GIF-XP160, Olympus), lubricated with lignocaine gel (Orion Laboratories, Australia), was passed through the nose into the second part of the duodenum. Once positioned (t = 0 min), 2 duodenal biopsies were collected using standard endoscopic biopsy forceps and placed immediately in Allprotect® Tissue Reagent (Qiagen, Australia). Following this, an ID infusion of 10% Intralipid® (Fresenius Kabi AB, Sweden; 2 kcal/min) commenced via the endoscope infusion channel for 30 min. This design was based on our previous endoscopic studies combining nutrient infusion and biopsy collection, and establishing that expression of small intestinal glucose sensors was modulated within that time (i.e., 30 min) in humans[1]. At the conclusion of the ID infusion (t = 30 min), two additional biopsies were collected, and the endoscope removed. Participants were provided with a meal and discharge instructions and then permitted to leave the hospital.

3.3.2.2 Part B: Intraduodenal lipid infusion

Participants attended the Discipline of Medicine at 0830 h following a standardised evening meal and overnight fast, as described above. Anaesthetic spray and gel was administered

into the nasal cavity (as above) prior to insertion of a small-diameter (3.5 mm) catheter (Dentsleeve International, Mui Scientific), which was allowed to pass via peristalsis through the pylorus into the second part of the duodenum. Accurate positioning of the catheter across the pylorus was achieved by monitoring the transmucosal potential difference using a Red Dot monitoring electrode (3M Healthcare) placed on the forearm as a reference[155]. Once positioned, an IV cannula was inserted into a forearm vein, and a baseline blood sample (10 ml) was collected (t = 0 min). ID infusion of 10% Intralipid® then commenced at a rate of 2 kcal/min for 120 min (t = 0 - 120 min), during which blood samples were collected every 15 min and placed in ice-chilled EDTA-treated tubes. Plasma was separated by centrifugation for 15 min at -4°C within 15 min of collection. Our study design purposefully omitted a control (saline) arm, as we have shown previously, under the same study conditions that ID infusion does not affect GI hormone secretion[133,150,156,157]. Mindful of the burden on participants, we considered that repeating this was unjustified.

3.4 Measurements

3.4.1 RNA extraction

Frozen duodenal biopsies were disrupted using a bead-based tissue homogeniser (TissueLyser LT, Qiagen) and homogenised through Qiashredder columns (Qiagen). Total cellular RNA was isolated using the PureLink™ MicroKit (Invitrogen, Thermo Fisher Scientific), which included an on-column DNase digestion, as per manufacturer's instructions. RNA quantity was determined using a Nanodrop™ Lite Spectrophotometer (Thermo Fisher Scientific) and purity assessed using A_{260}/A_{280} ratio.

3.4.2 Quantification of gene expression by relative RT-PCR

Real-time RT-PCR was performed using the 7500 fast Real-Time PCR system (Applied Biosystems, Thermo Fisher Scientific). Taqman® primers (Life Technologies, Thermo Fisher Scientific) were used to determine the expression of FFAR1 (Hs03045166_s1, product# 4453320), FFAR4 (Hs00699184_m1, product# 4453320), GPR119 (Hs02825719_s1, product# 4453320) and CD36 (Hs01567185_m1, product# 4448892) relative to expression of the housekeeper β2M (HS00984230_m1, product# 4331182). All targets were assessed in triplicate according to manufacturer's instructions.

3.4.3 Gut hormones

Plasma CCK. Plasma CCK-8 concentrations (pmol/L) were measured by radioimmunoassay using a protocol adapted from Santangelo and colleagues[158]. Samples were extracted in 66% ethanol; extracts were dried down and resuspended in assay buffer (50 mM phosphate, 10 mM EDTA, 2 g/L gelatin, pH 7.4). Standards were prepared using synthetic sulphated CCK-8 (Sigma Chemical) with antibody (C2581, Lot 041K4838, Sigma Chemical) added at a working dilution of 1:17,500. Sulphated CCK-8 ^{125}I-labeled with Bolton and Hunter reagent (Perkin Elmer, USA) was used as tracer. Incubation was for 7 days at 4˚C. The antibody-bound fraction was separated by the addition of dextran-coated charcoal containing gelatin (0.015 g gelatin, 0.09 g dextran, 0.15 g charcoal in 30 ml assay buffer) and the radioactivity determined in the supernatants following centrifugation. The antibody binds all CCK peptides containing sulphated tyrosine residue in position 7, shows a 15% cross-reactivity with non-sulphated CCK-8, ≤ 2% cross-reactivity with human gastrin I, 0.1% with CCK (30-33) and 1% with human Big Gastrin, and does not bind structurally unrelated peptides. Intra-assay CV was 5.2% and inter-assay CV was 15.4%. The detection limit was 1 pmol/L.

Plasma GLP-1. Plasma total GLP-1 concentrations (pg/mL) were analysed using a multiplex assay (Milliplex® MAP Human Metabolic Hormone Magnetic Bead Panel, HMHEMAG-34K) using the Bio-plex® MAGPIX™ Multiplex Reader (Luminex®, Millipore Corporation) and xPONENT® software (Luminex®, Millipore Corporation, version 4.2) according to manufacturer's instructions. There was negligible antibody cross-reactivity. Intra-assay CV was ≤ 10%, and inter-assay CV was ≤ 15%. The detection limit was 2.5 pg/mL.

3.4.4 Habitual dietary intake

A FFQ was completed by each participant to assess their average daily energy and fat intakes over the previous 12 months (DQES v2; Cancer Council Victoria, Carlton, Victoria, Australia[159]). This questionnaire has been validated for use in Australian adults[160].

3.4.5 Data and statistical analyses

Sample size was based on power functions derived from our *a priori* data[153] using within-subjects contrasts with $P \leq 0.05$ and statistical power $(1-\beta) = 0.8$. Statistical analysis was performed using SPSS® software (SPSS Inc, IBM®, version 20), and all graphs were generated using GraphPad Prism 6 (GraphPad Software Inc). One-way analysis of variance (ANOVA) with post-hoc Bonferroni testing was used to compare basal expression of all receptor targets. Paired samples t-tests were used to compare post-infusion (t = 30 min) expression with baseline (t = 0 min) for each of FFAR1, FFAR4, GPR119 and CD36. Relationships between receptor expression and habitual energy and fat intake were determined by correlation, with Pearson's r values presented. Paired samples t-tests were also used to compare gut hormone stimulation at t = 120 min with baseline concentrations (t = 0 min). Blood samples were collected on the longer infusion day for logistical reasons, as our research has shown that plasma CCK[161] and GLP-1[162] concentrations are comparable across multiple, identical study day visits. Hormone data were expressed as AUCs

(calculated using the trapezoidal rule from t = 0 min to 120 min). This AUC value (pmol.L min^{-1} or pg.mL min^{-1}) was divided by the time of last measurement to obtain a final weighted average (AUC, pmol/L or pg/mL) to account for occasions (in n = 2 participants) when samples could not be collected (e.g., bathroom breaks). Relationships between receptor expression at t = 0 min and t = 30 min with plasma hormone AUC, were determined by correlation, Pearson's r values presented. Data are expressed as means ± standard error of the mean (SEM), with statistical significance accepted at $P \leq 0.05$.

3.5 Results

Endoscopic and infusion procedures were well tolerated, and biopsies were successfully collected from all 20 participants.

3.5.1 Expression of fat sensors in the proximal human duodenum following acute ID lipid exposure

Expression of FFAR1, FFAR4, GPR119 and CD36 was detected in all duodenal biopsies at baseline, with relative abundance of CD36>>FFAR1>FFAR4>GPR119 (all $P \leq 0.05$). ID lipid infusion increased duodenal expression of GPR119 ($P \leq 0.05$), while other transcripts were unchanged. β2M was stably expressed in all biopsies and unchanged by the experimental paradigm (**Figure 3.1**).

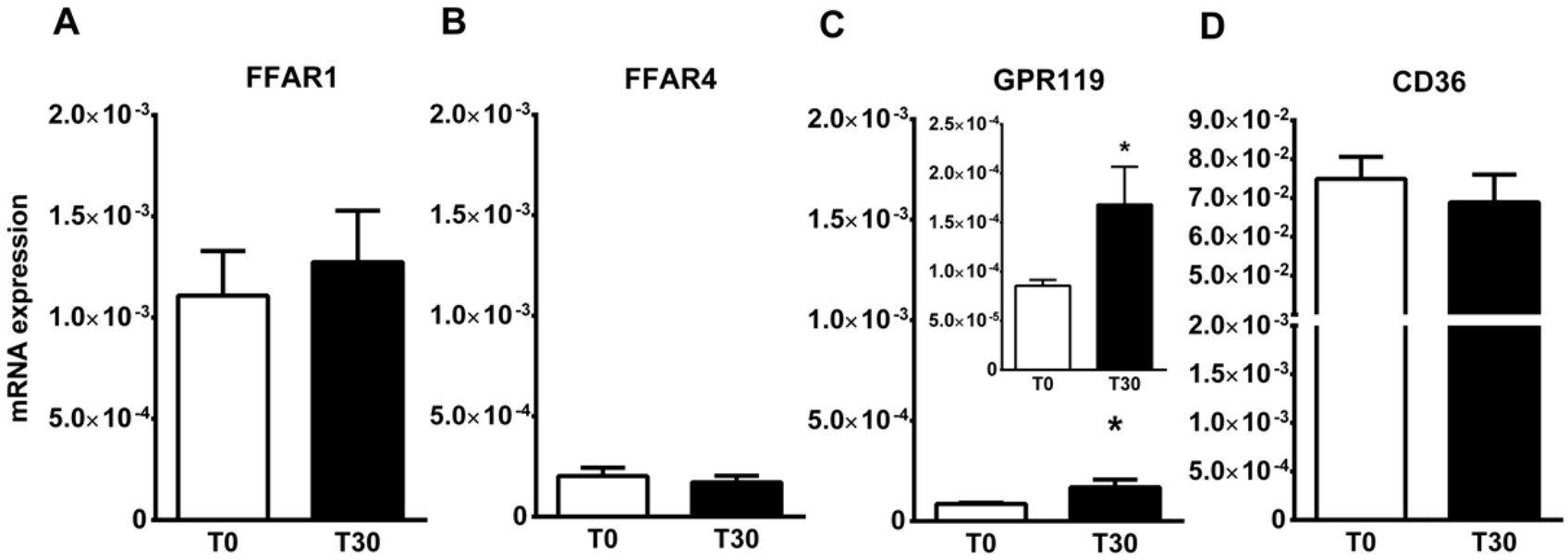

Figure 3.1: Relative transcript expression of FFAR1 (A), FFAR4 (B), GPR119 (C), and CD36 (D) in human duodenal biopsies at baseline (T0) and following 30 min ID Intralipid® infusion (T30) (2 kcal/min). Expression of **A**) FFAR1, **B**) FFAR4 and **D**) CD36 mRNA expression was unchanged by ID lipid infusion. **C**) GPR119 mRNA expression increased at t = 30 min (inset: optimised axes, *P ≤ 0.05) following ID lipid infusion. Data are expressed as mean ± SEM, n = 20.

3.5.2 Gut hormones

ID lipid stimulated CCK release ($P \leq 0.05$, **Figure 3.2**). The time to maximum concentration (TMax, group median), maximum concentration (CMax, group mean) and total AUC are detailed in **Table 3.1**. There was no correlation between FFAR1, FFAR4, CD36 and GPR119 expression at baseline, or following ID lipid, and CCK AUC.

ID lipid also stimulated release of GLP-1 ($P \leq 0.05$, **Figure 3.2**). The TMax, CMax and total AUC are indicated in **Table 3.1**. There was no correlation between FFAR1, FFAR4, GPR119 and CD36 expression at baseline, or following ID lipid, and GLP-1 AUC.

3.5.3 Relationship between habitual fat intake and duodenal expression of FFARs and CD36

Habitual dietary intake assessed from FFQs is summarized in **Table 3.2**. Three participants had incomplete questionnaires, which were rejected by the automated analysis. There were no relationships between baseline, or post-lipid duodenal expression, of FFAR1, FFAR4, GPR119 or CD36 with habitual energy, total fat, saturated fat, monounsaturated fat, protein, or carbohydrate intakes. Habitual PUFA consumption (13 ± 1 g/day) was negatively correlated with basal duodenal GPR119 expression ($r = -0.5$, $P \leq 0.05$, **Figure 3.3**).

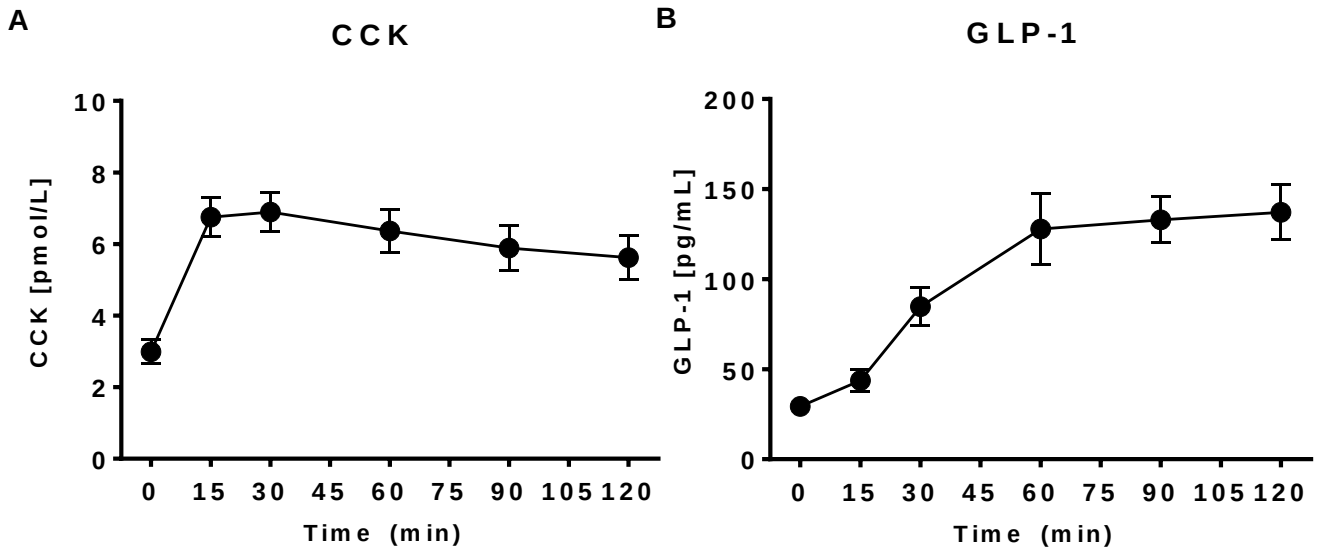

Figure 3.2: Plasma CCK (A) and GLP-1 (B) concentrations during 120 min ID Intralipid® infusion (2 kcal/min). Plasma CCK and GLP-1 were both increased by ID lipid infusion (both $P \leq 0.05$)**.** Data are mean ± SEM, n = 19 (CCK) and n = 17 (GLP-1).

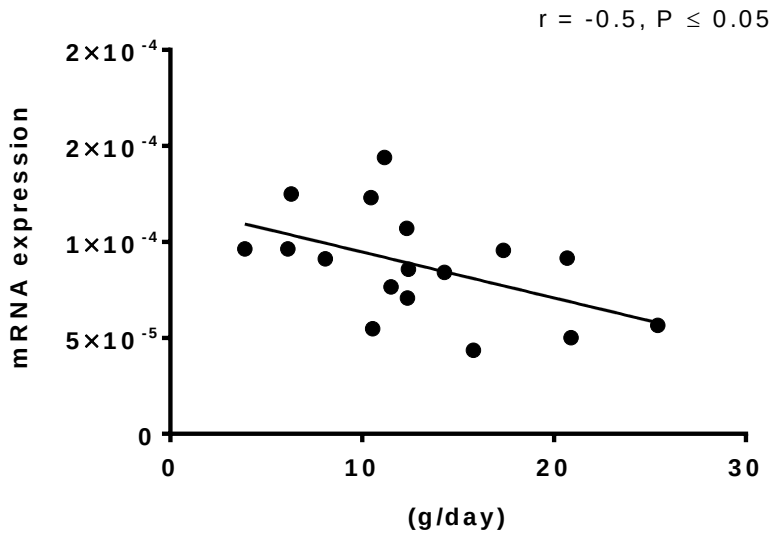

Figure 3.3: Relationship between habitual consumption of PUFAs (g/day) and duodenal mRNA expression of GPR119. Habitual consumption of PUFA was negatively correlated to basal expression of GPR119 ($r = -0.5$, n = 17, $P \leq 0.05$).

Table 3.1: Plasma gastrointestinal hormone concentrations in healthy, lean humans during 120-min ID Intralipid® infusion (2 kcal/min).

