Investigating the mechanisms responsible for the effect of protein on appetite

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Abstract

Obesity, its associated complications and mortality are major worldwide public health issues. Current treatments are poorly effective and are limited by risks and side-effects, and diet and lifestyle measures are poorly adhered to in the long-term. Protein has a satiating effect superior to that achieved with other macronutrients. High protein diets reduce food intake, increase weight loss and improve body composition in animal models and humans. However, they are difficult to adhere to in the longer-term and the exact mechanisms by which a high protein diet regulates food intake remain poorly understood. There is evidence that the amino acid products of protein digestion may regulate appetite and satiety.

In vivo studies in rodents from our group have shown that specific amino acids, including L-arginine, can reduce food intake and stimulate the release of anorectic gut hormones. The work carried out in this thesis looked at the effect of L-arginine on appetite in humans. Further work was carried out to determine the metabolites that drive early gut hormone release in humans in response to meals rich in different macronutrients.

Oral administration of the maximum dose of L-arginine considered to be a foodstuff by the Medicine Healthcare products Regulatory Agency was well-tolerated with no reported side-effects in a study carried out to determine tolerability. In combination with an *ad libitum* meal, L-arginine significantly elevated levels of the anorectic gut hormones glucagon-like peptide-1 and peptide YY in healthy human volunteers compared to vehicle treatment. There were possible delayed effects on energy intake and fat intake following oral L-arginine administration subsequent to the study period as observed in self-reported food diaries.

Determining the specific metabolites generated by digestion of a high protein meal associated with gut hormone release could guide the targeting of future treatment options such as the creation of functional foods which manipulate intrinsic hunger and satiety mechanisms. Thus a study was carried out using metabolomics to analyse the metabolites present in the stomach and duodenum following ingestion of a high protein meal, and their correlation with gut hormone release. A high protein meal significantly elevated GLP-1 and glucagon. The increase in certain metabolites, some of which may be the products of microbial action, correlate with the increase in GLP-1 release following a high protein meal. Some of the metabolites identified in the duodenum were unexpected. The presence of SCFAs following the high protein meal was one of the findings from the metabolomics analysis, opening questions as to the importance of the gut microbiome in the upper gastrointestinal tract following a high protein meal. This study highlighted the utility of using metabolomics as a

mechanistic tool to explore how particular diets may exert biological effects. Differences in metabolic profiles between the pre-meal and post-meal period could better our understanding of how diet influences appetite. Using this type of approach may provide a novel approach to the management of chronic conditions such as obesity.

These studies demonstrate the effects of both L-arginine and a high protein meal on appetite-regulating hormones, and contribute to understanding the mechanism of how protein regulates appetite and its effect on glucose homeostasis. Further studies are now necessary to determine the potential of specific amino acids and protein metabolites as treatment options in the management of obesity.

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Declaration of Contributors

The majority of work presented in this thesis was performed by the author. All collaboration and assistance are described below:

Chapter 2:

Both studies were performed with assistance from Dr Niamh Martin and Dr Christina Neophytou. Gut hormone assays were performed with assistance from Dr Amin Alamshah and Dr Elly Spreckley.

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Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
7TM	Seven transmembrane
AAV	Adeno-associated virus
ACC	Acetyl coenzyme A carboxylase
AgRP	Agouti-related peptide
ALT	Alanine aminotransferase
AMPK	5' adenosine monophosphate-activated protein kinase
ANOVA	Analysis of variance
AP	Area postrema
APC	Anterior piriform cortex
ARC	Arcuate nucleus
ASL	Argininosuccinate lyase
Asp	Aspartate
AST	Aspartate aminotransferase
ASS1	Argininosuccinate synthase 1
ATF4	Activating transcription factor 4
BMI	Body mass index
BSA	Bovine serum albumin
CART	Cocaine and amphetamine-regulated transcript
CaSR	Calcium sensing receptor
CAT	Cationic amino acid transporter
CCK	Cholecystokinin
CCK-1R	Cholecystokinin receptor 1
CCK-2R	Cholecystokinin receptor 2
CNS	Central nervous system
CoA	Coenzyme A
CP	Carbamoyl phosphate
CPS I	Carbamoyl-phosphate synthase I
CPS1	Carbamoylphosphate synthetase 1
CPT1	Carnitine palmitoyl transferase 1
CRD	Cysteine rich domain
CRF	Clinical research facility
CTIMP	Clinical trial of an investigational medicinal product
DEBQ	Dutch eating behaviour questionnaire
DIT	Dietary induced thermogenesis
DMN	Dorsomedial nucleus
DMX	Dorsal motor nucleus of the vagus
DPP-4	Dipeptidyl peptidase 4
DVC	Dorsal vagal complex
EAT	Eating attitudes test

EIA	Enzyme immunoassay
elf2α	Eukaryotic initiation factor 2 alpha
ELISA	Enzyme-linked immunosorbent assay
ENS	Enteric nervous system
ERK1/2	Extracellular signal-regulated kinases 1 and 2
FFA	Free fatty acid
FFAR1	Free fatty acid receptor 1
FFAR2	Free fatty acid receptor 2
FFAR3	Free fatty acid receptor 3
FTO	Fat mass and obesity-associated protein
GABA	Gamma-aminobutyric acid
GCGR	Glucagon receptor
GCK	Glucokinase
GCN2	General control nonrepressed 2 protein
GDP	Guanosine diphosphate
GE	Glucose-excited
GGT	Gamma-glutamyl transpeptidase
GH	Growth hormone
GHSR	Growth hormone secretagogue receptor
GI	Glucose-inhibited
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide 1
GLP-1R	Glucagon-like peptide 1 receptor
Glu	Glutamate
GLUT	Glucose transporter
GOAT	Ghrelin O-acyltransferase
GPCR	G-protein coupled receptor
GPR93	G-protein receptor 93
GPR120	G-protein receptor 120
GPRC6A	G-protein coupled receptor group C member 6A
GTP	Guanosine triphosphate
HPLC	High pressure liquid chromatography
ICV	Intracerebroventricular
IL	Interleukin
Kcal	Kilocalories
KG	Ketoglutarate
LCFA	Long-chain fatty acid
LDL	Low-density lipoprotein
LHA	Lateral hypothalamic area
MBH	Mediobasal hypothalamus
MCFA	Medium-chain fatty acid
MEF	Mouse embryonic fibroblast

MHRA	Medicines and Healthcare products Regulatory Agency
mRNA	Messenger ribonucleic acid
MSH	Melanocyte stimulating hormone
mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1
NAcc	Nucleus accumbens
NMDA	N-methyl-D-asparatate
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOS	Nitric oxide synthase
NPY	Neuropeptide Y
NTS	Nucleus tractus solitarius
OAT	Ornithine aminotransferase
Ob-R	Leptin receptor
OCT	Ornithine carbamoyltransferase
OPLS	Orthogonal Projections to Latent Structures
OPLS-DA	Orthogonal Projections to Latent Structures-Discriminant Analysis
OXM	Oxyntomodulin
PCA	Principal component analysis
PDG	Phosphate-dependent glutaminase
PLS-DA	Partial Least Square Discriminant Analysis
PO	Proline oxidase
P5C	L-Δ1-pyrroline-5-carboxylate
P5CS	L-Δ1-pyrroline-5-carboxylate synthetase
PEG	Poly ethylene glycol
PKC	Protein kinase C
POMC	Proopiomelanocortin
PP	Pancreatic polypeptide
PROHD	Proline dehydrogenase
PVN	Paraventricular nucleus
PYY	Peptide YY
QC	Quality control
RIA	Radioimmunoassay
SCFA	Short-chain fatty acid
SCOFF	Sick control one fat food
SD	Standard deviation
SEM	Standard error of the mean
SGLT1	Sodium glucose co-transporter 1
SLC	Solute-linked carrier
SNAT2	Sodium-coupled neutral amino acid transporter 2
STOCSY	Statistical TOtal Correlation SpectroscopY
STORM	SubseT Optimization by Reference Matching

T1R	Taste 1 receptor
tRNA	Transfer ribonucleic acid
TRPM5	Transient receptor potential cation channel subfamily M member 5
TSC	Tuberous sclerosis
VAS	Visual analogue scale
VFT	Venus fly trap
VMN	Ventromedial nucleus
WHO	World Health Organization
WT	Wild-type

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Chapter 1:

General Introduction

1.1. **The obesity epidemic**

Obesity and its associated complications and mortality are a major worldwide public health concern. Estimates from the World Health Organisation (WHO) suggest that 39% of the world's adult population are overweight and 13% are obese, and that the prevalence of obesity has more than doubled in the period between 1980 and 2014 (WHO, 2016). In 2015, a total of 107.7 million children and 603.7 million adults were obese (Afshin et al., 2017). Once considered to be a problem of the developed world, it is clearly now a global problem also affecting low- and middle-income countries, especially in urban settings. It is estimated that up to 58% of the world's adult population will be overweight or obese by 2030 (Kelly et al., 2008). If current trends continue, the chances of achieving the worldwide obesity target (that is, halting the rise in obesity) are virtually zero (NCD-RisC, 2016).

Worldwide, 2.8 million people are estimated to die annually as a result of being overweight or obese and an estimated 35.8 million (2.3%) of global disability-adjusted life years are caused by overweight or obesity (WHO, 2009). Obesity is associated with cardiovascular disease, type 2 diabetes and certain forms of cancer, and the rise in obesity levels has resulted in rises in these chronic diseases (Whitlock et al., 2009). The Foresight report highlighted appetite regulation as a major target in the dietary treatment of obesity (Butland, 2008). In particular, there is a move towards developing functional foods aimed at increasing satiety. The goal is to design foods or dietary regimens that cause an increased sense of fullness and encourage the individual to stop eating sooner and to delay their next meal and/or eat less at the next meal, thus reducing total energy intake (Hill and Peters, 2002).

1.1.1. Current treatments for obesity

Current treatments for obesity are poorly effective. They consist predominantly of lifestyle changes, medical and surgical treatment. Lifestyle treatment effectively means dietary changes and physical exercise, and although this has been shown to have some benefit, the difficulty lies in long-term adherence. The only licensed oral medication in the UK for the treatment of obesity is orlistat, a gastrointestinal lipase inhibitor that reduces fat absorption from the gut. Unfortunately the achievable weight loss is modest and the drug is poorly tolerated, mostly due to its gastrointestinal side-effects (Torgerson et al., 2004). More recently, new treatments in the form of glucagon-like peptide-1 (GLP-1) receptor agonists have now been licensed in the UK for weight management in adults with an initial body mass index (BMI) of ≥30kg/m², or ≥27kg/m² to <30kg/m² (overweight) in the presence of at least one weight-related comorbidity such as dysglycaemia (pre-diabetes or type

2 diabetes mellitus), hypertension, dyslipidaemia or obstructive sleep apnoea. These drugs do, however, have a significant side-effect profile, the most common being profound nausea, and result in at best 8.4kg of weight loss (Pi-Sunyer et al., 2015). Bariatric surgery is the most effective treatment for obesity, resulting in sustained weight loss (Sjostrom et al., 2007), but comes at a considerable cost and carries with it major surgical risk.

1.2. **Energy homeostasis**

Homeostatic mechanisms keep a balance between food intake and energy expenditure. Body weight maintenance is dependent on maintaining this balance. Central neuronal circuits interpret and integrate information from peripheral neural and humoral signals, providing a coordinated response to maintain energy homeostasis. Gastrointestinal hormones, for example, can activate circuits in the hypothalamus and brainstem, the main central nervous system (CNS) centres responsible for the regulation of energy homeostasis (Figure 1.1).

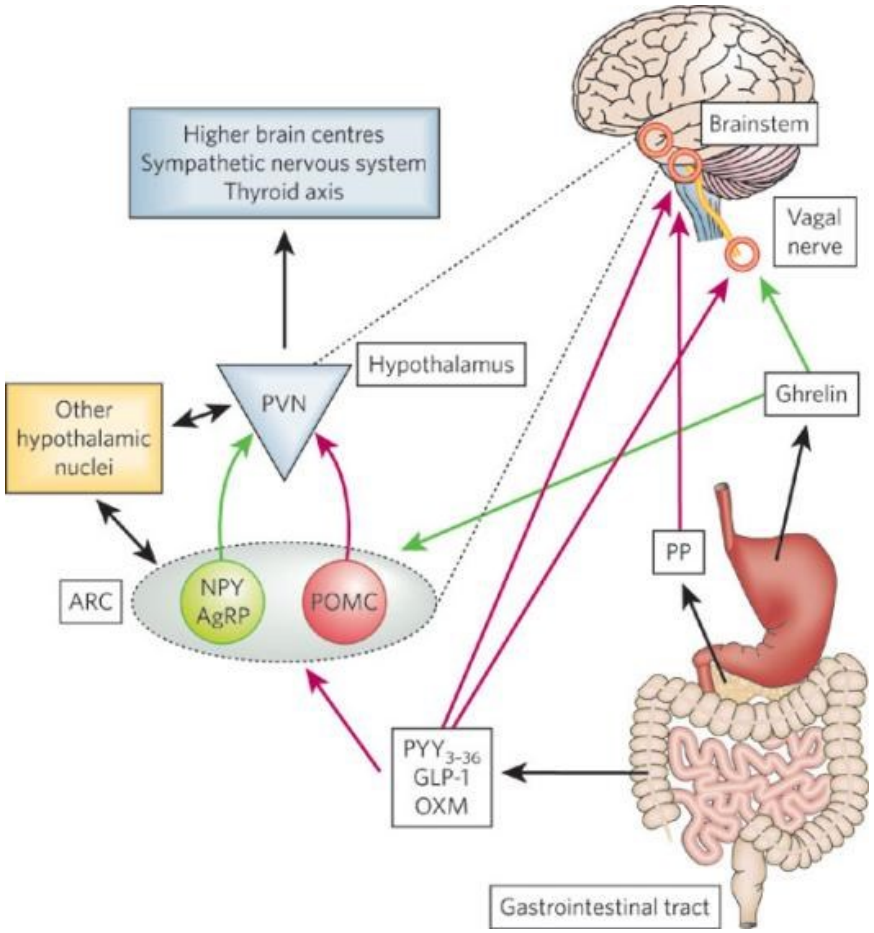


Figure 1.1. **Schematic diagram showing the pathways by which gut hormones regulate energy homeostasis**.
Gastrointestinal hormones can activate circuits in the hypothalamus and brainstem, the main CNS centres responsible for the regulation of energy homeostasis (Murphy and Bloom, 2006).

1.2.1. Central regulation of energy homeostasis

The principal areas of the brain involved in energy homeostasis are the hypothalamus and brainstem. The hypothalamus sits at the base of the brain, just under the thalamus, and receives afferent signals from the periphery as well as other parts of the CNS. It consists of several distinct nuclei, including the arcuate nucleus (ARC), dorsomedial nucleus (DMN), ventromedial nucleus (VMN), paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA). These nuclei communicate with each other and other brain regions in order to maintain energy homeostasis (Waterson and Horvath, 2015). The ARC is thought to be integral in coordinating signals involved in maintaining energy balance. It is located at the base of the hypothalamus and lies just adjacent to the median eminence, an area with incomplete blood-brain barrier. Thus the ARC is a target for circulating nutrients and metabolic signals (Schwartz et al., 2000). Two neuronal populations within the ARC are involved in energy homeostasis: the orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons (Broberger et al., 1997), which increase food intake, and anorexigenic neurons co-expressing proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART), which reduce food intake (Vrang et al., 1999). These neuronal populations interact with neurons in other hypothalamic nuclei and other brain regions to regulate energy homeostasis (Bouret et al., 2004, Elias et al., 1998).

The brainstem is located at the base of the brain, and is in continuum with the spinal cord. The main regions of the brainstem involved in maintaining energy homeostasis are the nucleus tractus solitarius (NTS), the dorsal motor nucleus of the vagus (DMX) and the area postrema (AP), which together make up the dorsal vagal complex (DVC) (Schwartz, 2006). The DVC receives neural afferents from the gut as well as circulating signals, as the AP is situated outside of the blood-brain barrier. In turn, the AP and NTS convey these peripheral satiety signals to the ARC and other brain regions (Bailey, 2008) via complex neuronal circuitry (Murphy and Bloom, 2006).

Peripheral signalling to the hypothalamus and brainstem occurs via neuronal or endocrine signals. Vagal pathways carry predominantly satiating signals from the gastrointestinal tract. Nutrients within the gastrointestinal tract trigger signals from gut epithelial cells, which then act via vagal afferents to have subsequent effects on the CNS. This signalling stimulates gastric vagal motor neurons resulting in relaxation of the proximal stomach, inhibition of gastric antral contractions and stimulation of tonic and phasic pyloric pressures (Horowitz and Dent, 1991, Azpiroz and Malagelada, 1987, Niijima, 2000). Interrupting this system, via vagotomy or afferent denervation, in both animals and humans can attenuate the inhibition of gastric emptying and food intake, which occurs when

nutrients enter the gut (Wilkinson and Johnston, 1973, Roze et al., 1977, Raybould and Holzer, 1992, Schwartz et al., 1993).