Hormone	T_{max} (min)	C_{max}	AUC
CCK (pmol/L) (n=19)	15	7.9 ± 0.6	7.5 ± 0.6
GLP-1 (pg/mL) (n=17)	90	168 ± 21	105 ± 10

Data are mean ± SEM. AUC: Area under the curve; CCK: Cholecystokinin; C_{max}; maximum concentration; GLP-1: glucagon-like peptide-1; T_{max}: time to maximum concentration

Table 3.2: Average daily energy and macronutrient intake assessed over the previous 12 months

	Mean ± SEM
Energy intake, kJ/day	9757 ± 785
Total fat, g/day	99 ± 9
Saturated fat, g/day	41 ± 4
Polyunsaturated fat, g/day	13 ± 1
Monounsaturated fat, g/day	36 ± 4
Protein, g/day	121 ± 13
Carbohydrates, g/day	241 ± 17
Sugars, g/day	103 ± 10
Starch g/day	137 ± 10
Fiber, g/day	27 ± 3

3.6 Discussion

This study has evaluated the expression of the fat sensors, FFAR1, FFAR4, GPR119, and the sensor/transporter, CD36, in the human duodenum, and the effects of acute ID lipid infusion. We showed expression of these targets in the duodenum of healthy participants, with relative abundance mirroring our earlier findings[153]. CD36 was the most abundant transcript expressed in the duodenum, consistent with its known localisation to the BBM of enterocytes and suggested FFA transport capabilities[153]. We further demonstrated that an acute, meal-relevant fat stimulus, known to trigger acute GI responses (as evidenced by gut hormone secretion), increased expression of GPR119, but not FFAR1, FFAR4 or CD36, in the duodenum of healthy participants. In addition, GPR119 expression was negatively correlated with habitual PUFA intake, supporting an ability of long-term dietary patterns to influence the availability of this sensor. These results are novel and bridge a gap in our understanding of how transcriptional control of these FFA-sensing GPRs is regulated in an acute *in vivo* setting.

Lipid-induced increases in GPR119 expression in our study, if linked to increased availability of the apical receptor, or its signalling, would support a capacity to respond to the ongoing presence of luminal fat. This, in turn, may augment secretion of gut hormones and/or increase satiety signalling. Although we did not detect significant associations between GPR119 and hormone secretion, this may have been due to a type 2 error owing to the relatively small sample size. Further, while the magnitude of the change in GPR119 expression was modest, previous work utilising RNA interference of GPR119 has demonstrated that even modest knockdown of GPR119 transcript (23%) in mGLUTag cells was sufficient to block the rise in cAMP in response to a GPR119-specific stimulus, and also resulted in a 45% reduction in GLP-1 secretion[46]. While the mechanisms linking luminal sensing and hormone secretion are largely unknown and cannot be derived from the current

data, there is extensive evidence supporting a role of GPR119 in mediating glucose-independent incretin secretion from enteroendocrine cells[50], and growing evidence for a role in satiation[46].

GPR119 is tuned to detect the oleic acid derivative, OEA, a potent trigger for GLP-1 secretion in human and rodent cell lines[46]. OEA and small molecule agonists of GPR119 are capable of suppressing food intake in animals, the former by prolonging latency between meals[163]. However, the hypophagic capacity of OEA is not established in humans, further complicated by the ability for OEA to signal through multiple mechanisms, for example via PPAR-α, a pathway implicated in the absorption, storage and utilisation of dietary fat[56,163]. Indeed, mice lacking CD36 (required for fat-induced OEA production in enterocytes) or PPARα show attenuated fat-induced satiety[56], suggesting that the appetite-suppressant effects of OEA may be GPR119- and CD36-dependent.

GPR119-mediated satiation may also be linked to GLP-1 release and subsequent activation of GLP-1 receptors on mucosal vagal afferents. As vagal afferent endings do not directly interact with luminal content, it is feasible that GPR119 may link luminal fats to secretion of hormones, such as GLP-1. While there is currently no direct evidence in humans of a functional role for GPR119 in energy intake regulation, ID infusion of the putative GPR119 ligand, 2-oleylgylcerol, in humans increases plasma GLP-1 and GIP[151], and the GLP-1 antagonist, exendin(9-39), attenuates the beneficial glucoregulatory effects of the GPR119 agonist, AR23145, in mice[50]. Therefore, a stimulus known to enhance incretin secretions *in vivo* also activates GPR119 *in vitro*, implicating GPR119 in this process. Our data show that acute fat exposure is not only sufficient to elicit a hormone response, but also triggers transcriptional regulation of GPR119. Together with previous work, our findings add support to the view that GPR119 activation may be linked to gut hormone secretion in humans.

We acknowledge that proteomics will be a critical step in accurately linking transcript changes seen in GPR119 to luminal receptor activity and intracellular/downstream signalling events. This remains a challenge for GPR119, as commercially available antibodies suitable for Western blotting for G-proteins are often found to be non-specific for the same target (for review see[164]). In addition, in-group anti-sera have also been shown to produce inconsistent immunolocalisation[165,166]. Nonetheless, transcript data here justify further proteomic investigations. GPR internalisation in the face of ongoing stimulus results in a loss of signalling fidelity, with evidence from cell-based assays revealing that FFAR1, FFAR4 and GPR119 show dose- and time-dependent internalisation in response to endogenous and synthetic agonists[28,167-169]. However, a sustained cAMP elevation has been noted in GPR119-containing HEK293 cells chronically exposed to physiological doses of OEA, or the synthetic GPR119 agonist, AR231435[170]. While discussion of these discrepancies is beyond the scope of this work, it highlights the fact that GPCR desensitisation in fat sensor systems is not well understood.

Our results provide support that basal GPR119 expression is driven, at least in part, by habitual PUFA consumption. While studies have not previously addressed the relationship between long-term habitual fat intake and GPR119 gene expression, chronic consumption of a HFD is associated with obesity, while consumption of PUFA is linked to lower levels of adiposity[171]. PUFAs (such as linoleic acid) are known to bind to PPARα to regulate various genes involved in fat metabolism[172], and although GPRs are uncommon targets of PPARα action, it is conceivable that factors which act upon this nuclear receptor may regulate GPR119 expression. Recent studies have also shown that the type and amount of habitual fat intake is an important predictor of intestinal OEA production, and therein, potential GPR119 activation. For example, a 1-week diet high in monounsaturated fatty acids (MUFAs), PUFAs, saturated and unsaturated fats in rodents attenuated basal jejunal

levels of OEA[173], while 1 week of diets high in MUFAs (olive oil) had no effect on basal OEA levels in the rat jejunum[174]. Accordingly, participants with higher habitual PUFA intake in our study may have a greater capacity for PPARα activation, and lower basal GPR119 expression. The reasons for such an effect remain elusive, but illustrate the potential for habitual fat intake to influence expression of genes relating to fat sensing. A limitation of our study is that the FFQ analysis did not have the specificity to reveal habitual intakes of different PUFAs. In addition, the questionnaire may not accurately account for participants' acute fat intake immediately prior to the study. Prospective studies are warranted to investigate the effects of dietary composition on fat sensor expression.

In summary, this study provides the first evidence of transcriptional upregulation of GPR119 in the face of an acute ID fat stimulus within the human duodenum. Further investigations that extend the postprandial period may reveal changes in other targets. We also revealed associations between GPR119 and habitual PUFA intake, indicating that fat sensing systems can be influenced by dietary fat intake. Further studies also need to determine the molecular mechanisms by which GPR119 operates in humans, and the significance of its endogenous ligands, such as OEA, in paracrine signalling within the duodenal mucosa. Finally, whether acute changes in GPR119 in response to fat are maintained under metabolic challenges, such as a HFD or in obesity, in humans should be the subject of further research, to determine the potential for fat sensing mechanisms to become dysregulated.

Chapter 4: Lipid-Stimulation of Fatty Acid Sensors in the Human Duodenum: Relationship with Gastrointestinal Hormones, BMI and Diet

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Overall percentage (%)	75		
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	27/03/2017

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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Signature		Date	14/12/2016

4.1 Abstract

Background and Aims: The small intestinal FFA sensors, FFAR1, FFAR4, GPR119 and CD36, mediate the fat-induced release of GI hormones. We investigated whether expression of duodenal FFA sensors in humans was (i) altered by ID lipid infusion, (ii) disordered in overweight or obese individuals, (iii) related to lipid-induced GI hormone secretion or (iv) affected by habitual dietary patterns.

Methods: Endoscopic duodenal biopsies were collected from 20 lean (BMI: 22 ± 1 $kg.m^2$), 18 overweight (BMI: 27 ± 1 $kg.m^2$) and 19 obese (BMI: 35 ± 1 $kg.m^2$) participants at baseline, and following a 30 min ID Intralipid® infusion (2 kcal/min); FFA sensor expression was quantified by RT-PCR. On a separate day, participants underwent ID Intralipid® infusion (2 kcal/min) for 120 min, to assess GI hormone responses. Habitual diet was evaluated using FFQs.

Results: Baseline FFAR1 and FFAR4 expression were lower, and CD36 higher, in obese compared to lean participants. ID lipid increased GPR119 and FFAR1 expression equally across study groups, but did not alter FFAR4 or CD36 expression. Increased FFAR1 expression correlated positively with GIP secretion ($r = 0.3$, $P \leq 0.05$), while there was no relationship between habitual diet with the expression of FFA sensors.

Conclusions: Obesity is associated with altered duodenal expression of FFAR1, FFAR4 and CD36, suggesting altered capacity for the sensing, absorption and metabolism, of dietary lipids. GPR119 and FFAR1 are early transcriptional responders to the presence of ID lipid, while FFAR1 may be an important trigger for lipid-induced GIP release in humans. This study was prospectively registered with the Australia and New Zealand Clinical Trial Registry (www.anzctr.org.au trial number: ACTRN12612000376842).

4.2 Introduction

Small intestinal sensing of FFAs potently triggers the release of GI hormones such as CCK and GLP-1, slows gastric emptying, and suppresses subsequent energy intake[150]. However, chronic consumption of a HFD promotes increased energy intake[175] and is associated with the development of obesity. In animal studies, chronic HFD consumption markedly attenuates GI sensing of dietary fat, GI hormone secretion, and motility responses (reviewed in[176,177]), and is likely to be an important mechanism underlying increased energy intake. While studies investigating the influence of HFD consumption on GI fat sensing in humans are limited, we previously reported that obese humans with high habitual fat and energy intakes had reduced stimulation of pyloric motility and CCK in response to ID infusion of the FFA, oleic acid (C18:1), and a higher energy intake at a subsequent *ad libitum* meal, when compared with lean individuals[127]. Collectively, this indicates that small intestinal sensitivity to fat may be reduced in human obesity, contributing to dysregulated energy intake. However, the mechanisms underlying these changes in GI fat sensing are poorly understood.

Rodent models and cell line studies have determined that the FFA sensors, FFAR1, FFAR4 and GPR119, localised on enteroendocrine cells, and the putative FFA transporter, CD36, localised on enterocytes, detect the presence of dietary FFAs in the small intestine and trigger the release of GI hormones[28,36,38-40,46,178]. We demonstrated that fasting duodenal expression of CD36 and FFAR4 were increased, and GPR119 decreased, with increasing BMI[153]. Furthermore, a 30-min ID lipid infusion upregulated duodenal expression of GPR119 in healthy, lean individuals, with the magnitude of this response reduced in individuals with a high habitual consumption of PUFAs[179]. Therefore, the expression of GPR119 is likely to be modulated both acutely by small intestinal nutrient exposure, and chronically by habitual dietary patterns. Small intestinal changes in FFA sensor expression would be likely to impact

downstream signalling events linked to GI hormone secretion and energy intake, but this has not been investigated in humans.

Therefore, the aims of the current study were to (i) evaluate the effects of acute ID lipid infusion on the duodenal expression of the FFA sensors, FFAR1, FFAR4, GPR119 and CD36, in healthy lean, overweight and obese participants, and (ii) determine relationships between expression of these sensors and habitual fat and energy intakes, BMI, GI hormone secretion and *ad libitum* energy intake.

4.3 Materials and methods

4.3.1 Participants

20 lean (10M: 10F, age: 28 ± 2 years; BMI: 22 ± 0.5 kg.m^2), 18 overweight (12M: 6F, age: 32 ± 3 years; BMI: 27 ± 0.3 kg.m^2) and 19 obese (12M: 7F, age: 30 ± 2 years; BMI: 35 ± 1 kg.m^2) volunteers were included in the study. Participants were weight-stable for at least 3 months prior to enrolment and were unrestrained eaters[154]. Participants had no GI symptoms or previous GI surgery, did not take medications or supplements known to affect GI motility or appetite, consumed ≤ 20g of alcohol per week, were non-smokers, and did not take fish oil supplements. At the screening visits, their HbA1c and iron levels were within normal ranges (**Table 4.1**). The study protocol was approved by the Royal Adelaide Hospital Research Ethics Committee and carried out in accordance with the Declaration of Helsinki. Each participant provided written informed consent prior to study inclusion.

Table 4.1: Participant demographics at initial screening

	Lean	Overweight	Obese	P
n	20	18	19	
Sex	10M : 10F	12M : 6F	12M : 7F	NS
Age (years)	28 ± 2	32 ± 3	30 ± 2	NS
BMI ($kg.m^2$)	22 ± 0.5	27 ± 0.3	35 ± 1	*#∧P ≤ 0.001
HbA1c %	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	NS
Iron (μmol/L)	21 ± 2	22 ± 1	20 ± 2	NS

Data are mean ± SEM. *Lean vs overweight; #Obese vs lean; ^Obese vs overweight

4.3.2 Study design and protocols

4.3.2.1 Part A: Endoscopic collection of duodenal mucosal biopsies

Participants attended the Gastrointestinal Investigation Unit at the Royal Adelaide Hospital, at 0830 h, following a standardized evening meal (400g lasagne, 2470 kJ; fat, 20g; protein, 20g; carbohydrate, 80g; McCain Foods, Australia) and an overnight fast (12 hrs for solids, 10 hrs for liquids). The protocol for endoscopic collection of mucosal biopsies has been previously described[179]. Briefly, duodenal biopsies were collected using standard biopsy forceps, and separate biopsies collected into Allprotect® Tissue Reagent (Qiagen, Australia) or archived at -20˚C. An ID infusion of 10% Intralipid® (Fresenius Kabi AB, Sweden; 2 kcal/min; 109 ml/hr) was then commenced via the infusion channel of the endoscope and maintained for 30 min, with two additional biopsies collected at the conclusion of the ID infusion (t = 30 min). The duration of infusion was based on a previous study from our group showing changes in intestinal expression of sweet taste receptors within 10 min of a 30-min ID infusion of glucose in humans[1].

4.3.2.2 Part B: GI hormone, appetite, and energy intake responses to ID lipid

Participants arrived at 0830 h following a standardised evening meal and overnight fast, as described above. A small-diameter (3.5mm) catheter was positioned in the second part of the duodenum, and an IV cannula inserted into a forearm vein and a baseline blood sample collected (t = 0 min), as previously described[179]. ID infusion of 10% Intralipid® was then commenced at a rate of 2 kcal/min for 120 min (t = 0 - 120 min). Blood samples were collected in ice-chilled EDTA-treated tubes every 15 min and separated by centrifugation (15 min at 4°C), within 15 min of collection. Visual analogue scales (VAS) were completed every 15 min from t = 0 – 120 min. At t = 120 min participants received a standardized, cold, buffet-style meal as previously described[161].

4.4 Measurements

4.4.1 RNA extraction

Frozen duodenal biopsies were disrupted using a bead-based homogeniser (TissueLyser LT, Qiagen) and homogenised through Qiashredder columns (Qiagen). Total cellular RNA was isolated using the PureLink™ MicroKit (Invitrogen, Thermo Fisher Scientific, Australia) with on-column DNase digestion, as per the manufacturer's instructions. RNA quantity was determined using a Nanodrop™ Lite Spectrophotometer (Thermo Fisher Scientific) and purity assessed using A_{260}/A_{280} ratio.

4.4.2 Quantification of FFA sensor expression by relative RT-PCR

Real-time RT-PCR was performed using a 7500 fast Real-Time PCR system (Applied Biosystems, Thermo Fisher Scientific). Taqman® primers (Life Technologies, Thermo Fisher Scientific) were used to determine the expression of FFAR1 (Hs03045166_s1, product# 4453320), FFAR4 (Hs00699184_m1, product# 4453320), GPR119

(Hs02825719_s1, product# 4453320) and CD36 (Hs01567185_m1, product# 4448892) relative to expression of the housekeeper gene β2M (HS00984230, product# 4331182). All targets were assessed in triplicate according to manufacturer's instructions.

4.4.3 Gut hormones

4.4.3.1 Plasma CCK

CCK-8 was measured by radioimmunoassay using a protocol adapted from Santangelo and colleagues[158], as described previously[179].

4.4.3.2 Total GLP-1 and GIP, PYY, insulin and leptin

Total GLP-1 and GIP, PYY, insulin and leptin were determined using a multiplex assay (Milliplex® MAP Human Metabolic Hormone Magnetic Bead Panel, HMHEMAG-34K, Millipore Corporation, USA) and analysed on a Bio-plex® MAGPIX™ Multiplex Reader (Luminex®, Millipore Corporation) using xPONENT® software (Luminex®, Millipore Corporation, version 4.2) according to the manufacturer's instructions. There was negligible antibody cross-reactivity. Intra-assay CV was ≤ 10%, and inter-assay CV was ≤ 15% for all analytes. The detection limits were: GLP-1, 2.5 pg/mL; GIP, 0.6 pg/mL; PYY 28 pg/mL; insulin 87 pg/mL; and leptin, 41 pg/ml.

4.4.4 Blood glucose

Venous blood glucose (mmol/L) was measured at collection by the glucose oxidase method using a portable glucometer (Medisense Precision QID; Abbott Laboratories, USA).

4.4.5 Appetite perceptions, and *ad-libitum* buffet meal intakes

VAS were used to determine perceptions of hunger, fullness, desire to eat, prospective consumption, nausea and bloating[180].