Endocrine signals also act centrally (Schwartz et al., 2000). Hormones specifically released from the gut can signal to the brain via vagal afferent pathways (Schwartz, 2000) that express specific gut hormone receptors (Steinert and Beglinger, 2011). Gut hormones can also act directly on central neurons (Kalra et al., 1999, Cone et al., 2001). .

1.2.2. Peripheral regulation of energy homeostasis

1.2.2.1. **Adiposity signals**

To maintain energy homeostasis, the brain coordinates peripheral signals regarding energy availability. The brain receives information on acute nutrient availability by sensing the release of gut hormones and metabolic factors, and on long term energy balance from hormones such as insulin and leptin, levels of which are dependent on adiposity (Baskin et al., 1999).

1.2.2.1.1. Insulin

Insulin is a peptide hormone produced by the β-cells of the pancreatic islets. It consists of two polypeptide chains linked together by disulfide bonds, and arises from the precursor preproinsulin, which is cleaved in a number of steps resulting in insulin formation. Insulin levels rapidly rise post-prandially to stimulate the uptake of excess glucose from the circulation by acting on tissues such as the liver, adipose tissue and skeletal muscle. This results in a number of physiological effects, including stimulation of intracellular glucose uptake, glycogen synthesis and decreased gluconeogenesis.

Although its main effects are in glucose homeostasis, insulin also plays a role in maintaining energy balance. Insulin has multiple roles in lipid metabolism, including stimulation of lipid uptake into adipocytes and inhibition of lipolysis in adipose tissue, thus lowering circulating free fatty acids (Dimitriadis et al., 2011, Begg and Woods, 2012). Insulin also plays a major role in protein metabolism, promoting the uptake of amino acids into muscle and the proteinogenesis. Circulating insulin is highly influenced by adiposity (Kahn et al., 1993) and can enter the brain via the blood-brain barrier using a saturable transporter (Banks et al., 2012), where it acts as an anorectic signal to central brain circuits (Schwartz et al., 2000). Insulin signalling via the insulin receptor is thought to play a role in energy homeostasis (Bruning et al., 2000) in areas such as the ARC (Marks et al., 1990) via effects on NPY and POMC (Begg and Woods, 2012).

1.2.2.1.2. Leptin

Leptin is a protein hormone produced predominantly in white adipose tissue, with circulating levels proportional to adiposity (Maffei et al., 1995, Considine et al., 1996). Leptin's structure consists of a four-helix bundle, similar to members of the long chain helical cytokine family such as interleukin-2 (IL-2), interleukin-12 (IL-12) and growth hormone (GH) (Zhang et al., 2005, Matarese, 2000). Leptin is the product of the *ob* gene and, in concert with the leptin receptor (Ob-R), is thought to play an important role in energy homeostasis (Lee et al., 1996, Chen et al., 1996). Absence of leptin results in profound hyperphagia and obesity, and leptin administration can suppress food intake in rodents and lean humans (Chen et al., 1996, Tartaglia et al., 1995, Lee et al., 1996, Pelleymounter et al., 1995, Corp et al., 1990, Heymsfield et al., 1999). Obesity is typically associated with high leptin levels and rodents and humans that become obese on a high-fat diet do not respond to leptin – reflecting so-called leptin resistance (Flier, 1998, Heymsfield et al., 1999).

Its role in appetite regulation is thought to occur primarily through direct effects on areas of the brain known to be involved in energy homeostasis and feeding, such as the hypothalamus and the mesolimbic dopamine system (Kelesidis et al., 2010).

1.2.2.2. **Enteric nervous system**

The enteric nervous system (ENS) is a distinct part of the nervous system which contains neural circuits involved in gastrointestinal motor function, blood supply, mucosal transport, and exocrine and endocrine secretions. The ENS plays an important role in communication between the intestine and the central nervous system, otherwise known as the gut-brain axis. Although the ENS contains 200-600 million neurons, the majority are found in two plexuses, the myenteric and submucosal plexuses (Furness et al., 2014). Vagal and spinal sensory neurons transfer information in a bi-directional fashion between the CNS and the ENS, and thus play an important role in the gut-brain axis. Vagal afferents are more concentrated in the proximal gut and are activated by mechanical, thermal and chemical stimuli and relay sensory information to the NTS, whilst spinal afferents are primarily in the distal gut and are mostly activated by mechanical stimuli (Berthoud et al., 2004, Furness et al., 2004). Vagal afferents also respond to gut hormones such as GLP-1, peptide YY (PYY), cholecystokinin (CCK) and ghrelin (Koda et al., 2005, Date et al., 2002, Abbott et al., 2005a, Moran et al., 1997) (Section 1.2.2.3). In addition, some gut hormones with effects on food intake and gastric emptying such as GLP-1, PYY, CCK and ghrelin, have been shown to require vagal pathways to facilitate their actions (Koda et al., 2005, Date et al., 2002, Abbott et al., 2005a, Moran et al., 1997),

though there is also evidence that GLP-1, PYY and ghrelin work through other pathways as well (Holst, 2007a, Gutzwiller et al., 1999, Batterham et al., 2003, Wren et al., 2001a, Wren et al., 2001b).

1.2.2.3. **Gut hormones**

The gut is the largest endocrine organ in the body, releasing a multitude of bioactive peptides (Ahlman and Nilsson, 2001). Gut hormones are secreted from a variety of enteroendocrine cells that are present in the gut epithelial wall throughout the entire gastrointestinal tract. These cells are sensitive to luminal nutrient content, pH and luminal distension. The cells may sense directly or via other cell types, and regulate their secretion of appetite-regulating hormones in response to these changes (Fig 1.1) (Murphy and Bloom, 2006).

1.2.2.3.1. Ghrelin

Ghrelin is an orexigenic gut peptide hormone primarily synthesised in X/A-like enteroendocrine cells in the stomach and proximal gut (Kojima et al., 1999, Date et al., 2000). The gene for ghrelin encodes the 117 amino acid pre-proghrelin, which is cleaved and processed to produce the 28 amino acid ghrelin. The third amino acid in its structure (which is usually a serine but in some species is a threonine) is O-acylated by a post-translational modification via the enzyme ghrelin O-acyl transferase (GOAT) to generate acyl-ghrelin (Kojima and Kangawa, 2005, Yang et al., 2008), the active form of the hormone capable of activating its cognate receptor, the growth hormone secretagogue receptor (GHSR) (Bednarek et al., 2000).

Ghrelin has a number of different roles. It stimulates food intake, which is thought to be mediated by NPY and AgRP neurons within the CNS (Chen et al., 2004). Ghrelin administration increases food intake in both animals and humans (Wren et al., 2001a, Wren et al., 2000b). In rodents, there is an acute increase in food intake and a chronic increase in adiposity when ghrelin is given centrally or peripherally (Wren et al., 2000a, Tschop et al., 2000). It has a role also in glucose homeostasis, where in concert with other factors, it maintains fasting blood glucose by suppressing insulin release (Scott et al., 2012). In addition, ghrelin promotes gastric motility (Perboni and Inui, 2010) and adipogenesis (Thompson et al., 2004).

Ghrelin is the only known orexigenic hormone, and thus makes it a potential target in the development of an anti-obesity treatment. Obese humans, despite having low circulating ghrelin levels (Tschop et al., 2001), are also unable to significantly suppress ghrelin post-prandially (le Roux et al., 2005), suggesting disrupted ghrelin signalling. Ghrelin and ghrelin receptor knockout mice models are resistant to diet-induced obesity (Sun et al., 2008), and thus blocking ghrelin or its

receptor would appear to be a potential treatment for obesity. However, thus far, using GHSR antagonists or neutralising ghrelin by the use of antibodies or oligonucleotides, has not provided promising results (Asakawa et al., 2003, Vizcarra et al., 2007, Shearman et al., 2006). It is possible that GOAT antagonists may be useful as targets for obesity treatment. They have been shown to reduce circulating acyl-ghrelin, and weight gain and fat mass in mice fed a high fat diet (Barnett et al., 2010), and to reduce early dark phase food intake (Teuffel et al., 2015). The reasons for the failure of the other treatments remain unclear. It may be due to the reduced ghrelin levels which occur in obesity, thus limiting the effectiveness of further attempts to suppress ghrelin signalling.