Energy intake (kJ), amount (g) and percentage energy from fat, carbohydrate and protein consumed at the buffet meal was analysed using commercial software (FoodWorks® 2009, Version 6, Xyris Software Pty Ltd).

4.4.6 Habitual dietary intake

Participants completed a FFQ to assess their average energy and macronutrient intake (fat, carbohydrate, protein) over the previous 12 months (DQES v2; Cancer Council Victoria, Carlton, Victoria, Australia[159]). This questionnaire has been specifically validated for use in Australian adults[160].

4.4.7 Data and statistical analyses

Sample size was based on power functions derived from our *a priori* data[153] using within-subject contrasts of $P \leq 0.05$ and statistical power $(1-\beta) = 0.8$. Statistical analysis was performed using SPSS® software (SPSS Inc, IBM®, Version 20), in collaboration with a professional biostatistician. Expression levels of FFA sensors at t = 0 min and t = 30 min were normalised to levels of β2M using delta CT comparison (cycle threshold - target of interest cycle threshold)[181]. One-way ANOVA was used to compare baseline (t = 0 min) expression of FFAR1, FFAR4, GPR119 and CD36, with BMI group (i.e., lean, BMI: 18-24 $kg.m^2$; overweight, BMI: 25-29 $kg.m^2$; or obese, BMI $\geq$ 30 $kg.m^2$) as the factor. Two-way repeated measures ANOVA was used to compare changes in receptor expression from baseline (t = 0 min) to post-infusion (t = 30 min), with BMI group as a between-subjects factor. Post-hoc pairwise comparisons, corrected for multiple comparisons using the

Bonferroni method, were performed if ANOVAs were significant. Baseline plasma hormone and blood glucose concentrations, and VAS scores, were calculated from mean values obtained at t = -15 min and t = 0 min. Blood samples were collected only on the longer infusion day for logistical reasons. We have previously demonstrated that plasma CCK and GLP-1 responses to nutrient do not differ across multiple, identical study days within an individual[161, 162]. iAUCs for gut hormones, blood glucose, and VAS were calculated using the trapezoidal rule from t = 0 min to 120 min (pmol.L min^{-1} or pg.mL min^{-1}), which was divided by the time of last measurement to obtain a final weighted average (iAUC, pmol/L or pg/mL) to account for occasions (in n = 8 participants) when samples could not be collected (e.g., during bathroom breaks). In occasional instances where the t = 15 min time point was below the detection limit of the assay (PYY, n = 5; insulin, n = 3, leptin n = 1), the minimum limit of detection was halved to obtain a baseline estimate for iAUC calculation. The maximum concentration of blood glucose and gut hormones (CMax), was calculated from t = 0 - 120 min. Associations between the transcript expression and BMI, plasma hormones, blood glucose, and acute and habitual dietary intakes were determined by Pearson's correlations, with r values presented, with transcript expression expressed as change from baseline (Δ, t30 - t0) due to variability in individual transcript data. Data are expressed as mean ± SEM, with statistical significance accepted at $P \leq 0.05$.

4.5 Results

Endoscopic procedures were well tolerated, and biopsies collected from 56 out of 57 participants. One obese participant did not complete the endoscopy for reasons unrelated to the study. Eight participants did not complete the full 120-min infusion protocol due to nausea (2 lean, 4 overweight, 1 obese; duration of infusion ranging from t = 15 min to t = 90 min) or catheter displacement (lean, n = 1); buffet meal intake was not measured in these

participants. Eight participants (3 lean, 4 overweight, 1 obese) had incomplete FFQ questionnaires, which were, therefore, rejected by the automated analysis.

4.5.1 Habitual dietary intake

Average daily energy and macronutrient intakes over the previous 12 months did not differ between the lean, overweight and obese groups (**Table 4.2**).

4.5.2 Duodenal FFA sensor expression at baseline and following the 30-min ID lipid infusion

FFAR1, FFAR4, GPR119 and CD36 expression was detected in all duodenal biopsies at baseline (i.e., during fasting), with relative abundance CD36 >> FFAR1 > FFAR4 > GPR119 seen in all subject groups (**Figure 4.1**). Expression of the housekeeper gene, β2M, was stable in all biopsies across time points and groups. Baseline FFAR1 expression was 62% lower in the obese compared with lean ($P \leq 0.05$), and 51% lower in the overweight ($P = 0.054$) compared with lean, with no difference between obese and overweight groups. Baseline FFAR4 expression was 62% lower in the obese compared with the lean group ($P \leq 0.05$), with no differences between overweight and obese, or overweight and lean groups. Baseline GPR119 expression was not different between the groups. CD36 expression was higher in the obese compared with both the lean (242-fold, $P \leq 0.001$), and overweight (202-fold, $P \leq 0.001$) groups, with no difference between lean and overweight (**Figure 4.2**).

Within each group, FFAR1 expression increased after ID lipid when compared to baseline; by 1.4 ± 0.2-fold in the lean ($P \leq 0.05$), by 1.6 ± 0.2-fold in the overweight ($P \leq 0.05$), and by 1.7 ± 0.3-fold in the obese ($P \leq 0.05$). GPR119 expression also increased from baseline after ID lipid; by 2.0 ± 0.3-fold in the lean ($P \leq 0.05$), by 2.4 ± 0.7-fold in the overweight

($P \leq 0.05$), and by 2.0 ± 0.3-fold in the obese ($P \leq 0.05$). There was no effect of ID lipid on expression of FFAR4 and CD36 in any group (**Figure 4.2**).

Table 4.2: Daily energy and macronutrient intakes over the previous 12 months, and *ad libitum* consumption at a buffet meal following 120 min ID Intralipid® infusion (2 kcal/min).

Habitual	**Lean**	**Overweight**	**Obese**	**P**
Energy intake (kJ/day)	9757 ± 785	9820 ± 857	9320 ± 1476	NS
Total fat (g/day)	99 ± 9	99 ± 10	101 ± 17	NS
Saturated fat (g/day)	41 ± 4	41 ± 5	43 ± 8	NS
Polyunsaturated fat (g/day)	13 ± 1	13 ± 2	13 ± 2	NS
Monounsaturated fat (g/day)	36 ± 4	36 ± 4	37 ± 6	NS
Protein (g/day)	121 ± 13	126 ± 13	114 ± 15	NS
Carbohydrates (g/day)	241 ± 17	239 ± 20	219 ± 36	NS
Sugars (g/day)	103 ± 10	92 ± 11	87 ± 14	NS
Starch (g/day)	137 ± 10	146 ± 13	131 ± 22	NS
Fiber (g/day)	27 ± 3	25 ± 2	21 ± 3	NS
Buffet meal				
Energy intake (kJ)	4413 ± 434	4407 ± 443	4125 ± 383	NS
Amount (g)	1001 ± 88	1062 ± 78	931 ± 89	NS
Fat (g)	38 ± 4	36 ± 5	40 ± 4	NS
Fat (%)	32 ± 1	29 ± 2^	36 ± 2^	^ ≤ 0.01
Protein (g)	60 ±7	57 ±7	56 ± 6	NS
Protein (%)	23 ± 1	22 ± 1	23 ± 1	NS
Carbohydrate (g)	115 ± 11	123 ± 11	99 ± 10	NS
Carbohydrate (%)	43 ± 2	48 ± 3^	40 ± 2^	^ ≤ 0.05

^Obese vs overweight; Data are mean ± SEM; Habitual, lean, n = 17; overweight, n = 14; obese, n = 18; Buffet meal, lean, n = 17; overweight, n = 14; obese, n = 18.

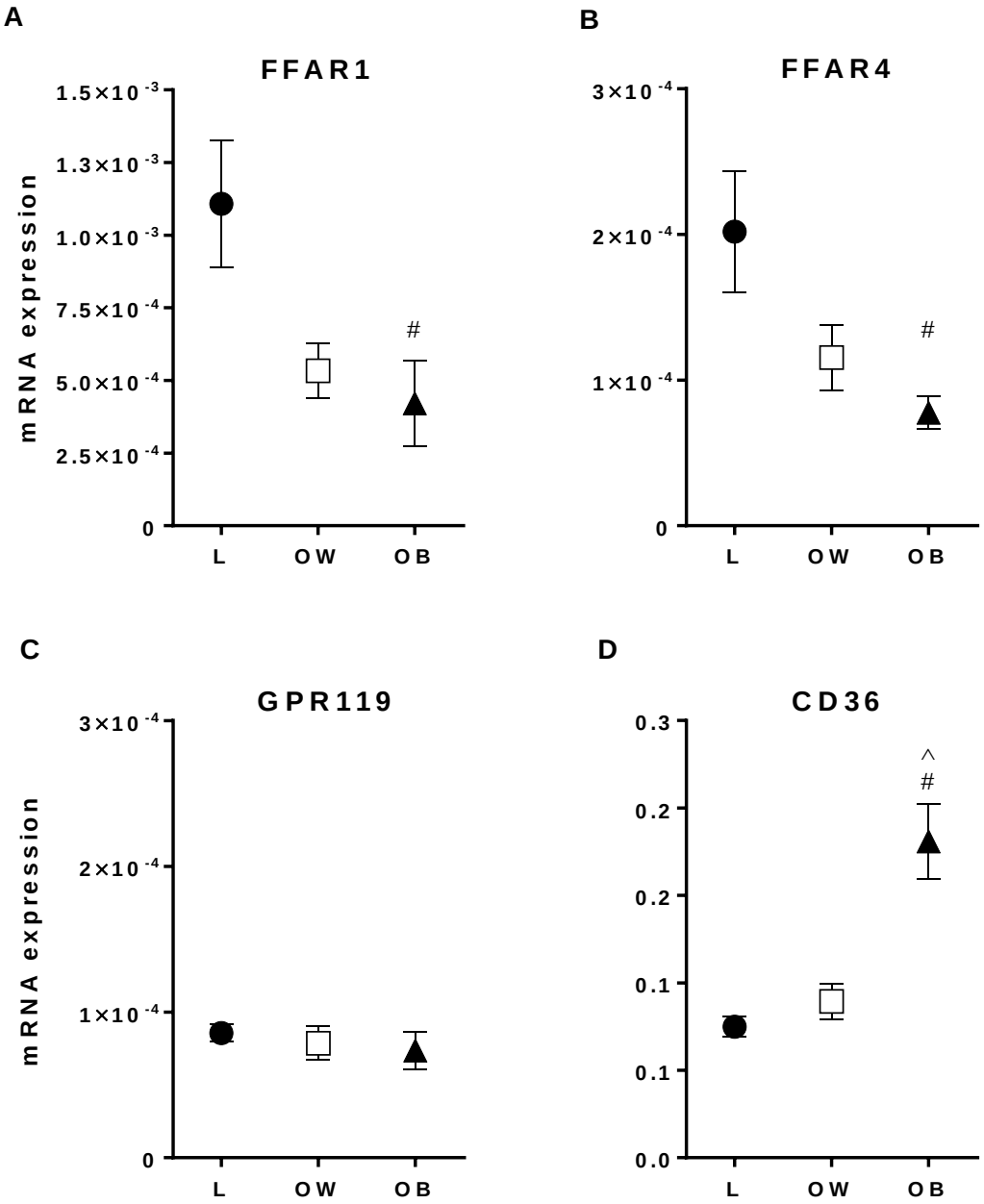

Figure 4.1: Baseline (fasting) duodenal expression of FFAR1, FFAR4, GPR119 and CD36 in lean, overweight and obese humans. A) FFAR1 and **B**) FFAR4 expression was lower at baseline in obese compared to lean subjects ($^{\#}P \leq 0.05$), but did not differ between lean and overweight participants. There were no differences in expression of **C**) GPR119 between study groups, while **D**) CD36 expression was higher in obese compared to both lean and overweight participants ($^{\#\wedge}P \leq 0.001$). Data are mean ± SEM. Lean (L, n = 20), overweight (OW, n = 18), obese (OB, n =18).

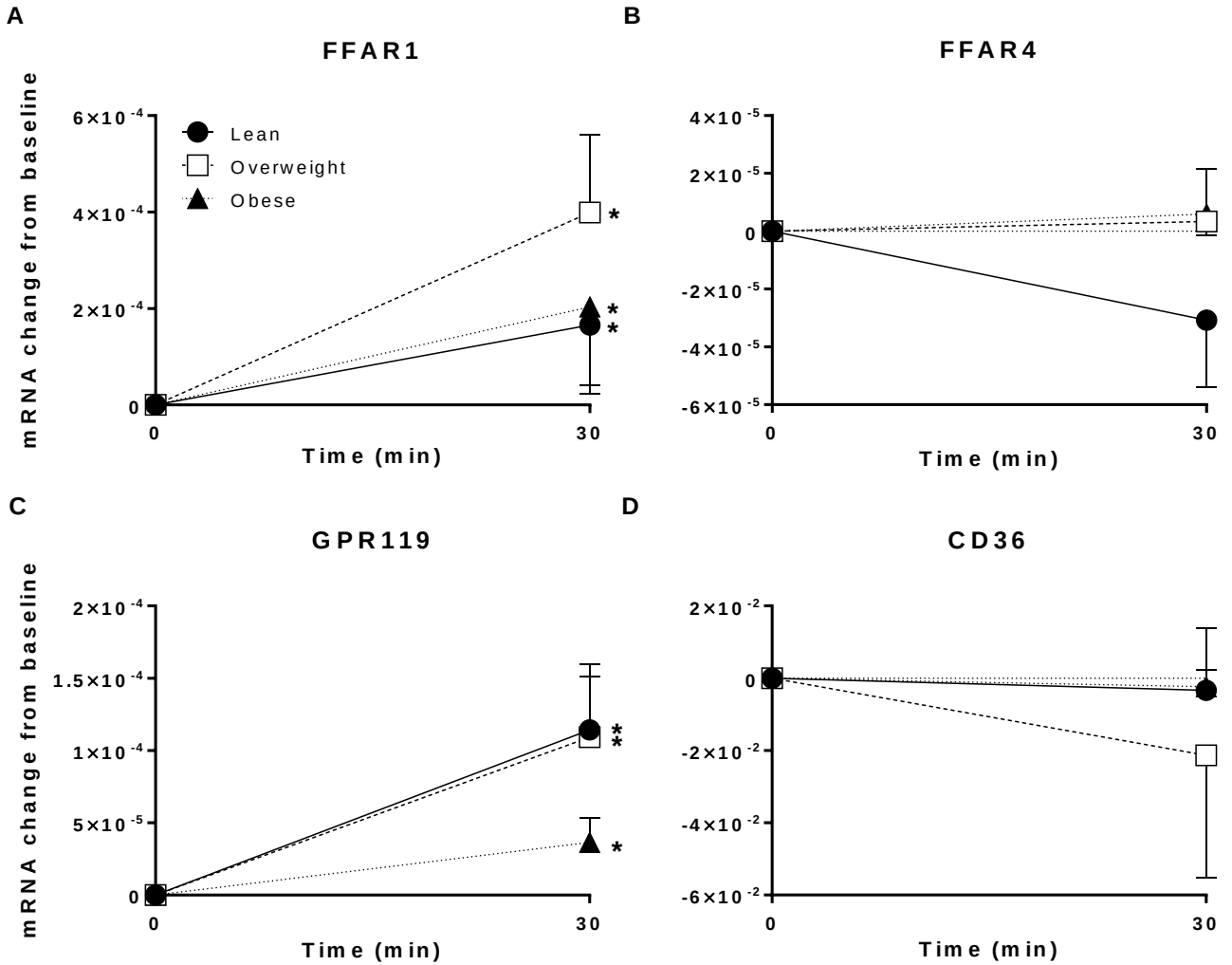

Figure 4.2: Intralipid®-induced changes in duodenal expression of FFAR1, FFAR4, GPR119 and CD36. Expression of **A**) FFAR1 and **C**) GPR119 was increased in response to lipid infusion in all groups (*P ≤ 0.05), whereas **D**) CD36 and **B**) FFAR4 expression was unchanged. Data are mean ± SEM. Lean (n = 20), overweight (n = 18), obese (n = 18).

4.5.3 Blood glucose and plasma GI hormone concentrations

Baseline blood glucose and plasma GLP-1, insulin and leptin concentrations were higher in the obese compared with lean and overweight groups (**Table 4.3**), without any difference between the latter groups. Baseline CCK, PYY and GIP concentrations did not differ between the groups. Blood glucose and plasma leptin concentrations were unchanged after ID lipid, but plasma CCK, PYY, GLP-1, GIP and insulin concentrations increased in all groups (iAUC $P \leq 0.05$, **Table 4.4, Figure 4.3**), with no difference between the groups (**Table 4.4**). Peak blood glucose, plasma insulin and leptin concentrations in response to ID lipid were higher in the obese compared with the lean (CMax, $P \leq 0.01$ for all) and the overweight ($P \leq 0.01$ for all) groups, with no difference between lean and overweight. Peak CCK, PYY, GLP-1, and GIP concentrations did not differ between the groups (**Table 4.4**).