1.2.2.3.2. Cholecystokinin

CCK is an anorexigenic peptide hormone secreted primarily by I-cells in the proximal small intestine (Buchan et al., 1978, Beinfeld et al., 1981, Hutchison et al., 1981). There are several forms of CCK, each of different lengths, including CCK-58, CCK-39, CCK-33, CCK-8 and CCK-5, generated by a series of post-translational modification steps from the precursor molecule, preprocholecystokinin (Johnsen, 2001). CCK-8 is the most predominant form in the brain, and CCK-58 is the most predominant systemic form in humans and rats (Eysselein et al., 1986, Reeve et al., 2003). The biological actions of CCK are mediated by the two CCK receptors CCK-1R (CCK-A) and CCK-2R (CCK-B). The effects of CCK on food intake occur primarily through the CCK-1R located on vagal afferents (Corp et al., 1993, Yox et al., 1992, Moran et al., 1990), and accordingly, subdiaphragmatic vagotomy and selective vagal deafferentation decrease the anorectic effects of peripheral CCK administration (Smith et al., 1981, Joyner et al., 1993). The receptor is also found in the pancreas, gallbladder and stomach where its activation mediates pancreatic secretion, gallbladder contraction (Tang et al., 1996), and slowing of gastric emptying, respectively (Scarpignato et al., 1993).

Peripheral administration of CCK in rodents and intravenous infusion of CCK into lean and obese humans, reduces food intake (Kissileff et al., 1981, Pi-Sunyer et al., 1982, Gibbs et al., 1973). Blocking the CCK-1R increases food intake in rodents (Yox et al., 1992) and humans (Lieverse et al., 1994), suggesting a physiological anorectic role for CCK in the control of food intake. Chronic CCK administration in rats reduces meal size, but results in increased meal frequency, resulting in no change in body weight (West et al., 1984). CCK administration decreases food intake acutely in humans (Muurahainen et al., 1988), but anorectic effects disappear after only 24 hours of treatment (Crawley and Beinfeld, 1983). As a treatment in itself, there is no current evidence of efficacy. There is, however, synergistic interaction between CCK and both leptin and amylin to reduce short-term food intake in rodents (Barrachina et al., 1997, Bhavsar et al., 1998), suggesting a possible role for dual therapy in the management of obesity.

1.2.2.3.3. Peptide YY

PYY is a 36 amino acid anorexigenic peptide hormone of the pancreatic polypeptide (PP)-fold family, secreted from enteroendocrine L-cells of the gastrointestinal tract following a meal. There are two major circulating forms: PYY_{1-36} and a truncated form, PYY_{3-36} (Eberlein et al., 1989), which results from enzymatic N-terminal cleavage of PYY_{1-36} by dipeptidyl peptidase-4 (DPP-4) (Mentlein et al., 1993). Both these forms have PP-fold structural motifs. PYY binds to the Y family of receptors, with PYY_{1-36} binding to Y1, Y2 and Y5 receptors and PYY_{3-36} binding selectively to Y2 receptors (Abbott et al., 2005b). PYY_{1-36} has no effect on food intake (Sloth et al., 2007), whilst PYY_{3-36} reduces food intake in both lean and obese rodents and humans following peripheral administration (Challis et al., 2003, Batterham et al., 2002, Batterham et al., 2003). PYY_{3-36} also delays intestinal transit times and inhibits gastric emptying (Savage et al., 1987).

The anorectic effects of PYY_{3-36} are abolished in Y2 receptor knockout mice (Batterham et al., 2002) and Y2 specific receptor antagonists attenuate the effect of endogenous and exogenous PYY_{3-36} on food intake, suggesting the anorexigenic effects of PYY_{3-36} are mediated by the Y2 receptor (Abbott et al., 2005b). The precise pathways by which PYY_{3-36} works are unclear but appear to include NPY/AgRP neurons within the ARC (Dumont et al., 1995), vagal afferents and the brainstem, all of which express the Y2 receptor (Koda et al., 2005); the effects of peripheral administration of PYY_{3-36} both on food intake and the activation of hypothalamic arcuate feeding neurons are abolished following vagotomy or brainstem-hypothalamic pathway transectioning (Abbott et al., 2005a). Peripheral administration of PYY_{3-36} activates neurons within the ARC and decreases hypothalamic NPY messenger ribonucleic acid (mRNA) expression; injection of PYY_{3-36} directly into the ARC reduces food intake in rats and electrophysiological studies have shown that PYY_{3-36} inhibits NPY neurons (Batterham et al., 2002). Other studies have shown that intracerebroventricular (ICV) injection of PYY_{3-36} increases food intake (Clark et al., 1987, Corp et al., 2001), which may reflect the pharmacological activation of the Y1 receptor and/or Y5 receptor in the PVN at high concentrations. In addition, action on the limbic system (including the amygdala) may be of significance when considering modulation of hedonic pathways by PYY_{3-36} (Batterham et al., 2007, De Silva and Bloom, 2012).

PYY is expressed in enteroendocrine L-cells throughout the entire gastrointestinal tract, with concentrations of PYY immunoreactivity highest in the distal part of the gut, particularly in the terminal ileum, colon and rectum (Adrian et al., 1985a, Ekblad and Sundler, 2002). PYY is released in response to a meal, with levels proportional to calories consumed; circulating levels are reduced by fasting (Adrian et al., 1985a). PYY knockout mice are obese and hyperinsulinaemic, suggesting a

physiological role for PYY in energy balance and glucose homeostasis (Batterham et al., 2006b, Boey et al., 2006).

The utility of exogenous PYY_{3-36} as a therapeutic agent in the treatment of obesity seems limited due to its rapid degradation in circulation (Lluis et al., 1989). In addition, supraphysiological concentrations of PYY_{3-36} can cause nausea without greater food reduction (le Roux et al., 2008). Similar to GLP-1, long acting PYY_{3-36} analogues may be a more viable treatment option than the endogenous hormone.

1.2.2.3.4. Glucagon-like peptide-1

GLP-1 is an anorexogenic peptide hormone secreted from enteroendocrine L-cells of the gastrointestinal tract following a meal. The two bioactive forms of GLP-1, $GLP\text{-}1_{7\text{-}36\ amide}$ and $GLP\text{-}1_{7\text{-}37}$, are formed by a number of post-translational modifications to its precursor molecule, preproglucagon. $GLP\text{-}1_{7\text{-}36\ amide}$ is thought to be the most biologically active circulating form (Orskov et al., 1994). Both forms are present in the gut, whilst it is $GLP\text{-}1_{7\text{-}36\ amide}$ that is found in the CNS, namely within the AP and NTS of the brainstem in neurons projecting to the PVN and DMN of the hypothalamus (Larsen et al., 1997).

Circulating levels of GLP-1 increase post-prandially (Herrmann et al., 1995), stimulating the release of insulin, delaying gastric emptying and inhibiting food intake and appetite (Drucker, 2006, Holst, 1999). Central or peripheral administration of $GLP\text{-}1_{7\text{-}36\ amide}$ reduces food intake in animal models (Turton et al., 1996). Chronic central administration of $GLP\text{-}1_{7\text{-}36\ amide}$ results in significant weight loss in rodents (Meeran et al., 1999). In humans, peripheral administration of $GLP\text{-}1_{7\text{-}36\ amide}$ reduces food intake (Flint et al., 1998).

Both forms of GLP-1 are potent agonists of the GLP-1 receptor (GLP-1R) (Vahl et al., 2003). There are other agonists of the GLP-1 receptor, including other endogenous peptide hormones such as oxyntomodulin, as well as other peptides such as Exendin-4, which was first isolated from venom of the Gila monster lizard, *Heloderma suspectum* (Goke et al., 1993). Both the anorectic and insulinotropic effect of GLP-1 are thought to be mediated by the GLP-1R. ICV $exendin_{9\text{-}39}$ (an antagonist of the GLP-1R) increases food intake in satiated rats, while ICV co-administration of $GLP\text{-}1_{7\text{-}36\ amide}$ and $Exendin_{9\text{-}39}$, abolishes the anorexigenic effects of $GLP\text{-}1_{7\text{-}36\ amide}$ (Turton et al., 1996). The GLP-1R is highly expressed in a number of hypothalamic nuclei involved in appetite regulation, including the ARC and PVN (Shughrue et al., 1996), and in the brainstem. Both central and peripheral administration of $GLP\text{-}1_{7\text{-}36\ amide}$ induces expression of c-fos (a marker of neuronal activation) in the PVN, but only peripheral administration induces c-fos expression and increases neuronal activity as

measured by manganese-enhanced magnetic resonance imaging in the brainstem (Turton et al., 1996, Baggio et al., 2004b, Parkinson et al., 2009). In addition, the inhibitory effects of peripheral GLP-1 administration on food intake are attenuated by vagotomy or transectioning of the brainstem-hypothalamic pathway (Abbott et al., 2005a). Thus it is possible that peripheral GLP-1 either acts directly or via vagal afferents on the brainstem, which then signals to the hypothalamus. Interestingly GLP-1R knockout mice have normal food intake and body weight, but have disordered glucose metabolism (Gallwitz and Schmidt, 1997, Scrocchi et al., 2000). This suggests the main role for the GLP-1R is in glucose homeostasis rather than energy homeostasis, but may be a reflection of compensatory changes in other appetite-regulating systems.

There is much research into exploiting the appetite-reducing effects of GLP-1 for use as an anti-obesity agent. One of the challenges with using GLP-1 for this purpose is its very short half-life in circulation due to its rapid degradation by circulating DPP-4 (Holst, 2004). However, GLP-1 analogues with substitutions of the penultimate N-terminal amino acid residue may be resistant to DPP-4 (Deacon et al., 1998), and a number of these have been licensed in the treatment of type 2 diabetes and more recently for obesity management. Exenatide and liraglutide are both long-acting GLP-1 analogues that have been used in overweight/obese patients with type 2 diabetes, and a version of Liraglutide is now licensed for the treatment of obesity or overweight in adults with an initial BMI of ≥30kg/m², or ≥27kg/m² to <30kg/m² (overweight) in the presence of at least one weight-related comorbidity such as dysglycaemia (pre-diabetes or type 2 diabetes mellitus), hypertension, dyslipidaemia or obstructive sleep apnoea. Clinical trials have shown significant weight loss with these agents, although the exact mechanism of these effects remains unclear.

Another class of drugs targeting the GLP-1 receptor signalling pathway is the DPP-4 inhibitors. These drugs inhibit the catalytic activity of DPP-4, thus maintaining endogenous GLP-1 levels. They have had a more modest effect on weight loss and glycaemia than the GLP-1 analogues, but do have a role in combination treatment in patients with type 2 diabetes.

1.2.2.3.5. Oxyntomodulin

Oxyntomodulin (OXM) is a 37 amino acid anorexogenic peptide hormone secreted from enteroendocrine L-cells of the gastrointestinal tract following a meal. Like GLP-1, OXM results from post-translational processing of the preproglucagon precursor and, also like GLP-1, it delays gastric emptying and reduces food intake in humans and reduces food intake in rodents (Cohen et al., 2003, Dakin et al., 2004). The mechanisms behind its anorectic effects remain unclear. No specific receptor for OXM has been identified, although it does bind to the glucagon receptor (GCGR) and

the GLP-1R at a lower affinity than native GLP-1 (Fehmann et al., 1994). The effects on food intake are thought to be mediated by the GLP-1R; a GLP-1R antagonist (Exendin$_{9-39}$) blocks the anorectic effects of OXM (Dakin et al., 2001) and GLP-1R knockout mice do not respond to the anorectic effects of OXM (Baggio et al., 2004a). Even though it has a relatively weak affinity for the GLP-1R, OXM has a more potent anorectic effect than GLP-1 at similar doses (Dakin et al., 2004), which suggests there may be additional unknown mechanisms involved in mediating its effect on food intake.

OXM also increases energy expenditure in rodents and humans, (Wynne et al., 2006), an effect thought to be mediated by the GCGR (Pocai et al., 2009). The combined effects on food intake and energy expenditure make OXM an attractive target for obesity therapeutics. Unfortunately, like GLP-1, OXM has a short half-life in circulation due to its degradation by DPP-4, which limits its use as a treatment for obesity, although the development of DPP-4 resistant OXM or long acting analogues of OXM may be a promising approach for the future. Hijacking the mechanisms by which OXM exerts its effects on food intake and energy expenditure may be useful in the development of novel anti-obesity agents.

1.3. **Nutrient sensing**

Nutrient sensing is the ability of a cell within an organism to recognise and respond to the presence of substrates such as glucose, fatty acids and amino acids, usually in an attempt to optimise metabolic function. For example, chemosensory cells are present within the gut epithelium which sense luminal contents. These cells express specific cell-surface receptors that detect the products of nutrient digestion. In order to prevent nutrient excess, the body relies on various nutrient sensors that detect nutrient availability and signal to maintain energy homeostasis. Alterations in nutrient-sensing mechanisms are likely to cause disruption of various homeostatic responses and could contribute to the pathogenesis of metabolic diseases. The hypothalamus and the gastrointestinal tract play an important role in nutrient sensing. Activating specific nutrient sensing receptors within the gut has been shown to stimulate anorectic gut hormone release (Wellendorph et al., 2009a). Nutrient sensing receptors which stimulate endogenous anorectic gut hormone production are thus potential new targets for the treatment of metabolic diseases such as obesity and type 2 diabetes.

1.3.1. Macronutrient diets

One of the most important ways of maintaining energy homeostasis is the control of food intake. Nutritional studies have focused on both reducing energy intake and maintaining a balanced diet in

order to promote weight loss. Despite initial positive results, in the long-term these measures are difficult to adhere to. Most of the strategies designed to aid weight loss in overweight and obese individuals are associated with increased hunger levels and adaptations in energy expenditure. Thus newer dietary intervention programs focus not only on initial weight loss, but also on sustainable long-term weight maintenance and improvement in obesity-related metabolic disturbances (Abete et al., 2010).

Diets with a low glycaemic index have been shown to be effective in longer-term weight maintenance. These diets result in weight loss, improved glycaemia, a reduction in blood pressure and in total and low-density lipoprotein (LDL) cholesterol (Goff et al., 2013, Evans et al., 2017, Juanola-Falgarona et al., 2014). Diets rich in omega-3 fatty acids have been shown to improve markers of cardiovascular health, blood pressure and glycaemia (Due et al., 2008, Balk and Lichtenstein, 2017). However, high protein diets have been shown to be the most effective compared to diets rich in other macronutrients in facilitating weight loss and maintenance (Halton and Hu, 2004).

1.3.2. Central nutrient sensing

Nutrients can signal within the CNS in order to maintain energy homeostasis. The CNS has been suggested to act as a rheostat of acute nutrient availability (Blouet and Schwartz, 2010). The hypothalamus contains a high concentration of nutrient-sensing machinery and is the main centre for the coordination of nutrient-related signals. The ARC-median eminence area is incompletely isolated by the blood-brain barrier and therefore represents an important location of interplay between the periphery and the brain. Subsets of neurons in specific hypothalamic nuclei (such as the ARC, VMN and LHA) can sense nutrients such as glucose, fatty acids, amino acids and other fuel-related stimuli. Within these neuronal populations, nutrients can act as signalling molecules to directly elicit neurochemical responses influencing energy intake and nutrient utilisation (Blouet and Schwartz, 2010).

1.3.3. Peripheral nutrient sensing

The gastrointestinal tract is an important site of peripheral nutrient sensing. The series of factors that signal between the gut and the brain together make up the gut-brain axis (Stanley et al., 2005, Chaudhri et al., 2006, Badman and Flier, 2005). The hypothalamus and the brain stem are the main areas of the brain involved in the regulation of appetite and body weight, although other areas also play a role (Chaudhri et al., 2008a). The brain receives neural and hormonal signals from the gut in

response to food intake. These signals are integrated in the CNS with signals from other organs to coordinate physiological responses.

Intestinal nutrient-sensing mechanisms are highly complex, involving specialised chemosensory cells, the enteric nervous system and the gastrointestinal immune system. The gut responds to multiple signals in the lumen, including nutrient and non-nutrient chemicals, mechanical factors, and microorganisms. Intestinal nutrient sensing plays an important role in digestion, initiating metabolic responses to luminal nutrients. Sensors within the mucosal lining of the gut drive peripheral endocrine and neural signalling to regulate intestinal motility, digestion and absorption, and signal to the CNS to regulate food intake.

Specific nutrients are detected by the gastrointestinal tract to reduce food intake in animal models (Greenberg et al., 1990, Matzinger et al., 2000). There are numerous chemosensory cells within the gut epithelial lining, which are in direct contact with the intraluminal contents. Chemosensitive cells include enterocytes, brush cells and enteroendocrine cells. Enteroendocrine cells play a specialised role in luminal nutrient sensing, despite representing a very small minority (less than 1%) of epithelial cells within the gut (Figure 1.2). Peptide hormones are released from secretory granules located in the basal cytoplasm of this cell type (Hofer et al., 1999). The original one cell-one hormone concept is now no longer accepted; it is thought that a lineage of enteroendocrine cells can co-express members of a group of functionally related peptide hormones (Janssen and Depoortere, 2013, Habib et al., 2012, Egerod et al., 2012). Enteroendocrine cells that have processes (microvilli), which extend to the intestinal lumen where they can directly sense intraluminal contents are known as open-type cells, and directly sense gut contents to modulate the release of hormones that enter circulation and activate extrinsic or intrinsic afferent nerves or other nearby target cells. Those enteroendocrine cells that do not extend to the epithelial surface are controlled indirectly by the luminal content via neural and hormonal signalling, and directly by circulating nutrients absorbed from the gut, are known as closed-type cells. Vagal chemoreceptors sensitive to glucose, amino acids, lipids, pH and osmolality have also been identified, suggesting there is also post-absorptive vagal activation by nutrients absorbed from the gut (Raybould, 1998, Hofer et al., 1999).