Table 4.3: Fasted plasma gut hormone concentrations

Baseline	Lean	Overweight	Obese	P
Blood glucose (mmol/L)	5.4 ± 0.1	5.4 ± 0.1	6.0 ± 0.1 $^{\#\wedge}$	$^{\#} \leq 0.001$ $^{\wedge} \leq 0.001$
CCK (pmol/L)	3.0 ± 0.3	3.2 ± 0.4	2.6 ± 0.3	NS
GLP-1 (pg/mL)	33 ± 4	49 ± 6	72 ± 8 $^{\#\wedge}$	$^{\#} \leq 0.001$ $^{\wedge} \leq 0.05$
GIP (pg/mL)	23 ± 3	22 ± 3	25 ± 4	NS
PYY (pg/mL)	46 ± 8	62 ± 23	46 ± 6	NS
Insulin (pg/mL)	196 ± 21	223 ± 31	450 ± 74$^{\#\wedge}$	$^{\#} \leq 0.01$ $^{\wedge} \leq 0.01$
Leptin (pg/mL)	3076 ± 919	5464 ± 1233	17047 ± 3835$^{\#\wedge}$	$^{\#} \leq 0.001$ $^{\wedge} \leq 0.01$

CCK, cholecystokinin; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; PYY, peptide YY. $^{\#}$Obese vs lean; $^{\wedge}$Obese vs overweight. Data are mean ± SEM; lean, n = 19; overweight, n = 17; obese, n = 18.

Table 4.4: Plasma gut hormone and blood glucose concentrations in response to a 120 min ID infusion of 10% Intralipid® (2 kcal/min).

Post-infusion	Lean	Overweight	Obese	P
Blood glucose (mmol/L) *CMax (mmol/L)*	0.10 ± 0.03 5.8 ± 0.1	0.04 ± 0.02 5.6 ± 0.1	0.10 ± 0.05 6.4 ± 0.1$^{\#\wedge}$	NS $^{\#} \leq 0.01$, $^{\wedge} \leq 0.001$
CCK iAUC (pmol/L) *CMax (pmol/L)*	3.1 ± 0.4 8 ± 0.6	4.0 ± 0.5 9 ± 1	4.0 ± 0.3 8 ± 1	NS NS
GLP-1 iAUC (pg/mL) *CMax (pg/mL)*	75 ± 9 173 ± 21	89 ± 22 222 ± 49	75 ± 9 206 ± 19	NS NS
GIP iAUC (pg/mL) *CMax (pg/mL)*	148 ± 21 264 ± 34	150 ± 22 278 ± 35	163 ± 15 295 ± 22	NS NS
PYY iAUC (pg/mL) *CMax (pg/mL)*	28 ± 6 118 ± 12	29 ± 8 123 ± 18	49 ± 7 134 ± 10	NS NS
Insulin iAUC (pg/mL) *CMax (pg/mL)*	142 ± 45 395 ± 54	84 ± 20 425 ± 57	163 ± 35 868 ± 125$^{\#\wedge}$	NS $^{\#\wedge} \leq 0.001$
Leptin iAUC (pg/mL) *CMax (pg/mL)*	232 ± 117 3632 ± 1012	503 ± 195 6716 ± 1538	1432 ± 417$^{\#}$ 21001 ± 4893$^{\#\wedge}$	$^{\#} \leq 0.01$ $^{\#} \leq 0.001$, $^{\wedge} \leq 0.01$

CCK, cholecystokinin; CMax, concentration maximum; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; iAUC, incremental AUC; PYY, peptide YY. $^{\#}$Obese vs lean; $^{\wedge}$Obese vs overweight. Data are mean ± SEM; lean, n = 19; overweight, n = 17; obese, n = 18.

4.5.4 Appetite perceptions and *ad libitum* energy and macronutrient intake

Baseline appetite perception scores for hunger and fullness were similar in all study groups and did not change in response to ID lipid. The proportion of energy consumed as fat at the *ad libitum* buffet meal was higher in the obese compared with the overweight group (36 ± 2% vs. 29 ± 2%, $P \leq 0.01$), with no difference between the obese and lean, or the lean and overweight, groups. Lower carbohydrate consumption (% of energy) was also evident in the obese, compared with the overweight group ($P \leq 0.05$). Energy intake, amount consumed and protein intakes at the buffet meal did not differ between groups (**Table 4.2**).

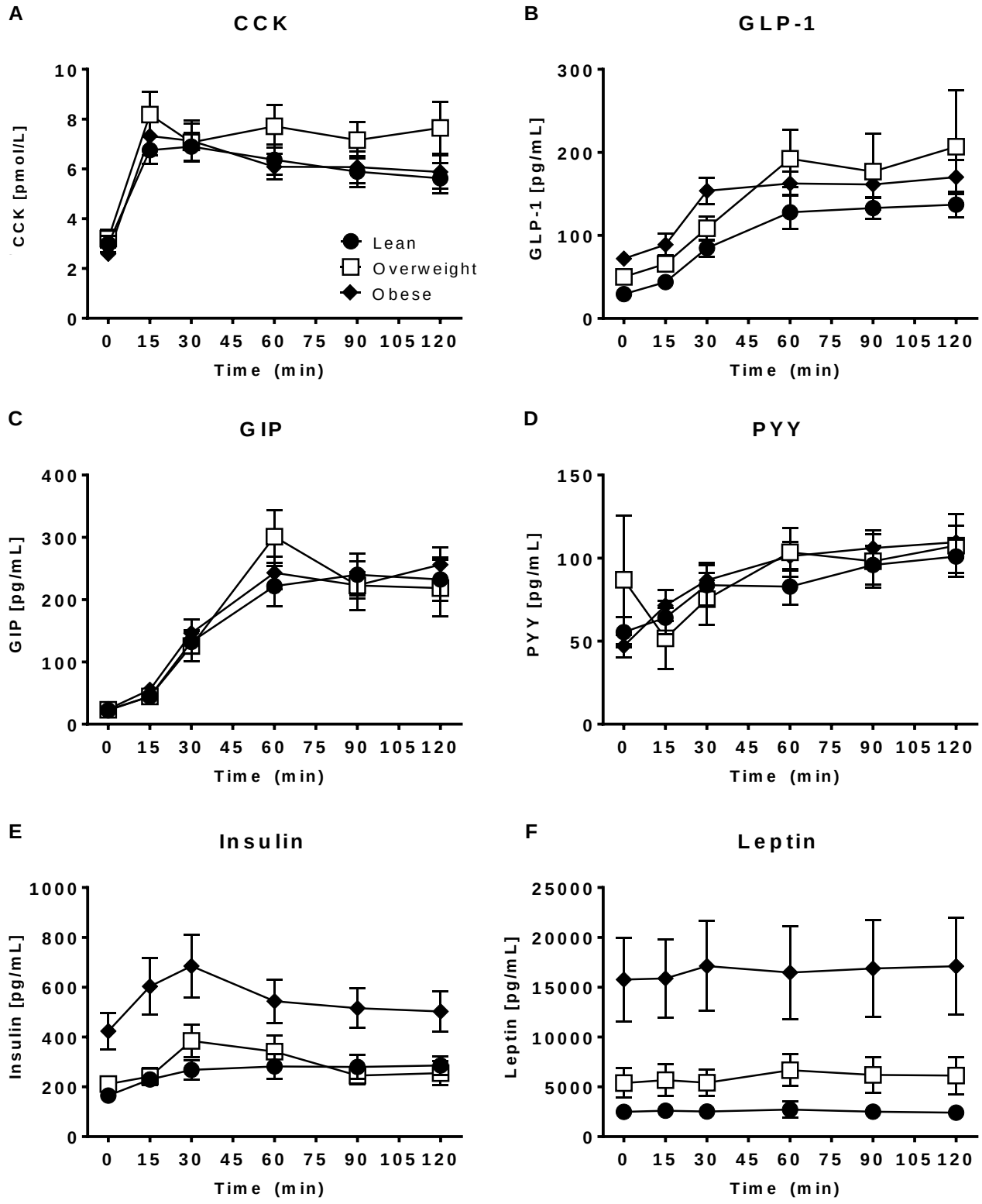

Figure 4.3: Intralipid®-induced changes in plasma hormones and gut peptides. Relative to baseline, concentrations of **A**) CCK, **B**) GLP-1, **C**) GIP, **D**) PYY and **E**) insulin were increased by lipid infusion over 120 min (group comparisons of baseline and iAUC values are presented in **Table 4.4** of the manuscript) with similar responses in all study groups. Plasma **F**) leptin concentrations were unchanged during lipid infusion. Data are mean ± SEM.

4.5.5 Relationships between BMI and duodenal FFA sensor expression

BMI was negatively related to baseline expression of both FFAR1 ($r = -0.4$, $P \leq 0.01$, **Figure 4.4A**) and FFAR4 ($r = -0.3$, $P \leq 0.01$, **Figure 4.4B**) but unrelated to baseline expression of GPR119 (**Figure 4.4C**). BMI was positively related to baseline expression of CD36 ($r = 0.5$, $P \leq 0.001$, **Figure 4.4D**). There was no relationship between BMI and the magnitude of the lipid-induced changes in duodenal FFA sensor expression.

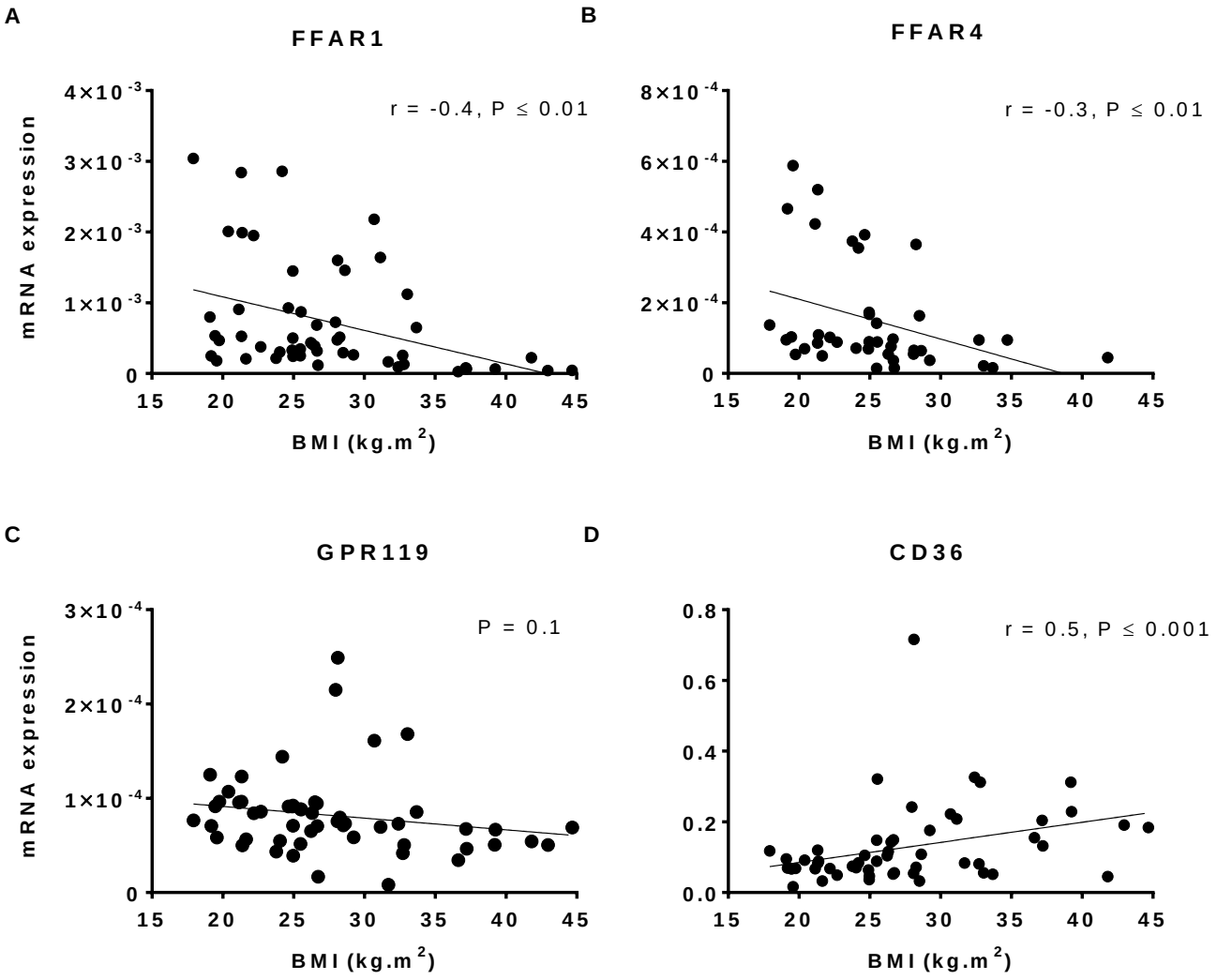

Figure 4.4: Relationships between BMI and duodenal expression of FFAR1, FFAR4, GPR119 and CD36. BMI was negatively associated with baseline expression of **A**) FFAR1 ($r = -0.4$, $P \leq 0.01$) and **B**) FFAR4 ($r = -0.3$, $P \leq 0.01$) and positively associated with baseline **D**) CD36 expression ($r = 0.5$, $P \leq 0.001$). **C**) GPR119 expression was unrelated to BMI.

4.5.6 Relationships between habitual dietary intake, and BMI and duodenal FFA sensor expression

No relationships were evident between habitual dietary energy or macronutrient intakes and BMI. There were also no relationships evident between habitual energy or macronutrient intakes and the expression of duodenal lipid sensors at baseline, or following ID lipid infusion.

4.5.7 Relationships of blood glucose and gut hormones, with BMI and duodenal FFA sensor expression

BMI was positively related to baseline concentrations of blood glucose ($r = 0.5$, $P \leq 0.01$), plasma GLP-1 ($r = 0.5$, $P \leq 0.01$), insulin ($r = 0.6$, $P \leq 0.001$) and leptin ($r = 0.7$, $P \leq 0.001$). In contrast, baseline plasma CCK, PYY and GIP concentrations were unrelated to BMI. Lipid-stimulated PYY concentrations were positively related to BMI (iAUC $r = 0.4$, $P \leq 0.05$, **Figure 4.5**), however, no relationships were evident between changes in CCK, GLP-1 or GIP and BMI. There was a positive relationship between lipid-induced changes in FFAR1 expression with plasma GIP across all study groups (iAUC, $r = 0.3$, $P \leq 0.05$, **Figure 4.6**). This correlation was largely due to the strength of the relationship in the overweight group ($r = 0.7$, $P \leq 0.05$), and was not apparent in analyses limited to the lean or obese groups. There were no other relationships between expression of duodenal FFA sensors, with baseline or lipid-induced hormone concentrations.

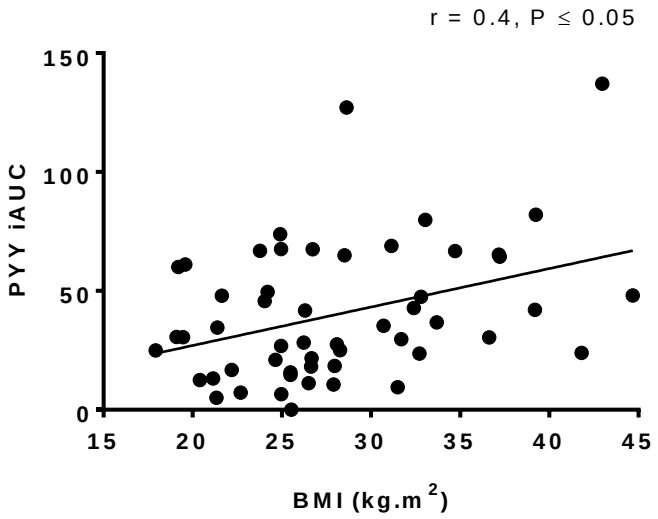

Figure 4.5: Relationship between Intralipid®-induced changes in PYY and BMI. As a cohort, the change in PYY secretion from baseline in response to ID lipid infusion was positively correlated with BMI ($r = 0.4$, $P \leq 0.05$).

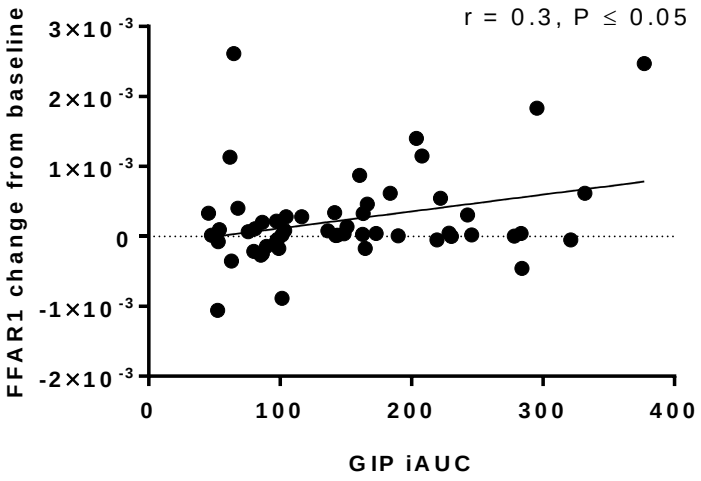

Figure 4.6: Relationship between Intralipid®-induced changes in duodenal FFAR1 expression and GIP secretion. As a cohort, the change in FFAR1 expression in response to lipid infusion was positively associated with plasma GIP concentration (iAUC $r = 0.3$, $P \leq 0.05$). This correlation emerged particularly from the overweight group ($r = 0.7$, $P \leq 0.05$), as both lean and overweight groups alone did not reach a significant correlation ($P = 0.1$ and 0.4 respectively).