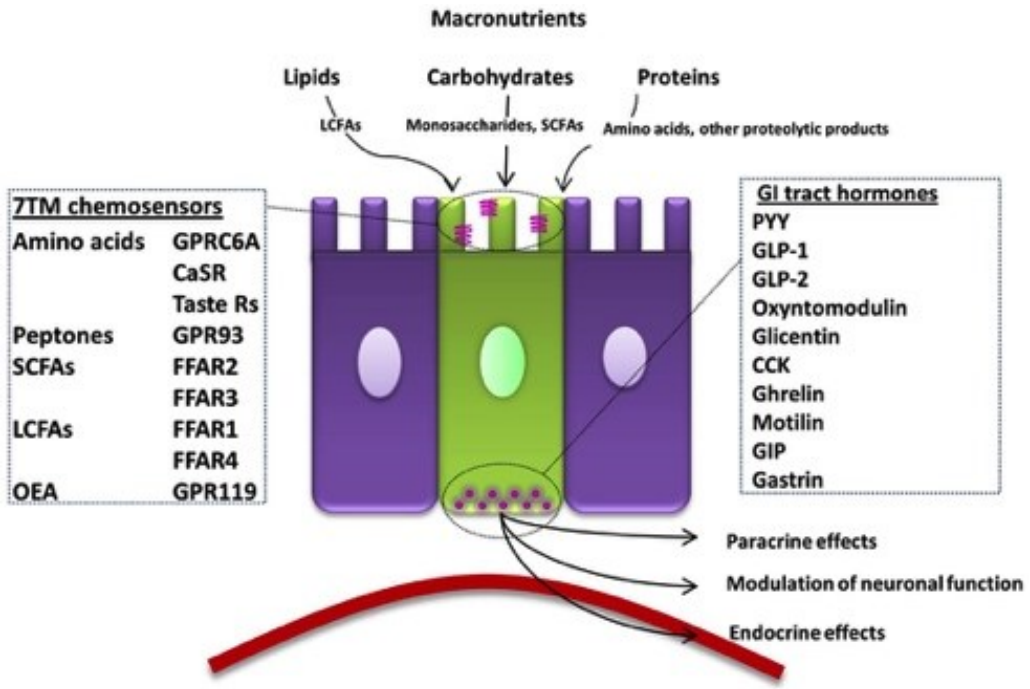


Figure 1.2. The role of the enteroendocrine cell in nutrient sensing. (Spreckley and Murphy, 2015)

1.3.4. Carbohydrate sensing

Over time, there has been an alteration in both the quality and composition of dietary carbohydrates in our food supply (Gross et al., 2004). There is higher consumption of readily digested refined carbohydrates, which has had an effect on obesity-related diseases. Carbohydrates consumed in the diet are digested to release monosaccharides (glucose, fructose, and galactose), which are absorbed by enterocytes in the gut (Wright et al., 2003). The type of sugar and the rate of digestion may have a significant impact on nutrient sensing pathways. Digestion and absorption of ingested carbohydrate is coordinated by sugar sensing in the gastrointestinal tract, which controls nutrient absorption, peptide hormone release and gastrointestinal motility, resulting in the formation of satiation signals to the brain that terminate a meal (Dyer et al., 2003, Raybould, 2007). Glucose is

the most utilized of the monosaccharides. The carbohydrate sensing mechanisms discussed below are summarised in figure 1.3.

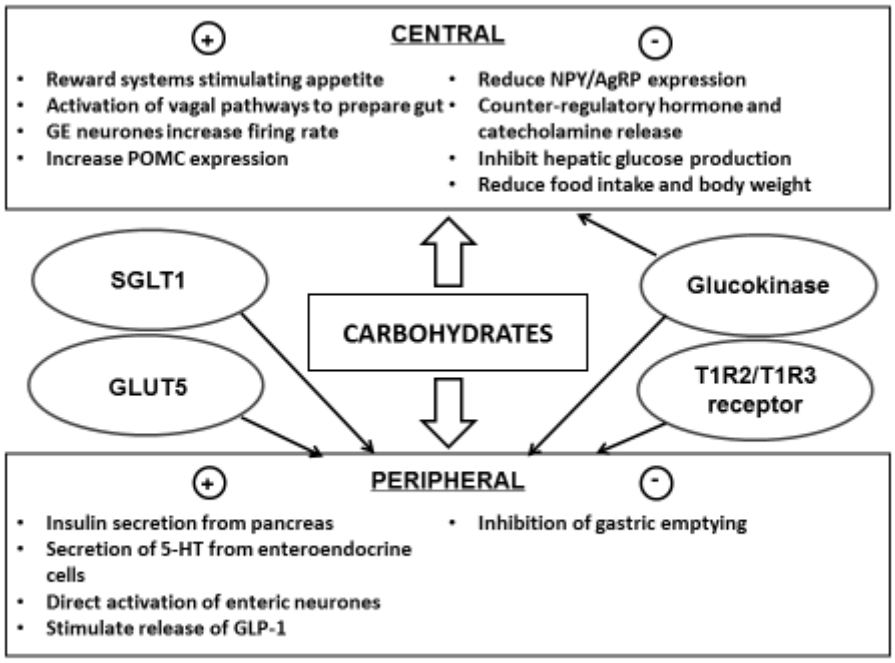


Figure 1.3. Central and peripheral effects of dietary carbohydrates on stimulatory (+) and inhibitory (-) pathways involved in appetite regulation.
Adapted from (Amin and Murphy, 2012)

1.3.4.1. **Central carbohydrate sensing**

Glucose homeostasis is achieved by both signalling to the gut and the pancreas in response to neural and hormonal signals, but also by indirect glial metabolism of glucose to pyruvate resulting in reduced hepatic glucose production (Lam et al., 2009). Sweet taste signaling within the brain activates central reward systems that stimulate sweet appetite (Berthoud, 2002). Brain autonomic centres signal via vagal pathways to prepare the gut for the incoming carbohydrate load (Berthoud, 2002). The CNS, in particular the hypothalamus, is highly sensitive to changes in blood glucose concentrations. The ARC, VMN and PVN contain high numbers of glucose-sensing neurons. There are two major types of glucose-sensing neurons: glucose-excited (GE) neurons, which are activated in response to increases in extracellular glucose concentrations, and glucose-inhibited (GI) neurons, which are activated in response to a reduction in extracellular glucose concentrations (Oomura and Yoshimatsu, 1984). Both types of neurons have been found throughout the brain, with most of the

GE neurons located in the VMN, ARC and PVN, whilst most of the GI neurons are situated in the LHA, the median ARC and PVN (Silver and Erecinska, 1998, Melnick et al., 2011). Within the ARC, increasing concentrations of extracellular glucose inhibit NPY/ AgRP neurons and excite anorectic POMC neurons (Ibrahim et al., 2003, Muroya et al., 1999, Mountjoy et al., 2007, Fioramonti et al., 2007). GE and GI neurons are also found in the brainstem, particularly in the AP, NTS and DMV (Marty et al., 2007). The glucose-sensing elements of the brainstem may then signal observed changes in glucose to the hypothalamus (Marty et al., 2007).

There are numerous effects of central glucose sensing, involving both central and peripheral tissues. Central injection of glucose in rodents inhibits hepatic glucose production, suggesting a role for glucose-sensing neurons in the control of blood glucose levels (Lam et al., 2005a). Infusing glucose directly into the hypothalamus reduces food intake and body weight (Davis et al., 1981, Panksepp and Rossi, 1981), suggesting a possible role in appetite regulation. This process may be mediated by modulation of an intracellular pathway involving 5' adenosine monophosphate-activated protein kinase (AMPK), a key enzyme in the regulation of cellular energy metabolism (Xue and Kahn, 2006).

Animal studies show a possible metabolism-independent mechanism by which GE and GI neurons might respond to local changes in extracellular glucose concentrations, and this involves sweet taste signalling pathways. The Taste 1 Receptor (T1R) receptor class is comprised of three subunits: T1R1, T1R2, and T1R3. Combinations of two of these subunits act as nutrient sensing receptors. The T1R2/T1R3 receptor complex is activated by sweet-tasting compounds and is expressed in the hypothalamus, suggesting a role for this receptor as a central glucose sensor (Ren et al., 2009).