4.5.8 Relationship of buffet meal energy and macronutrient intakes with BMI and duodenal FFA sensor expression

The proportion of energy consumed as fat at the buffet meal was positively related to BMI ($r = 0.4$, $P \leq 0.01$), however, total energy intake, total amount of food eaten, and protein or carbohydrate consumed at the buffet meal were unrelated to BMI. The expression of FFA sensors at baseline, or following ID lipid, was unrelated to total energy intake, total amount of food eaten, or protein, fat or carbohydrate consumed at the buffet meal.

4.6 Discussion

This study examined the effects of acute ID lipid on duodenal expression of the FFA sensors FFAR1, FFAR4, GPR119 and CD36 in lean, overweight and obese humans. Compared to lean participants, obese participants had increased CD36 expression, and decreased FFAR1 and FFAR4 expression at baseline. FFAR1 expression was also decreased in overweight compared to lean participants. GPR119 and FFAR1 were positive and early transcriptional responders to ID lipid infusion, and their expression was increased to a similar extent in all study groups. In contrast, expression of CD36 and FFAR4 was unchanged by ID lipid. Lipid-induced gut hormone secretion was comparable across study groups. Interestingly, participants with the largest lipid-stimulated increase in FFAR1 expression had higher plasma GIP levels, supporting a role for FFAR1 in GIP secretion in humans. Finally, the proportion of energy consumed as fat at the buffet meal was higher in the obese group. Further research is required to establish whether dysregulation of duodenal FFA sensors is intrinsic to human obesity or results from chronic overconsumption of fat.

The reduced FFAR1 and FFAR4 expression seen in obese participants may explain, in part, the lower intestinal fat sensitivity reported in human obesity[127]. It is known that knockdown of FFAR4 in cell lines and in rodents attenuates LCFA-induced GLP-1 and CCK secretion[27,28]. Furthermore, FFAR4 KO mice fed a HFD also develop obesity, glucose intolerance, and insulin resistance[27,28]. In humans, a FFAR4 loss-of-function polymorphism (R270H) increases the risk of developing obesity and insulin resistance in European populations, and when transfected into the human endocrine cell line, NCI-H716, attenuates GLP-1 secretion[35]. We previously demonstrated a positive association between FFAR4 expression and BMI[153], however, these outcomes, which appear contradictory to the outcomes in the current study, were probably due to notable differences in study design, including the use of a cross-section of patients attending the endoscopy unit with various, systemic co-morbidities (non-GI), including morbid obesity (BMI > 60) and differences in PCR methods. Nevertheless, these difference in FFAR4 data highlight the potential for plasticity of this FFA sensor in disease states.

FFAR1 stimulates insulin secretion via direct actions on pancreatic β-cells, and indirectly by augmenting incretin hormone release from the intestine[38,39]. We showed that obese individuals had the lowest FFAR1 expression at baseline, while lipid-induced increases in FFAR1 were associated with higher plasma GIP levels across the cohort, and were strongest in the overweight group, possibly a compensation to preserve FFAR1-GIP signalling at similar levels to those in lean participants. Low fasting FFAR1 may be an adaptation in obese individuals to the long-term negative effects of dietary FFAs on insulin secretion, to re-establish homeostasis under conditions of excess caloric intake[182,183]. Importantly, the protective effects of FFAR1 deletion against metabolic dysfunction in HFD-fed mice remains controversial[41,45], and consequences of chronic alterations in FFAR1 in humans require further investigation.

GPR119 was expressed independent of BMI in the current study. We showed earlier that baseline GPR119 expression was negatively related to habitual PUFA consumption in lean participants[179]. The present findings included one individual whose data weakened the otherwise strong trend to the same correlation ($r = -0.3$, $P = 0.06$). While further investigations are needed to link chronic HFD consumption with FFA-sensor expression, this finding indicates that the type of fat, rather than fat consumption *per se*, may influence duodenal GPR119 expression. Relationships between GPR119 and hormone secretion have described, for example, RNA interference of GPR119 resulting in modestly reduced expression (23%) in murine endocrine cell lines (GLUTag) attenuated GLP-1 secretion[46], while antagonising GLP-1 in mice attenuated the glucoregulatory effects of the GPR119 agonist, AR23146[50]. In humans, infusion of GPR119-specific ligands, including 2-oleoylglycerol, enhanced the secretion of GLP-1 and GIP[151].

While baseline CD36 expression was higher in the obese, several lines of evidence suggest that LCFA transport is not the primary mode of action of CD36, suggesting that alterations in CD36 may have broader implications for lipid metabolism. For example, CD36 protein expression on enterocytes is reduced as early as 1 hour following FFA exposure, as CD36 is rapidly ubiquinated[184]. Moreover, while deletion of CD36 from enterocytes in the proximal intestine of mice attenuates LCFA uptake[152], LCFA absorption is unaffected in CD36-deficient mice[184]. CD36, however, is vital in the formation of chylomicrons, which are critical for LCFA-induced CCK secretion, gastric emptying and food intake, and plasma lipid transport[184,185]. In mice with diet-induced metabolic syndrome, dysregulated sensing of lipid by CD36 results in altered chylomicron formation, and postprandial hypertriglyceridaemia[186]. Indeed, postprandial hyperlipidaemia in humans with CD36 deficiency has been primarily linked to an impairment of triglyceride metabolism[135].

Therefore increased CD36 expression may reflect post-absorptive defects in lipid metabolism, rather than enhanced LCFA uptake.

Limitations

Our study design of acute ID lipid infusion was based on our work investigating the effects of glycaemic state and glucose exposure on STRs in the human duodenum[1,61], which revealed transcriptional regulation of STRs within 30 min of ID glucose, with functional links to glucose absorption[1,61]. The current study, however, cannot exclude the possibility that larger changes in expression of FFA sensors may occur over a longer duration of postprandial exposure to dietary lipid and other nutrients, or in response to a higher nutrient load. While we assessed transcriptional changes in the current study, confirmation of these changes at the protein level will add further support. However, this analysis will require verified, commercial antibodies for human use, which are not currently available. Reported dietary intakes did not differ between groups, although there are known limitations with questionnaires such as FFQs, particularly the underreporting of fat and energy intakes in the obese[187].

Conclusions

This study demonstrated differences in the transcriptional regulation of FFA sensors, FFAR1, FFAR4, GPR119 and CD36 in the human duodenum in obesity. Further investigations into the *in vivo* consequences of altered expression of these targets in obesity will be necessary to provide causal links between luminal sensing and subsequent satiety signalling.

Chapter 5: Altered Expression of Sweet Taste Receptors and Glucose Transporters in Poorly Controlled Type 2 Diabetes

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Manuscript in preparation

STATEMENT OF AUTHORSHIP

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Name of Principal Author (Candidate)	Nada Cvijanovic		
Contribution to the Paper	Performed experiments, analysed data and interpreted results of experiments, prepared figures and drafted manuscript		
Overall percentage (%)	75		
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	27/03/2017

Co-Author Contributions

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

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

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5.1 Abstract

Background and aims: Enteroendocrine cells in the small intestine express STRs (a heterodimer of T1R2 + T1R3) which are tuned to detect all sweet stimuli, and can, in turn, regulate postprandial glycaemia by enhancing insulin secretion (i.e., the "incretin" effect) and increase glucose absorption by augmenting expression and function of the glucose transporter SGLT-1. In diet-controlled T2D patients with 'well' controlled glycaemia (WC-T2D), the intestinal STR system at euglycaemia responds similarly to healthy individuals, yet at hyperglycaemia, there is an impairment in transcriptional regulation of T1R2 and exaggerated glucose absorption. It is unknown, however, whether the prevailing hyperglycaemia in 'poorly' controlled T2D (PC-T2D), has more profound effects on the intestinal STR system with exaggerated consequences for postprandial glycaemic control.

Materials and methods: Twelve healthy individuals, 12 patients with WC-T2D (HbA1c 6.3 ± 0.2%), and 9 patients with PC-T2D (HbA1c 10.6 ± 0.5%) undertook an OGTT following an overnight fast, as previously described[1]. The participants were then studied during a euglycaemic clamp (5 ± 1 mmol/L), with duodenal biopsies collected at baseline (fasted) and after a 30-min ID glucose infusion (4 kcal/min). Copy numbers of T1R2, SGLT-1 and GLUT2 transcript were assessed at t = 0, 10 and 30 min by RT-PCR. Plasma concentrations of GIP, GLP-1, and C-peptide were measured at 10-min intervals from baseline (t = 0 min) for 60 min (t = 60 min). Plasma concentrations of 3-OMG were measured at t = 30 and 60 min, using mass spectrometry, to assess capacity for glucose absorption.

Results: PC-T2D patients had higher blood glucose concentrations during the OGTT, at all times, compared to WC-T2D and HC groups ($P \leq 0.001$), while WC-T2D patients had higher blood glucose beyond t = 30 min compared to the HC group ($P \leq 0.001$). Basal SGLT-1

transcripts were lower in PC-T2D patients than in WC-T2D (2-fold, $P \leq 0.01$), and GLUT2 transcripts 3-4 fold lower than in HC and WC-T2D groups (both $P \leq 0.01$). Transporter expression did not differ between HC and WC-T2D groups, while basal T1R2 expression was similar across all groups. Fasting concentrations of GLP-1 and C-peptide did not differ between groups, whereas GIP was higher in WC-T2D patients than in the HC group ($P \leq 0.01$). Glucose infusion increased SGLT-1 and decreased GLUT2 transcripts at 10 min (group × time interaction) in both HC and WC-T2D groups (both $P \leq 0.001$, $P \leq 0.05$ respectively), but not in PC-T2D patients. When corrected for baseline, T1R2 transcripts were lower in PC-T2D patients after 10 min compared to WC-T2D ($P \leq 0.05$). All transcript levels were similar to basal levels at 30 min, and did not differ between groups. ID glucose increased plasma GIP, GLP-1 and C-peptide in all groups (all $P \leq 0.001$). GIP concentrations, however, were higher in both T2D groups than in HC; at 20, 40, 50 and 60 min in WC-T2D ($P \leq 0.05$), and t = 10 to 30 min in PC-T2D ($P \leq 0.01$). Accordingly, GIP iAUC was increased in PC-T2D patients. GLP-1 concentrations were similar between PC-T2D and HC groups, but were higher in the WC-T2D group than in PC-T2D and HC groups between 30-50 min ($P \leq 0.05$), with correspondingly higher iAUC (WC-T2D vs. PC-T2D, $P \leq 0.05$). C-peptide concentrations were lower in both T2D groups at 30 min compared to the HC group ($P \leq 0.01$) and in PC-T2D at 60 min compared to both HC ($P \leq 0.01$) and WC-T2D groups ($P \leq 0.05$). C-peptide iAUC was higher in HC then either T2D group ($P \leq 0.05$).

Conclusions: Glucose absorption is increased in patients with PC-T2D at euglycaemia, as and is likely to arise due to a loss of transcriptional regulation of SGLT-1 and GLUT2, and augmented cellular accumulation of SGLT-1 and GLUT2 protein. Increased glucose absorption in PC-T2D patients, together with preserved GIP responses, but lower GLP-1 and C-peptide responses, could exacerbate hyperglycaemia, and may be linked to impaired sensing of luminal glucose by duodenal T1R2.

5.2 Introduction

The presence of carbohydrates within the small intestine generates neural and hormonal signals that play a key role in the regulation of postprandial glycaemia. In particular, the secretion of the incretin hormones GIP and GLP-1 from intestinal enteroendocrine cells accounts for ~70% of the insulin released in response to enteral glucose in healthy individuals, compared to insulin responses to an isoglycaemic IV glucose stimulus[188]. In patients with T2D, the incretin effect is impaired[189], due to a markedly diminished insulinotropic effect of GIP[190] and, in some cases, reduced GLP-1 secretion[107]. Importantly, there is also evidence of an enhanced capacity to absorb glucose in both animal models of T2D and in patients with T2D[61,64,191-193]. However, while the incretin hormones are a critical determinant of postprandial glycaemia, the contribution of glucose sensing and absorption within the gut and their subsequent effects on glycaemic control in patients with T2D, is poorly understood.

STRs, a heterodimer of the G-protein coupled receptors T1R2 and T1R3, are localised to the BBM of a subset of intestinal enteroendocrine cells[57,61]. Intestinal STRs detect the presence of luminal sweet stimuli and initiate a cascade of intracellular signaling events, resulting in cell depolarisation and the basolateral secretion of the incretin hormones, as well as the intestinotrophic peptide hormone, GLP-2[60,72,194]. Mice lacking either T1R3 or the taste-specific G-protein, α-gustducin, have attenuated glucose-stimulated GLP-1 secretion[59]. Although less established in humans, intestinal STRs have been shown to participate in glucose-stimulated GLP-1 secretion in humans as this secretion is dose-dependently attenuated by blockade of intestinal STRs by the carboxylic acid lactisole[30,195]. STRs have also been linked to regulation of the primary intestinal glucose transporter, SGLT-1, localised to the BBM of intestinal enterocytes. The secretion of GLP-2 is a likely mechanism, as it is co-secreted with GLP-1 in an STR-dependent manner, and positively regulates the

expression of SGLT-1 in rodents, and in patients with short-bowel syndrome[103,120,194,196]. Increased SGLT-1 expression increases the capacity for glucose absorption at the BBM[59,61,91,93,194], which is matched by an increase in facilitated glucose transport to the portal circulation via the basolaterally located, facilitative GLUT2 transporter[197]. As such, STRs have the capacity to direct glycaemia through actions on incretin hormone release, or by regulating SGLT-1 availability for glucose absorption, via GLP-2.

We recently demonstrated that duodenal expression of T1R2 was similar in healthy individuals and patients with diet-controlled T2D at baseline euglycaemia, and that in both groups, T1R2 expression was rapidly upregulated in response to luminal glucose infusion. In contrast, at hyperglycaemia, while T1R2 expression was downregulated by enteral glucose in healthy individuals, it remained elevated in patients with T2D. This was linked to augmented glucose absorption, as assessed by increased absorption of the non-metabolisable glucose analogue, 3-OMG, which serves as a marker of glucose absorption and is transported by SGLT-1[1]. Importantly, patients with PC-T2D commonly have elevated glycated haemoglobin (HbA1c) and hyperglycaemia, and remain at higher risk of diabetic complications, despite the provision of standardised treatments[198-200]. While HbA1C is strongly influenced by postprandial glycaemia in T2D patients[201], the contribution of intestinal glucose absorption is underappreciated and poorly understood. It is therefore of interest to investigate whether PC-T2D patients demonstrate more profound defects in the intestinal STR system, and whether this equates to increased risk of postprandial hyperglycaemia.

It is critical that we develop a better understanding of the influence of longstanding hyperglycaemia on the transcriptional regulation of the STR system, and of SGLT-1 and GLUT2, since this has the potential to identify new targets for therapy in patients with

PC-T2D. We, therefore, characterised the duodenal expression of T1R2, SGLT-1 and GLUT2 at euglycaemia in healthy individuals, and in patients with both WC-T2D and PC-T2D. Changes in plasma incretins, C-peptide (a marker of insulin production), and glucose absorption were also assessed, together with transcriptional changes. We hypothesised that in response to enteral glucose, patients with PC-T2D would exhibit further defects in the regulation of intestinal STRs, SGLT-1 and GLUT2, impaired incretin responses, exaggerated glucose absorption, and increased blood glucose, when compared with patients with WC-T2D and HC individuals.

5.3 Materials and methods

5.3.1 Participants

Twelve healthy individuals, 12 patients with WC-T2D (HbA1c 6.3 ± 0.2%), and 9 patients with PC-T2D (HbA1c 10.6 ± 0.5%) were recruited through existing departmental databases, newspaper advertisement, and flyers displayed at local universities and hospitals, and were screened for significant co-morbidities. World Health Organisation criteria were used to define those with WC-T2D (HbA1c ≤ 7%) from PC-T2D (HbA1C ≥ 9%, and ≤12%). Participant demographics are presented in **Table 5.1**. All had normal haemoglobin (> 135 g/L) and ferritin (> 10 mcg/L), no history of GI disease, and had normal renal and liver function. All WC-T2D patients and four PC-T2D patients were managed by diet alone. The remaining five PC-T2D patients were metformin treated, which was withheld for 48 hr prior to the study day due to known effects of metformin on GLP-1 release[202]. The study protocol was approved by the Royal Adelaide Hospital Research Ethics Committee and carried out in accordance with the Declaration of Helsinki. After receiving verbal and written study information, all participants provided written, informed consent.

Table 5.1: Demographic, anthropometric and metabolic characteristics of study participants

	HC	WC-T2D	PC-T2D	P
n	12	12	9	
Sex	10M : 2F	4M : 8F	9M	$P \leq 0.01$
Age (years)	31 ± 3	65 ± 2	59 ± 3	$^{*\#}P \leq 0.05$
BMI ($kg.m^2$)	25 ± 2	28 ± 1	31 ± 1	$^{\#}P \leq 0.05$
HbA1c (%)		6.3 ± 0.2	10.6 ± 0.5	$^{\wedge}P \leq 0.001$
Fasting blood glucose (mmol/L)	5.9 ± 0.1	7.5 ± 0.4	12.7 ± 1.2	$^{\#\wedge}P \leq 0.05$
Duration of T2D (years)		5 ± 1	7 ± 3	

HC, healthy controls; WC-T2D, well-controlled type 2 diabetes; PC-T2D, poorly-controlled type 2 diabetes. Data are mean ± SEM. *WC-T2D vs HC, $^{\#}$PC-T2D vs HC, $^{\wedge}$WC-T2D vs PC-T2D.

5.3.2 Oral glucose tolerance test

All participants underwent an OGTT at a screening visit, as previously described[1]. Briefly, each participant attended the laboratory at 0830 hr following an overnight fast. An IV cannula was inserted for blood collection and participants consumed a glucose drink consisting of 75 g glucose dissolved in 300mL water, within 5 min. Blood was collected at t = 0, 30, 60, 120 and 180 min following the glucose drink, with blood glucose concentrations measured by portable glucometer (Medisense Precision QID, Abbott Laboratories, Bedford, MA, USA).

5.3.3 Endoscopy protocol

Participants attended the Gastrointestinal Investigation Unit at the Royal Adelaide Hospital, at 0830 hr following a standardised evening meal (400g beef lasagne, McCain Foods, Australia) and overnight fast from 1900 hr. On arrival, an IV cannula was positioned into an

antecubital vein of each arm. Blood glucose was clamped at euglycaemia (5 mmol/L), first by administering a 50 mL IV bolus of 0.9% saline (Baxter Healthcare) for 1 min, then a continuous infusion at a rate of 150ml/hr, then 100 IU of insulin IV (Actrapid; Novo Nordisk, Baulkham Hills, NSW, Australia) in 500 mL of 4% succinylated gelatin solution (Gelofusine; B, Braun Australia, Bella Vista, NSW, Australia) administered at a variable rate to maintain euglycaemia. A 25% dextrose solution was administered if blood glucose fell below 5 mmol/L.

Once blood glucose concentrations were at stable euglycaemia for 30 min, a small-diameter video endoscope (GIF-XP160, Olympus, Tokyo, Japan) was inserted into an anaesthetised nostril to the second part of the duodenum, and 2 duodenal biopsies collected using standard biopsy forceps, and placed in RNAlater (Qiagen, Sydney, Australia). After baseline collection (t = 0 min), an ID glucose infusion was commenced via the biopsy channel of the endoscope (30g glucose with 3g 3-OMG, Sigma-Aldrich, St. Louis, MO; in water to a total volume of 150 mL, 4 kcal/min (total: 120 kcal)), and maintained for 30 min. At t = 10 min, the infusion was paused for 1 min and 2 additional biopsies taken. At t = 30 minutes, 2 final biopsies were taken and the endoscope removed. Blood samples (20 mL) were collected every 10 min for 1 hour (t = 0 - 60 min), with IV insulin and glucose infusions terminated at t = 60 min. Plasma or serum were separated from whole blood by centrifugation (15 min at 4°C), within 15 min of collection, and stored at -80˚C for later analysis. Participants were then given a meal and blood glucose was checked to exclude hypoglycaemia (≤ 4 mmol/L), prior to them leaving the hospital.

5.4 Measurements

5.4.1 Quantification of T1R2, SGLT-1 and GLUT2 expression by RT-PCR

Total RNA was extracted from a biopsy at each time point using the PureLink™ MicroKit (Invitrogen, Thermo Fisher Scientific, Australia) as per the manufacturer instructions. The other biopsy was placed into archival storage. RNA quantity was determined using a Nanodrop™ Lite Spectrophotometer (Thermo Fisher Scientific, Australia) and purity assessed using A_{260}/A_{280} ratio. Absolute standard curves were generated by including known copy number standards for each target in each RT-PCR assay (**Table 5.2**), as described previously[61]. RT-PCR was performed using a QuantiTect® SYBR Green® one-step RT-PCR kit (Qiagen) and 7500 fast Real-Time PCR system (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's specifications. Each assay was performed in triplicate and included no-template, and no reverse-transcription controls. Validated human primers for T1R2, SGLT-1 and GLUT2 were used (**Table 5.3**) (QuantiTect®, Qiagen). All replicates were averaged for final mRNA copy number, which was expressed as copies per 25 ng of total RNA.

Table 5.2: Human primers used to generate RT-PCR products containing the target amplicon to create absolute standard curves

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Amplicon (bp)
T1R2	TACCTGCCTGGGGATTAC	AAATAGGGAGAGGAAGTTGG	390
SGLT-1	TGGAATGCCCTGGTTTTGGT	GGAAGATGTGGAAGGAGTCGG	493
GLUT2	ACCCTGGTTTTCACTGTCATCA	AATTAGCCCACAATATAGTCCTGA	480

T1R2, taste receptor type 1, member 2; SGLT-1, sodium-glucose co-transporter-1; GLUT2, glucose transporter-2; bp, base pairs.

Table 5.3: Human primers used for absolute quantification of SGLT-1, T1R2 and GLUT2 by RT-PCR

Gene	Primer information	Amplicon (bp)
T1R2	QT01026508	94
SGLT-1	QT00001246	81
GLUT2	QT01008399	88

QT = QuantiTect® primer assay (Qiagen)

5.4.2 Plasma GLP-1, GIP, C-Peptide and serum 3-OMG assays

Total plasma GLP-1 was measured by RIA (GLPIT-36HK, Millipore, Billerica, MA). The minimum detectable limit was 3 pmol/L, and intra-, and inter-, assay CVs were 5.3% and 8.1%, respectively. Plasma GIP was measured by radioimmunoassay using a modified version of a previously published method[203]. The standard curve was prepared in buffer and the radio-iodinated label was supplied by Perkin Elmer (Boston, MA). The minimum detectable limit was 2 pmol/L, and intra- and inter-assay CVs were 9.6% and 8.6%, respectively. C-peptide was 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 5.2% and 5.7%, respectively. Serum 3-OMG concentrations were measured by commercial liquid chromatography mass spectrometry with assay sensitivity of 10 pmol/L[1, 204].

5.4.3 Data and statistical analyses

Statistical analysis was performed using SPSS® software (SPSS Inc, IBM®, version 20). A one-way ANOVA, with group as a factor, was used to determine differences in age, BMI, and fasting blood glucose at the screening visit. Sex distribution across groups was assessed using Fishers Exact test (Chi-squared). Differences in HbA1C (%) between WC-T2D and

PC-T2D groups was assessed using an independent samples t-test. Basal transcripts, and transcript changes in response to glucose of T1R2, SGLT-1 and GLUT2 in response to ID glucose were assessed by two-way repeated measures ANOVA, with group, and time, as factors. Due to inter-subject variability in transcript levels, the responses to ID glucose were also assessed as change from baseline (t = 30 – 0 min), with group as the between-subjects factor. The iAUC for blood glucose, GIP, GLP-1 and C-Peptide was calculated using the trapezoidal rule from t = 0 - 180 min (blood glucose) and t = 0 - 60 min (gut hormones), and analysed by one-way ANOVA. A two-way, repeated measures ANOVA was also performed on these variables with time and group as factors. The maximum concentration (Cmax) of gut hormones were calculated from t = 0 - 120 min. Post-hoc pairwise comparisons, corrected for multiple comparisons using the Sidak method, was performed for all ANOVA's that were significant. Relationships between transcript expression and blood glucose, GIP, GLP-1 and C-Peptide (iAUCs) were evaluated by Pearson's correlation coefficient (r). GIP assays were incomplete at the time of thesis preparation (PC-T2D, n = 3). There was low signal detection for GIP in n = 2 HC, and n = 2 WC-T2D participants, which were excluded from analysis. GLP-1 was below signal detection for n = 1 HC participant, which was similarly excluded. Statistical significance was accepted at $P \leq 0.05$. Data are expressed as mean ± SEM.

5.5 Results

The procedures were well tolerated and biopsies were collected from all participants.

5.5.1 Blood glucose concentrations during the OGTT

Blood glucose increased in response to the glucose drink in all groups ($P \leq 0.001$), and was highest in PC-T2D patients compared with HC participants and WC-T2D patients at all time points ($P \leq 0.001$). Blood glucose in the WC-T2D group was also higher than in the HC group beyond t = 30 min ($P \leq 0.001$, **Figure 5.1**). Blood glucose iAUC was higher in PC-T2D patients than in both HC and WC-T2D groups ($P \leq 0.001$), with the HC group also lower in comparison to the WC-T2D group ($P \leq 0.05$).

5.5.2 Baseline T1R2, SGLT-1 and GLUT2 expression in the proximal human duodenum

T1R2, SGLT-1 and GLUT2 transcripts were detected in all duodenal biopsies at baseline, with abundance SGLT>>GLUT2>T1R2 in all groups (**Figure 5.2**). T1R2 transcript levels at baseline did not differ between groups. Baseline SGLT-1 transcript levels were 1.8-fold lower in PC-T2D, compared to WC-T2D ($P \leq 0.01$). Baseline GLUT2 transcript levels were 3.8-, and 3.4-fold lower in PC-T2D compared to HC ($P \leq 0.01$) and WC-T2D ($P \leq 0.01$), respectively. Baseline transcript levels of T1R2, SGLT-1 and GLUT2 did not differ between HC and WC-T2D groups.

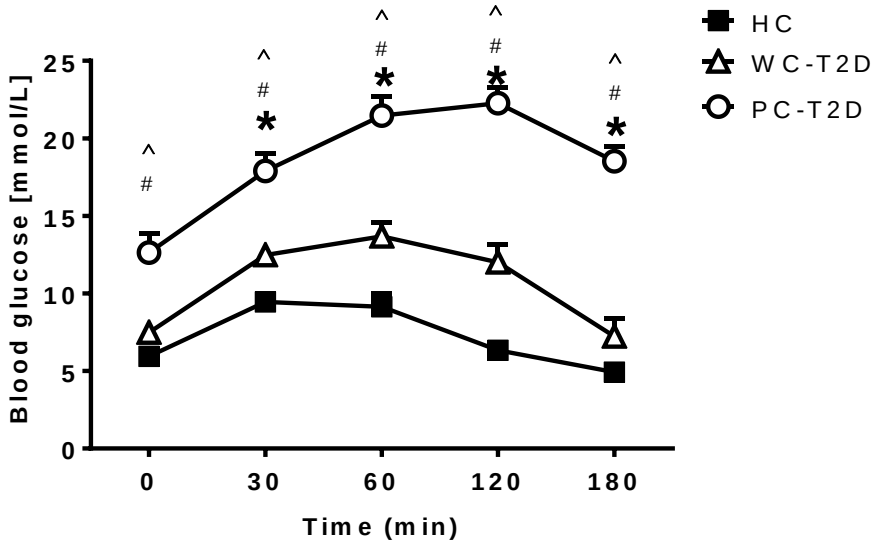

Figure 5.1: Blood glucose concentrations during the OGTT. Consumption of glucose drink increased blood glucose concentrations in all groups ($P \leq 0.001$), with higher concentrations in PC-T2D patients at all time points compared with both HC and WC-T2D groups ($P \leq 0.001$), and in WC-T2D compared with HC from t = 30 min onwards ($P \leq 0.001$). Data are mean ± SEM. *WC-T2D vs HC, #PC-T2D vs HC, ^WC-T2D vs PC-T2D. HC, healthy controls; PC-T2D, poorly-controlled type 2 diabetes; WC-T2D, well-controlled type 2 diabetes. HC, n = 12; WC-T2D, n = 12; PC-T2D, n = 9.

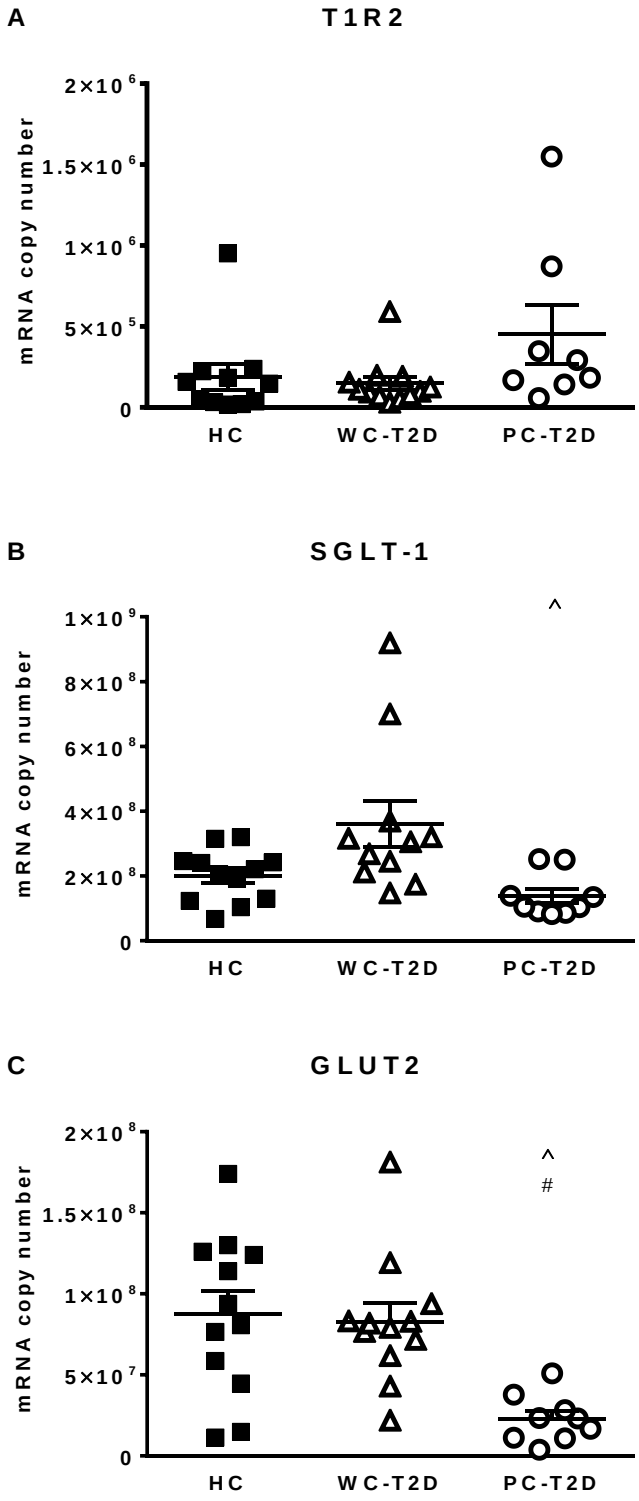

Figure 5.2: Duodenal transcript levels of T1R2, SGLT-1 and GLUT2 in HC, WC-T2D and PC-T2D groups at baseline. A) T1R2 transcripts did not differ between groups at baseline. **B**) SGLT-1 transcripts were 1.8-fold lower in PC-T2D compared to the WC-T2D group ($P \leq 0.01$). **C**) GLUT2 transcripts were 3.8 and 3.4-fold lower in PC-T2D compared to HC ($P \leq 0.01$) and WC-T2D ($P \leq 0.01$) groups, respectively. Data are mean ± SEM. HC, healthy controls; PC-T2D, poorly controlled type 2 diabetes; WC-T2D, well controlled type 2 diabetes. #PC-T2D vs HC; ^WC-T2D vs PC-T2D. HC, n = 11; WC-T2D, n = 12; PC-T2D, n = 9 (T1R2, n = 8).

5.5.3 Effects of ID glucose on expression of T1R2, SGLT-1 and GLUT2

T1R2 transcript levels did not change in response to ID glucose in subject groups, but when assessed as change from baseline, were lower in the PC-T2D group at 10 min compared to other groups (group × time interaction, $P \leq 0.05$). T1R2 levels were unchanged from baseline at t = 30 min, and were not different between groups (**Figure 5.3A**).

SGLT-1 transcript levels were higher in HC and WC-T2D subjects after 10 min of ID glucose than in PC-T2D subjects (group × time interactions, $P \leq 0.001$), whereas levels were similar across groups at 30 min. The increase in SGLT-1 transcripts from baseline was larger in the HC group at t = 10 min compared to the WC-T2D ($P \leq 0.05$) and PC-T2D groups ($P \leq 0.01$), with no difference between both T2D groups. SGLT-1 levels were unchanged across all study groups at 30 min (**Figure 5.3B**).

GLUT2 transcripts were similar and lower in HC ($P \leq 0.01$) and PC-T2D subjects ($P \leq 0.05$) compared to WC-T2D subjects after 10 min of ID glucose (group × time interactions). GLUT2 levels in HC subjects were similar to levels in WC-T2D subjects by 30 min, but remained lower in PC-T2D in comparison to these two groups (both $P \leq 0.05$). When assessed as baseline changes, GLUT2 transcripts were significantly lower in both the HC ($P \leq 0.001$) and WC-T2D groups ($P \leq 0.05$) compared to the PC-T2D group at 10 min, however GLUT2 levels were unchanged across all study groups at 30 min (**Figure 5.3C**).