Glucokinase (GCK) (also known as hexokinase IV) catalyses the conversion of glucose to glucose-6-phosphate but it is also thought to act as an intracellular nutrient sensor controlling systemic glucose homeostasis (unlike other hexokinases). This unique property occurs because, unlike the other hexokinases, which have dissociation constants for glucose much below the minimum physiological level of glucose, GCK has a significantly lower affinity and is only active when glucose levels are relatively high (~7 mM and greater) (Efeyan et al., 2015). Thus GCK is only active when glucose is abundant. GCK is expressed in neurons and also within the hypothalamus. Glucokinase activity in the ARC is thought to regulate glucose intake, with glucose being an important driver of overall food intake; this may represent a CNS mechanism for "sweet tooth" and carbohydrate craving (Hussain et al., 2015). The most clearly defined role of neuronal GCK is in the regulation of glucose homeostasis, which appears to be mediated mainly by GCK in the VMH and medial nucleus of the amygdala through modulation of the counterregulatory response (De Backer et al., 2016). The role of GCK in appetite regulation remains poorly understood.

Evidence from humans shows that a centrally detected reduction in blood glucose may cause changes to peripheral glucose management by a number of ways, including secretion of counter-regulatory hormones from the pancreas and release of catecholamines from the adrenal glands (Mitrakou et al., 1991, Taborsky et al., 1998), as well as activation of the autonomic nervous system to drive hepatic glucose production (Perseghin et al., 1997).

Macronutrients have varying effects on the hedonic pathways of food reward. Sugar intake induces specific neurochemical changes in brain areas involved in reward, suggesting a role for carbohydrate sensing in the regulation of hedonic pathways (Levine et al., 2003).

1.3.4.2. **Peripheral carbohydrate sensing**

Peripheral organs including the pancreas, gut and liver can sense sugars. The majority use glucose transporters (GLUTs) to absorb glucose from the blood. The sodium glucose co-transporter 1 (SGLT1) is involved in the absorption of glucose and galactose in the gastrointestinal tract, whereas fructose is passively absorbed by a member of the facilitative GLUT family called GLUT5 (Burant et al., 1992). The increasing consumption of fructose and sucrose has been suggested to be more associated with weight gain than consumption of glucose (Bray et al., 2004), and ingestion of these sugars is also linked to increases in circulating triglycerides, systolic blood pressure and insulin resistance (Johnson et al., 2007).

Within the gut, glucose acts as a signalling molecule to initiate a number of regulatory mechanisms. It stimulates the secretion of 5-hydroxytryptamine (5-HT) from enteroendocrine cells, which activates enteric neuronal activity. Glucose can also act directly on these neurons (Pfannkuche and Gabel, 2009).

GCK is also thought to play a role in peripheral glucose sensing. It is thought to control systemic glucose fate via effects on the liver and pancreas. The liver maintains glycaemia through gluconeogenesis and glycogen breakdown when glucose levels are low, or by storing glucose in the form of glycogen when glucose supply is plentiful. GCK is the most abundant hexokinase in the liver and because it is inactive when glucose is scarce, it allows export of un-phosphorylated glucose from the liver in order to supply the demands of the central nervous system and muscles (Efeyan et al., 2015). When hepatic glucose levels are high, GCK-mediated conversion of glucose to the metabolic intermediate glucose-6-phosphate allows its shunting into glycolysis or glycogen synthesis. GCK is also expressed in beta cells, where it acts as a glucose sensor (Matschinsky, 2002).

GLP-1 is released by L-cells following food ingestion. It has a potent incretin effect in both animals and humans, and inhibits food intake and appetite (Drucker, 2006). It is released in a biphasic manner with an early and late phase response; the early phase generally occurs within 30 minutes of food ingestion (Brubaker and Anini, 2003, Schirra et al., 1996, Herrmann et al., 1995). The majority of L-cells are located in the distal intestine. Nutrients are unlikely to reach these L-cells within the early phase, which suggests other factors regulate the secretion of GLP-1 from L-cells in the distal intestine. The early phase is thought to be mediated indirectly through other gastrointestinal neural and hormonal pathways, whilst the late phase reflects direct sensing of the intraluminal contents by L-cells (Roberge and Brubaker, 1993). Hormones secreted from the proximal small intestine, such as CCK or gastric inhibitory polypeptide (GIP), may stimulate L-cells (Beglinger et al., 2010, Brubaker and Anini, 2003). Neural pathways are also thought to play a role in GLP-1 release (Brubaker and Anini, 2003, Anini et al., 1999, Anini and Brubaker, 2003). Intraduodenal infusion of nutrients into humans stimulates the release of GLP-1 by activation of afferent vagal pathways to the brain (Lavin et al., 1998).

Naturally occurring sweet substances such as glucose, fructose, sucrose, sweet tasting D-amino acids and synthetic sweeteners such as aspartame and saccharin have been shown to be sensed by the sweet taste receptor T1R2/T1R3 in animals (Nelson et al., 2001, Li et al., 2002). T1R2/T1R3 is expressed in taste buds of the oral cavity and in the gut of humans and animals (Nelson et al., 2001, Dyer et al., 2005, Bezencon et al., 2007, Mace et al., 2009). The gut is thus capable of sensing sweet stimuli, potentially mediated by receptors such as the T1R2/T1R3. Isolated islets from T1R3 knockout mice show delayed insulin secretion in response to glucose (Geraedts et al., 2012). Dietary sugars are thought to act via gastrointestinal taste sensors to regulate glucose absorption and the release of GLP-1 in rodents (Le Gall et al., 2007, Mace et al., 2007, Margolskee et al., 2007, Jang et al., 2007b). The early-phase release of GLP-1 is thought to be due to direct stimulation of L cells by luminal glucose in the proximal intestine (Jang et al., 2007a, Kokrashvili et al., 2009). The mechanism behind the late phase release is likely to be related to direct signalling; enteroendocrine cells are glucose sensitive *in vitro* (Abello et al., 1994), and proximal enteroendocrine cells in animals express chemosensory receptors such as the sweet taste receptors which sense glucose, and the molecules required for the transduction of taste signalling such as α-gustducin (Jang et al., 2007a). The duration of the GLP-1 profile is dependent on the stimulating nutrient. The glucose-stimulated GLP-1 profile is more rapid and shorter in duration (within 15-30 minutes of ingestion) than the lipid-stimulated profile (from 60-180 minutes following ingestion) in man (Elliott et al., 1993). The mechanisms underlying these secretion patterns remain unclear, but may be related to relative

sensitivities of the sensing mechanisms and the time taken for gut transit times and absorption of different nutrients.

1.3.5. Lipid sensing

Following ingestion, fat is digested to form fatty acids and monoglycerides. The length of the hydrocarbon chain in a fatty acid determines whether it is described as short (tails of <6 carbons), medium (6-12 carbons) or long (13-21 carbons). Short-chain fatty acids (SCFAs) and medium-chain fatty acids (MCFAs) enter the portal circulation directly through intestinal capillaries, but long-chain fatty acids (LCFAs) enter the systemic circulation via the lymphatic system, bypassing the portal circulation, and thus their measurement may be more representative of acute lipid intake.

Free fatty acids (FFAs) have a number of physiological effects, mostly mediated by their intracellular metabolic effects. Several cell surface receptors activated by free fatty acids, belonging to the seven transmembrane domain G-protein coupled receptor family A, have been recently characterised; these may represent an alternative mechanism of action for FFAs (Wellendorph et al., 2009a). Fatty acids acting on these cell surface receptors are thought to be involved in appetite regulation. These receptors have been identified in a number of tissues, including the brain and the gastrointestinal tract (Briscoe et al., 2003, Ma et al., 2007), with each receptor having a varied tissue expression pattern. Free Fatty Acid Receptor 1 (FFAR1) and G-protein receptor 120 (GPR120) are activated by MCFAs and LCFAs whilst Free Fatty Acid Receptor 2 (FFAR2) and Free Fatty Acid Receptor 3 (FFAR3) are activated by SCFAs (Hara et al., 2013). The lipid sensing mechanisms discussed below are summarised in figure 1.4.