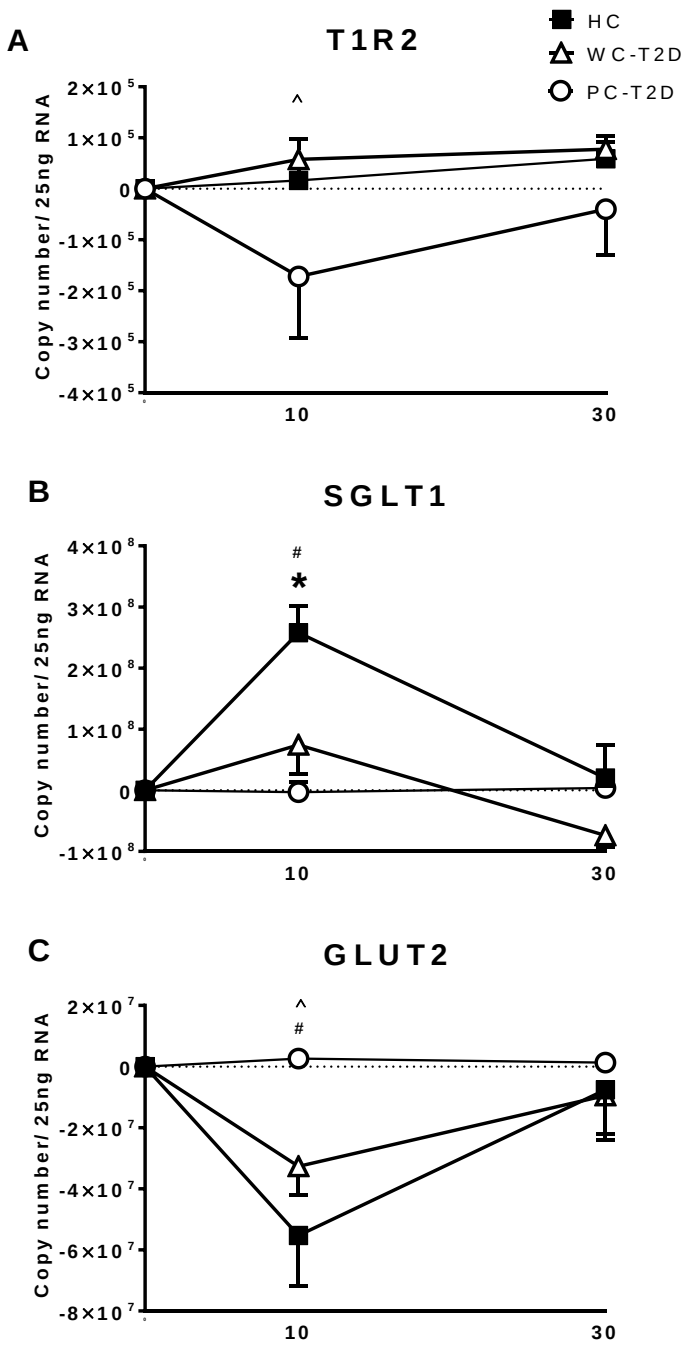

Figure 5.3: Glucose-induced changes in duodenal T1R2, SGLT-1 and GLUT2 transcript levels in HC, WC-T2D and PC-T2D groups. A) The decrease in T1R2 transcript copy number from baseline was larger in PC-T2D than in HC and WC-T2D at 10 min (both $P \leq 0.05$). **B**) At the same time SGLT-1 transcript numbers increased from baseline in the HC group compared with WC-T2D ($P \leq 0.05$) and PC-T2D ($P \leq 0.01$) groups, with no difference between the two T2D groups. **C**) GLUT2 transcripts decreased further in the HC ($P \leq 0.001$) and WC-T2D groups ($P \leq 0.05$) at 10 min than in the PC-T2D group. Transcripts of all targets were unchanged from baseline at 30 min. Data are mean ± SEM. HC, healthy controls; PC-T2D, poorly-controlled type 2 diabetes; WC-T2D, well-controlled type 2 diabetes. *WC-T2D vs HC; #PC-T2D vs HC; ^WC-T2D vs PC-T2D.

5.5.4 Plasma GIP, GLP-1 and C-peptide concentrations

GIP concentrations were higher in the WC-T2D group at baseline compared to both the HC ($P \leq 0.01$) and PC-T2D groups ($P \leq 0.05$, **Table 5.4**), but similar in HC and PC-T2D groups. GIP increased in response to ID glucose in all groups ($P \leq 0.001$, **Figure 5.4A**), and was higher in WC-T2D at t = 20 and between t = 40-60 min compared with the HC group (group × time interaction, $P \leq 0.05$). GIP concentrations were also higher in the PC-T2D group than in the HC group from t = 10 to 30 min ($P \leq 0.01$), and, accordingly, GIP iAUC was increased in PC-T2D ($P \leq 0.05$, **Table 5.5**). GIP concentrations did not differ between the WC-T2D and PC-T2D groups during the infusion (**Figure 5.4A**). Peak GIP concentration (Cmax) did not differ between the T2D groups, but both WC-T2D and PC-T2D had higher peak concentrations than the HC group ($P \leq 0.05$) (**Table 5.5**).

Plasma GLP-1 concentrations were not different at baseline (**Table 5.4**) but increased in response to ID glucose in all groups ($P \leq 0.001$, **Figure 5.4B**). GLP-1 concentrations were higher in the WC-T2D group between t = 30 to 60 min than in HC (group × time interaction, $P \leq 0.05$) and between t = 30 to 50 min than in the PC-T2D group ($P \leq 0.01$); GLP-1 concentrations were not different between PC-T2D and HC groups throughout the study. The iAUC for GLP-1 was higher in WC-T2D than in the PC-T2D group ($P \leq 0.05$; **Table 5.5**), and showed a trend to be higher than in HC ($P = 0.06$). Peak GLP-1 concentration was higher in WC-T2D compared to HC and PC-T2D groups (both $P \leq 0.05$), while peak levels in HC and PC-T2D groups did not differ (**Table 5.5**).

Plasma C-peptide concentrations did not differ between groups at baseline (**Table 5.4**), but increased in response to ID glucose in all groups ($P \leq 0.001$, **Figure 5.4C**). C-peptide was higher in the HC group than WC-T2D (group × time interaction, $P \leq 0.01$) or PC-T2D

($P \leq 0.001$) groups at 30 min, with no differences between the T2D groups. At t = 60 min, C-peptide concentrations were not different between HC and WC-T2D groups, and significantly higher than concentrations in the PC-T2D group ($P \leq 0.05$, $P \leq 0.01$ respectively, **Figure 5.4C)**. The iAUC of C-peptide was higher in the HC group than WC-T2D ($P \leq 0.05$) and PC-T2D groups ($P \leq 0.01$), but did not differ between T2D groups. Peak C-peptide concentrations were higher in HC than the PC-T2D group ($P \leq 0.01$), but were not different compared to WC-T2D, or between T2D groups (**Table 5.5**).

Table 5.4: Baseline plasma concentrations of GIP, GLP-1, and C-peptide

	HC	WC-T2D	PC-T2D	P
GIP[1] (pmol/L)	9 ± 2	16 ± 2	10 ± 1	*^P ≤ 0.01
GLP-1[2] (pmol/L)	21 ± 1	23 ± 2	22 ± 2	NS
C-peptide[3] (pmol/L)	313 ± 49	327 ± 37	294 ± 38	NS

HC, healthy controls; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; PC-T2D, poorly-controlled type 2 diabetes; WC-T2D, well-controlled type 2 diabetes. Data are mean ± SEM. *WC-T2D vs. HC; ^WC-T2D vs PC-T2D. [1]HC, n = 10; WC-T2D, n = 10; PC-T2D, n = 6, [2]HC, n =11; WC-T2D, n = 12; PC-T2D, n = 8; [3]HC, n = 12, WC-T2D, n = 12, PC-T2D, n = 8.

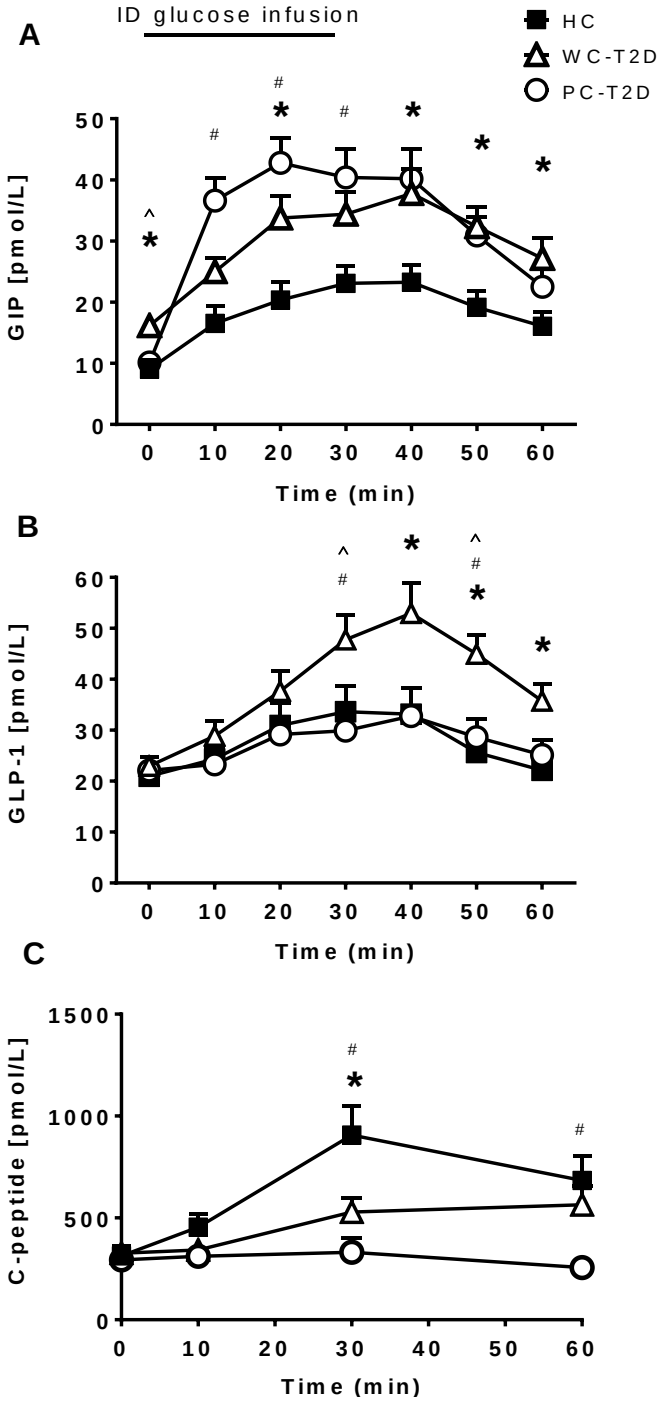

Figure 5.4: Effects of ID glucose on plasma GIP, GLP-1 and C-peptide concentrations in HC, WC-T2D and PC-T2D groups. A) GIP **B**) GLP-1 and **C**) C-peptide concentrations increased in response to ID glucose in all groups (group × time interaction: $P \leq 0.001$, for all). **A**) GIP was higher in WC-T2D ($P \leq 0.01$) and PC-T2D ($P \leq 0.05$) groups at baseline compared with the HC group. GIP concentrations remained elevated at t = 20, and between t = 40-60 min in WC-T2D compared with HC ($P \leq 0.05$), and between t = 10-30 min in PC-T2D compared with HC ($P \leq 0.01$). **B**) GLP-1 was higher in WC-T2D than the HC group between t = 30-60 ($P \leq 0.05$), and higher than the PC-T2D group between 30-50 min ($P \leq 0.01$). **C**) C-peptide concentrations were higher in HC than WC-T2D ($P \leq 0.01$) or PC-T2D groups at t = 30 min ($P \leq 0.001$), but similar in HC and WC-T2D groups at 60 min, when both concentrations were higher than in the PC-T2D group ($P \leq 0.05$, $P \leq 0.01$ respectively). Data are mean ± SEM. HC, healthy controls; PC-T2D, poorly-controlled type 2 diabetes; WC-T2D, well-controlled type 2 diabetes. *WC-T2D vs HC; #PC-T2D vs HC; ^WC-T2D vs PC-T2D.

Table 5.5: Effects of 30-min ID glucose infusion (4 kcal/min) on plasma GIP, GLP-1 and C-peptide concentrations.

	HC	WC-T2D	PC-T2D	P
GIP iAUC (pmol/L.min)[1]	765 ± 104	1008 ± 131	1464 ± 234	$^{\#}P \leq 0.05$
CMax (pmol/L)	26 ± 3	40 ± 4	43 ± 5	$^{*\#}P \leq 0.05$
GLP-1 iAUC (pmol/L.min)[2]	466 ± 183	1045 ± 174	369 ± 131	$^{\wedge}P \leq 0.01$
CMax (pmol/L)	36 ± 5	56 ± 6	35 ± 5	$^{*\wedge}P \leq 0.05$
C-peptide iAUC (pmol/L.min)[3]	22540 ± 4017	9621 ± 3439	2060 ± 978	$^{*}P \leq 0.01$, $^{\#}P \leq 0.001$
CMax (pmol/L)	932 ± 140	611 ± 86	361 ± 60	$^{\#}P \leq 0.01$

CMax, concentration maximum; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; HC, healthy controls; iAUC, incremental AUC; PC-T2D, poorly-controlled type 2 diabetes; WC-T2D, well-controlled type 2 diabetes. Data are mean ± SEM; *WC-T2D vs HC; #PC-T2D vs HC; ^WC-T2D vs PC-T2D. [1]HC, n = 10, WC-T2D, n = 10, PC-T2D, n = 6; [2]HC, n = 11, WC-T2D, n = 12, PC-T2D, n = 8; [3]HC, n = 12, WC-T2D, n = 12, PC-T2D, n = 8.

5.5.5 Serum 3-OMG concentrations

Serum 3-OMG increased in response to ID glucose in all groups ($P \leq 0.001$, **Figure 5.5**), but was significantly higher in PC-T2D after 30 min than in HC ($P \leq 0.05$) and WC-T2D ($P \leq 0.01$) groups (group × time interactions). The iAUC for 3-OMG was higher in PC-T2D than in the WC-T2D group ($P \leq 0.05$).

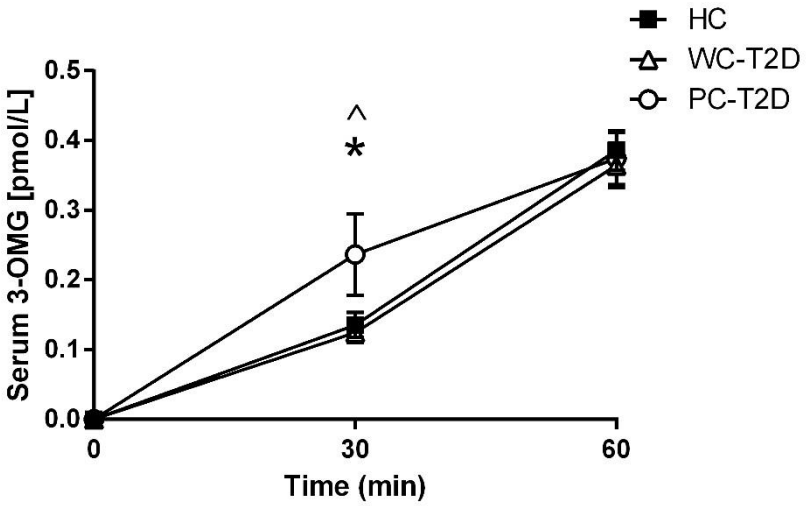

Figure 5.5: Effect of ID glucose on serum 3-OMG concentrations. ID infusion increased serum 3-OMG concentrations in all groups ($P \leq 0.001$), with higher levels in PC-T2D at 30 min than HC ($P \leq 0.05$) or WC-T2D groups ($P \leq 0.01$). Data are mean ± SEM. HC, healthy controls; PC-T2D, poorly-controlled type 2 diabetes; WC-T2D, well-controlled type 2 diabetes. HC, n = 11; WC-T2D, n = 12; PC-T2D, n = 8. *WC-T2D vs HC, ^WC-T2D vs PC-T2D.

5.5.6 Relationships between variables

Fasting blood glucose concentrations were positively related to HbA1C in both T2D groups ($r = 0.7$, $P \leq 0.01$), and with age ($r = 0.4$, $P \leq 0.05$) and BMI ($r = 0.4$, $P \leq 0.01$) across the entire cohort. The iAUC of blood glucose also positively associated with HbA1C levels ($r = 0.7$, $P \leq 0.01$). There were no associations between fasting blood glucose with GIP, GLP-1 or C-peptide at baseline. HbA1C was negatively related to basal GLUT2 ($r = -0.7$, $P \leq 0.01$) and SGLT-1 transcript levels ($r = -0.6$, $P \leq 0.01$) in T2D groups. A similar negative

association also existed between fasting blood glucose and basal GLUT2 transcript level ($r = -0.5$, $P \leq 0.01$). Basal T1R2 transcript levels did not relate to any demographic factors. Baseline transcript levels of T1R2, SGLT-1 or GLUT2 were not associated with GIP, GLP-1 or C-peptide concentrations.

Levels of T1R2 or GLUT2 transcript at baseline, or their change during ID infusion, were unrelated to GIP, GLP-1 or C-peptide iAUC. GIP concentrations at 10 min were, however, were negatively related to basal SGLT-1 transcripts ($r = -0.6$, $P \leq 0.05$), as was the degree of SGLT-1 transcript change during ID infusion ($r = -0.5$, $P \leq 0.05$). C-peptide and blood glucose were negatively associated across the cohort (iAUCs, $r = -0.5$, $P \leq 0.01$). There were no other associations between GIP, GLP-1, C-peptide, or blood glucose iAUCs.

Serum 3-OMG concentrations were unrelated to duodenal transcript expression at 10 or 30 min into glucose infusion across study groups. However, the change in T1R2 at 10 min was positively related to 3-OMG after 30 min in HC participants ($r = 0.7$, $P \leq 0.05$). 3-OMG concentrations at 30 and 60 min were also positively associated with C-peptide concentration across the cohort ($r = 0.4$, $P \leq 0.05$ and $r = 0.5$, $P \leq 0.01$, respectively).

5.6 Discussion

This study investigated the effects of ID glucose on transcriptional regulation of intestinal T1R2, SGLT-1 and GLUT2, and whether these effects differed in patients with WC-T2D and PC-T2D, and had consequences for incretin secretion and glycaemic control. We demonstrated that T1R2 transcripts were similarly abundant in HC, WC-T2D and PC-T2D groups at baseline, extending our previous findings[1]. To our knowledge, our study is the first

to show that patients with PC-T2D had lower basal expression of SGLT-1 and GLUT2 than WC-T2D patients. In response to ID glucose, transcriptional regulation of SGLT-1 and GLUT2 was also attenuated in PC-T2D patients. By contrast, T1R2 expression decreased in response to ID glucose in PC-T2D, but not in HC or WC-T2D groups. Given that 1) glucose absorption (assessed by 3-OMG) was higher in PC-T2D patients, and 2) GIP responses were preserved, while 3) GLP-1 and C-peptide responses were lower, we propose that a loss of intestinal STR signalling fidelity in PC-T2D patients may impair their ability to appropriately regulate glucose absorption, and promote a GIP-led incretin response, both of which could exacerbate hyperglycaemia.

Differences in prevailing glycaemia between HC participants, WC-T2D and PC-T2D patients did not influence intestinal T1R2 transcription at baseline. However, the transient downregulation of T1R2 upon ID glucose infusion in PC-T2D patients at euglycaemia paralleled T1R2 responses to ID glucose we had previously observed in HC participants during hyperglycaemia[1]. This may indicate a failure of glycaemic status to direct transcription of T1R2 by luminal glucose in PC-T2D patients, leading to altered luminal sensing by T1R2, and potentially, impaired coordination of glucose transport via SGLT-1.

The lower expression of SGLT-1 at baseline in PC-T2D patients did not correspond with reduced glucose absorption. Rather, glucose absorption was higher in PC-T2D participants early and over the first hour, supporting a role for post-transcriptional modification of SGLT-1 as an early determinant of postprandial glucose absorption in these patients. This is supported by findings in Sprague-Dawley rats, where intestinal infusion of D-glucose for up to 3 hours upregulated SGLT-1 protein, but did not change mRNA expression[65]. Accordingly, post-transcriptional modification of SGLT-1 may have an under-appreciated

role in functional absorption in patients with PC-T2D, which will need to be confirmed in follow-up proteomic experiments. The loss of transcriptional regulation of SGLT-1 in PC-T2D patients contrasted the early upregulation of SGLT-1 in HC participants and WC-T2D patients, which paralleled glucose absorption. While further studies of SGLT-1 protein kinetics in response to nutrient stimulation are required, a dissociation between luminal sweet sensing and transporter control may occur in PC-T2D, leading to a T1R2-independent, and post-transcriptional, gain in SGLT-1 absorptive capacity, and hyperglycaemia. Our evidence of exaggerated blood glucose excursions and lower C-peptide secretion in patients with PC-T2D is consistent with a progressive failure of GSIS with T2D disease progression.

Together with a loss of transcriptional control of SGLT-1 in PC-T2D patients, glucose-induced GIP secretion is preserved in the presence of augmented glucose absorption. This mechanism may also underlie the attenuated GLP-1 secretion in patients with PC-T2D, due to reduced luminal glucose exposure to L-cells in the distal small intestine. In contrast, the increased glucose-induced GLP-1 response seen in WC-T2D patients with normal absorption supports the existence of increased duodenal L-cell density and augmented glucose-stimulated GLP-1 secretion, as reported in newly diagnosed T2D patients[205]. As such, an increase in L-cell density, and glucose-induced GLP-1 secretion, may be limited in patients with more severe diabetes, as evidenced in the current study and supported by an earlier report where 4 weeks of insulin therapy failed to restore postprandial GLP-1 secretion in a similar and poorly controlled T2D patient cohort[206]. While several studies have described attenuated GLP-1 secretion as a feature of patients with WC-T2D[107], a recent meta-analysis has identified that patient-specific characteristics (e.g., age, body weight, fasting glucagon concentrations) exert a stronger influence on GLP-1 secretory responses. Such patient diversity may underpin reports of normal, and even augmented,

GLP-1 secretion in response to nutrient intake across T2D patient cohorts[207]. The GIP-led incretin response observed in our PC-T2D group, coupled with augmented glucose absorption, supports findings of studies in morbidly obese, non-diabetic participants exposed to a similar acute ID glucose stimulus[208]. Glucose absorption in these participants was markedly increased, in association with increased GIP secretion, but attenuated GLP-1 secretion, and suppression of glucagon. This occurred in concert with increased SGLT-1 expression, and led to hyperglycaemia and hyperinsulinaemia[208]. Although the directionality of SGLT-1 responses to glucose differed between non-diabetic, morbidly obese participants and non-obese diabetic participants, in both cases a GIP-led incretin response, attenuated GLP-1 secretion and augmented glucose absorption posed an increased risk for hyperglycaemia. Moreover, this combination with a loss of GSIS in patients with PC-T2D (attenuated C-peptide response) highlights multiple challenges for ongoing glycaemic homeostasis.

We found no association between GLUT2 and incretin secretion, in accord with studies showing that incretin secretion is largely unaltered in GLUT2 KO mice[98]. However, based on evidence of similar GLUT2 changes at transcript and protein levels in islets of a mouse model of T2D[209], the reduced intestinal GLUT2 expression after ID glucose in HC participants and WC-T2D patients may attenuate glucose egress from enterocytes. This could, in part, limit postprandial glycaemic excursions, as occurs in GLUT2 KO mice following intragastric gavage of glucose[98]. Together with SGLT-1 changes described in patients with PC-T2D, the absence of such a GLUT2 response could promote further hyperglycaemia, and indicate a failure to control glucose uptake and egress from intestinal enterocytes.

Limitations

Our study had a number of limitations. We did not quantify changes in protein levels of T1R2, SGLT-1 or GLUT2 in parallel to those of transcripts, however, similarly rapid changes in these proteins occur in the BBM of the rat jejunum in response to STR activation, while glucose absorption assessed by 3-OMG is expected to parallel function of SGLT-1[93]. We also acknowledge that euglycaemia does not reflect a physiologically normal state for PC-T2D patients, and although recruitment of these patients was challenging, future studies should establish whether changes in the STR system also occurs during hyperglycaemia. This would enable determination of the significance of prevailing glycaemia on the intestinal STR system. However, even at the therapeutically desired euglycaemia, we showed that PC-T2D patients respond differently to luminal glucose cues with dysregulated control of intestinal T1R2, SGLT-1 and GLUT2 transcription. The short infusion time used was chosen to minimise discomfort to participants, but further transcript and protein changes for these targets may occur into the postprandial period.

Conclusions

This study has demonstrated that patients with PC-T2D have reduced basal expression of glucose transporters SGLT-1 and GLUT2 which then fail to undergo transcriptional regulation by ID glucose, in contrast to the responses observed in HC and WC-T2D participants. Despite this, PC-T2D patients showed augmented glucose absorption supporting a role for post-transcriptional control mechanism(s), and augmented cellular accumulation of SGLT-1 and GLUT2 as the basis of these absorptive gains. Together with preserved glucose-induced GIP release, but attenuated GLP-1 and C-peptide responses, these findings indicate that hyperglycaemia in patients with PC-T2D arises due to a convergence of augmented glucose absorption with an impaired, GIP-led incretin and insulin

response, which may be linked to impaired sensing of luminal glucose by duodenal T1R2. Future studies should investigate these responses under hyperglycaemic conditions in PC-T2D, including proteomics, to investigate the influence of prevailing glycaemia on the intestinal STR system, and absorptive and glycaemic control.

Chapter 6: Conclusions

The studies presented in this thesis have examined the roles of intestinal sensors and transporters for fatty acids in the impaired appetite control in obesity, and intestinal sensors and transporters for carbohydrates in the glycaemic dysregulation of patients with T2D, in comparison to healthy participants. These studies have revealed fundamental and underlying differences in their transcriptional regulation that improve understanding of connections between intestinal nutrients and energy/glycaemic homeostasis. Novel therapeutics that capitalise on these unique pathways of metabolic control hold the potential to improve management of these chronic human diseases.

The study presented in **Chapter 3** characterised the expression of the intestinal fat sensors FFAR1, FFAR4, GPR119, and the fatty acid transporter CD36, in the duodenum of healthy individuals before and after an acute ID lipid infusion. Associations between fasted, and lipid-induced changes in these targets with the secretion of GLP-1 and CCK, was also investigated. To determine the influence of habitual energy and macronutrient intake on fasting fat sensor expression, and the potential impact on intestinal fat sensitivity, analysis of habitual dietary patterns were also undertaken. This results of this study revealed that all targets were expressed in the duodenum, with their order of transcript abundance matched to their cellular distribution (e.g., highly abundant CD36 expression on intestinal enterocytes[153]). We also observed that GPR119 expression was negatively associated with habitual consumption of PUFAs in healthy individuals. Although long-term consumption of habitual fat and GPR119 expression in humans has not been studied, consumption of PUFA is linked with lower levels of adiposity[171]. Future investigations should look to characterise the role of dietary fatty acid composition on these intestinal FFAR pathways, and to determine whether dietary modifications may enhance their sensitivity and improve fat metabolism.

The study presented in **Chapter 4** expanded on the findings in **Chapter 3**, by investigating the duodenal expression of FFAR1, FFAR4, GPR119 and CD36 across a broad BMI range in otherwise healthy individuals, and in response to acute ID lipid. The purpose of this design was to determine whether there was a reduced sensitivity to lipid with increasing BMI, as observed through the attenuation of satiety hormone secretion, and reduced suppression of appetite and energy intake, and to determine whether this was associated with transcriptional changes in duodenal fat sensors. The study revealed that BMI was positively associated with basal expression of CD36, but negatively associated with basal FFAR1 and FFAR4 expression, while GPR119 expression was independent of BMI. As such, human obesity may lead to a progressive and reduced ability to adequately respond to ingested LCFAs, through reduced availability of FFAR1 and FFAR4. This is supported by human studies of a loss-of-function-variant of FFAR4, shown to be associated with increased risk of obesity and insulin resistance[35]. Furthermore, FFAR4 KO mice that are fed a HFD develop obesity, glucose intolerance, and insulin resistance[27,28], indicating that a loss of FFAR4 has implications for obesity development. However, the metabolic implications for reduced FFAR1 in obesity remains unclear, as FFAR1 deletion in mice does not consistently protect against metabolic dysfunction in HFD-fed mice[41,45], and as such requires further investigation.

Although we found CD36 expression was increased in obese humans, the consequences for fatty acid transport is unclear, as knockdown of this transporter in mice does not affect overall LCFA absorption[152,184]. However, alterations in CD36 may impair postprandial triglyceride metabolism, as CD36 plays a vital role in the formation of lipid-rich chylomicrons, which are critical for the secretion of CCK in response to LCFAs, as well as gastric emptying, food intake, and plasma lipid transport[52,135]. Future experiments that will

analyse postprandial triglycerides in plasma are planned for this cohort, and will be related to changes in CD36 observed in the current dataset.

Chapter 4 also confirmed that while basal FFAR1 expression was lower in obese participants, it increased in response to lipid in a BMI-independent manner. Moreover, this study provided the first evidence that FFAR1 expression was positively associated with augmented GIP secretion following ID lipid. This adds support to the previously reported role for FFAR1-stimulated insulin secretion from pancreatic β-cells in rodents[38], and highlights a potential dual role for FFAR1 to augment postprandial insulin secretion in humans via direct (pancreatic) and indirect (intestinal GIP secretion) mechanisms. As in **Chapter 3**, GPR119 expression increased in response to ID lipid in a BMI-independent manner. As such, acute changes in GPR119 expression should be explored in future investigations, particularly in relation to the effect of lipid on plasma OEA, as OEA is a ligand for GPR119 and triggers the secretion of GLP-1 in both humans and rodent cell lines[46], and has been linked to between-meal satiety in rodents[163]. Previous work has shown that the type and amount of habitual fat consumption is a predictor of intestinal OEA production, and thus capacity for GPR119 activation[173]. Analysis of the plasma OEA content, along with lipid content of the duodenal biopsies is planned for future studies by our group, and will provide a vital piece of information about the link between GPR119, OEA, and satiety in humans.

Although we observed differences in the expression of FFAR1, FFAR4 and CD36 across BMI, this did not alter secretion of GLP-1, CCK and PYY across lean, overweight and obese participants. We did, however, note an increase in the proportion of energy consumed as fat at the buffet meal with increasing BMI, suggesting that individuals in the current study

represent an early phase of metabolic disruption, where they are still able to adequately compensate for increases in luminal fat exposure. It is possible that these responses would be altered in metabolically unhealthy obese, or T2D cohorts. As such, it would be worthwhile to determine in future studies the effects of chronic HFD consumption on GI responses, and fat sensor expression, and whether fat sensors are susceptible to modifications to habitual dietary intake, and as such whether they represent novel targets for obesity treatment and prevention.

The study presented in **Chapter 5** focussed on the intestinal sweet taste system across patients with a range of T2D disease control. Our group had previously established that transcriptional regulation of the intestinal STR system was similar at euglycaemia in patients with diet-controlled T2D disease and healthy participants during ID glucose, but became disordered in T2D patients at hyperglycaemia[1]. This study investigated whether patients with prevailing hyperglycaemia and more advanced T2D disease had further impairments in the intestinal STR system and incretin responses, predisposing to worsened postprandial glycaemia. This study extended findings that intestinal T1R2 expression was unaffected by diabetic status, and revealed a profound loss of basal SGLT-1 and GLUT2 expression in PC-T2D patients. Moreover, intestinal SGLT-1 and GLUT2 was upregulated by ID glucose in HC participants and patients with WC-T2D at euglycaemia, but not in patients with PC-T2D. From this study it is also clear that post-transcriptional modification of SGLT-1, and GLUT2, may play a prominent role in functional glucose absorption in patients with PC-T2D. However, this must be confirmed in follow-up proteomic experiments. PC-T2D patients also experienced a rapid and early downregulation of T1R2, and the potential uncoupling of SGLT-1 regulation from luminal sweet sensing. This was supported by evidence of increased glucose absorption and GIP secretion in patients with PC-T2D, along with the absence of an augmented, and potentially compensatory, GLP-1 secretion, which

occurred in patients with WC-T2D. Overall, postprandial hyperglycaemia in patients with PC-T2D may arise due to enhanced glucose absorption and an altered, GIP-led incretin response that fails to adequately stimulate insulin release, all of which may occur secondary to impaired sensing of luminal glucose by duodenal T1R2. This is in line with previous studies of morbidly obese humans, in which associations emerged between enhanced glucose absorption and the augmented secretion of glucagon and GIP, but reduced GLP-1, contributing to observed hyperglycaemia[208]. However, whether enhanced glucose absorption in PC-T2D is associated with attenuated glucagon suppression is unknown, and will form the basis of future analysis. Investigation of this system in PC-T2D under hyperglycaemic conditions will be necessary, to elucidate the influence of prevailing glycaemia on this system, as well as reflecting a more physiological state for PC-T2D patients.

Future investigations should also investigate whether non-caloric (i.e., 'artificial') sweeteners produce similar results. Animal studies have shown that non-caloric sweeteners activate STRs, and upregulate SGLT-1 expression in several species[29,58,65]. The use of non-caloric 'artificial' sweeteners, particularly in diet beverages, is emerging as a key contributor to the rising incidence of T2D, particularly amongst Western populations[109]. Importantly, while sweeteners such as sucralose, aspartame and acesulfame-K were originally marketed as suitable calorie-free sugar substitutes, particularly for those with T2D[110], these animal studies provide evidence that these substances may not be metabolically inert, and under conditions of high intake, may worsen glycaemic control.

Limitations

There were limitations to these studies which must be considered in data interpretation. First, the time frame of ID infusion of both Intralipid® and glucose was based on previous studies

which used ID glucose to examine intestinal regulation of STRs[1,61]. We cannot, therefore, exclude the possibility that transcriptional changes may occur further into the postprandial period. Secondly, the changes in mRNA expression in response to either lipid or glucose may not necessarily correlate with changes in cell-surface protein, and as such, proteomics will be a critical focus of future investigations. Due to the inconsistent quality of commercial antibodies targeting GPRs in pilot protein expression experiments (Western blots) through my candidature, the decision to commit regular protein experiments was postponed. Finally, the use of habitual dietary questionnaires to establish dietary consumption patterns is inherently flawed, as they do not determine acute consumption prior to the study, and intakes are commonly underreported by obese individuals[187]. Future studies in which diets are controlled are clearly needed. Despite these limitations, the studies presented in this thesis provide novel insights into intestinal nutrient sensing mechanisms, and open up new pathways of investigation.

In conclusion, the studies presented in this thesis provide important new knowledge to the field of intestinal nutrient sensing. Study findings have the potential to direct future investigations to establish the basis of functional connections between luminal nutrient sensing, GI hormone secretion and subsequent regulation of energy intake and glycaemia. These intestinal nutrient sensors for fats and carbohydrates represent a unique system that could be strategically targeted to re-establish energy, and glycaemic homeostasis in conditions such as obesity and T2D. The development of specific therapeutic interventions that capitalise on their unique localisation and distribution within the GI tract, hold the potential for less-invasive and more targeted therapies than those currently available, for the treatment of obesity and T2D.

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