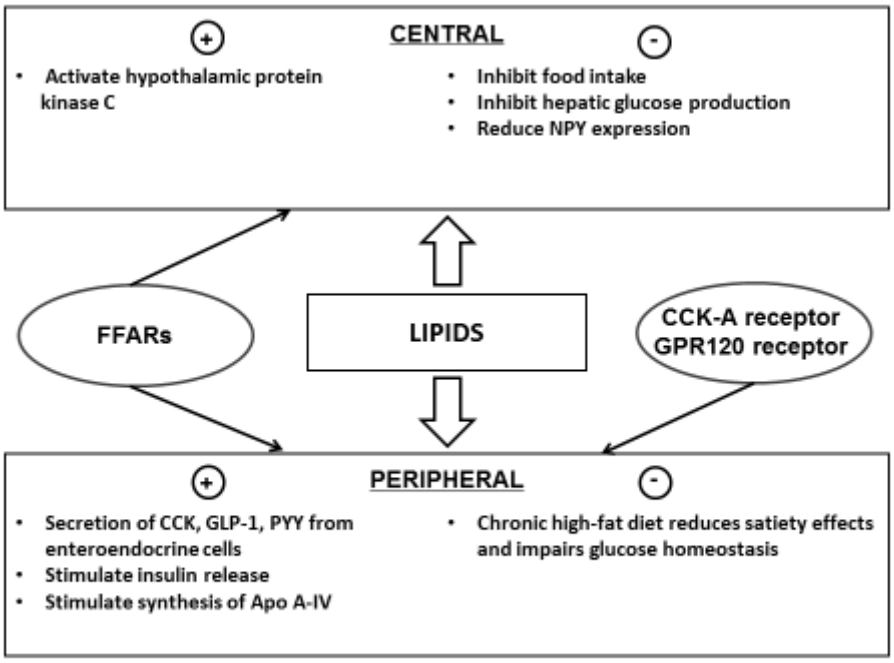


Figure 1.4. Central and peripheral effects of dietary lipids on stimulatory (+) and inhibitory (-) pathways involved in appetite regulation.
Adapted from (Amin and Murphy, 2012)

1.3.5.1. **Central lipid sensing**

The exact role of the receptors that respond to free fatty acids in brain lipid sensing remains unclear. Increasing the concentration of LCFAs within the brain, in particular within the hypothalamus, inhibits food intake and hepatic glucose production (Lam et al., 2005c, Caspi et al., 2007). Central administration of LCFAs decreases food intake and hypothalamic NPY expression in rats (Obici et al., 2002). Circulating LCFAs are taken up by the brain and esterified by acyl-CoA (acyl-Coenzyme A) synthetase to form LCFA-CoAs (Kamp et al., 2003). LCFA-CoAs regulate brain phospholipids and provide the substrate for brain fatty acid β-oxidation. One of the key enzymes involved in fatty acid metabolism is malonyl-CoA, which inhibits β-oxidation which in turn increases cellular concentrations of LCFA-CoA (McGarry, 2002). Animal models overexpressing malonyl-CoA decarboxylase (an enzyme involved in the degradation of malonyl-CoA) have low central levels of malonyl-CoA and are hyperphagic, suggesting malonyl-CoA may mediate the effects of LCFAs on appetite (He et al., 2006). The metabolism of fatty acids within the hypothalamus can thus influence feeding behaviour, suggesting a role for central nutrient sensing of fatty acids in energy homeostasis.

The protein kinase C (PKC) family of enzymes transduces cellular signals that promote lipid hydrolysis. Circulating PKC isoforms are activated by lipid-derived metabolites, reducing insulin

action in the liver and muscle (Lam et al., 2002), and impairing the actions of insulin and leptin in the brain (Benoit et al., 2009). Adeno-associated virus (AAV) knockdown of the theta isoform of PKC in the NPY/AgRP neurons of the ARC or in the DMN sensitises the hypothalamus to the actions of insulin and prevents mice from developing diet-induced obesity and abnormal glucose tolerance (Benoit et al., 2009). Hypothalamic PKC is also thought to play a role in peripheral glucose homeostasis (Ross et al., 2008, Morgan et al., 2004). Thus, genetic manipulation of PKC can have profound effects on energy homeostasis and metabolism. Hypothalamic lipid sensing in rodents regulates hepatic glucose production via efferent vagal pathways (Pocai et al., 2005). Peripheral lipid administration results in an increase in hypothalamic lipid concentration ultimately lowering glucose production (Lam et al., 2005b). In mice fed a high-fat diet, the ability of hypothalamic administration of specific LCFAs to lower hepatic glucose production is impaired (Morgan et al., 2004). This is thought partly to be related to the inability of LCFAs to increase cellular levels of LCFA-CoAs in the hypothalamus (Pocai et al., 2006). Carnitine palmitoyl transferase 1 (CPT1) is a mitochondrial outer membrane transporter involved in the β-oxidation of fatty acids, which transports LCFA-CoA into the mitochondria. Rats fed a high-fat diet have an increase in hypothalamic CPT1 activity following the activation of PKC, suggesting a role for CPT1 in central nutrient sensing. AMPK is also thought to be involved in the regulation of fatty acid metabolism in the brain. The synthesis of malonyl-CoA is regulated by acetyl CoA carboxylase (ACC), the activity of which is regulated by AMPK. Hypothalamic AMPK thus regulates fatty acid metabolism in the brain by reducing levels of malonyl-CoA and LCFA-CoA, resulting in effects on energy and glucose homeostasis (Kahn et al., 2005). In addition, hypothalamic AMPK may mediate the effect of glucose and gut hormones on the control of food intake (Minokoshi et al., 2004), though the mechanisms by which AMPK coordinates different nutrient signals remain poorly understood.

Factors involved in fatty acid metabolism are thought to be linked to the action of appetite signals within the hypothalamus (Minokoshi et al., 2004, Lopez et al., 2008). In addition, high-fat diets influence opioid and dopaminergic signaling in brain regions associated with food reward (Kelley et al., 2002, Pelchat et al., 2004).

1.3.5.2. **Peripheral lipid sensing**

Ingested triglycerides are metabolised to fatty acids and monoacylglycerol by pancreatic lipase. The mechanisms by which lipid is sensed in the gut are poorly characterised, but there are several proposed mechanisms. The fatty acid receptors may play a role in gut lipid sensing. High levels of GPR120 are expressed in both the human and mouse gastrointestinal tract and in mouse enteroendocrine cells (Hirasawa et al., 2005, Miyauchi et al., 2009). The presence of lipids in the gut

results in the release or suppression of specific gut hormones. LCFAs within the gastrointestinal tract result in the release of CCK from duodenal I-cells (Moran and Kinzig, 2004). This likely reflects direct sensing by enteroendocrine cells. Mouse enteroendocrine cells release CCK following incubation with free fatty acids (Sidhu et al., 2000). LCFAs also stimulate the release of PYY and GLP-1 from human enteroendocrine cells (Stoeckel et al., 2008). GPR120 may stimulate the late phase of gut hormone release mentioned previously in response to dietary lipid (Hirasawa et al., 2005). Oral or direct gut administration of FFAs increases circulating GLP-1 and insulin levels in animal models, an effect which is thought to be mediated by GPR120 (Hirasawa et al., 2005). The effects of LCFAs on satiation are reduced in animals fed on high-fat diets for greater than 2 weeks (Covasa et al., 2000). Why this occurs remains poorly understood, although it may relate to the adaptation of digestive processes or a change in the sensitivity of the lipid-sensing mechanisms mediating the satiating effects of lipids. However, the regulation of glucose homeostasis is also impaired in rats after just three days on a high-fat diet (Wang et al., 2008), a phenomenon also observed in humans (Lovejoy et al., 2001).

Short chain fatty acids (SCFAs) are the major fermentation product of fibre, and are known to influence appetite in humans and animals (Hamer et al., 2008). SCFAs stimulate the FFAR2 and FFAR3 *in vitro* (Brown et al., 2003, Le Poul et al., 2003, Nilsson et al., 2003). Both FFAR2 and FFAR3 have been localised to enteroendocrine cells (Karaki et al., 2006), where their exact roles are yet to be determined. Many of the beneficial metabolic effects of prebiotics can be linked to their digestion and the resulting SCFA production by gut microbiota. The gut microbiota is increasingly thought to play an important role in the development of obesity (Ley et al., 2006, Turnbaugh et al., 2006). The SCFAs sensed in the gut are acetate, proprionate and butyrate. The highest concentration of L-cells is found in the large intestine where SCFAs can be found at relatively high concentration (Eissele et al., 1992). It is thus possible that gut hormone release may be a vital host response to microbial fermentation and the subsequent production of SCFAs. Acetate and propionate both stimulate the release of GLP-1 *in vitro*, an effect attenuated by deletion of FFAR2 (Tolhurst et al., 2012). Intra-colonic administration of propionate stimulates the concurrent release of both GLP-1 and PYY in rats and mice (Psichas et al., 2015). Studies using mice lacking FFAR2 show that inulin (a fermentable fibre and good source of SCFAs) acts via FFAR2 to drive an increase in PYY producing cells, reduce food intake, and prevent diet-induced obesity (Brooks et al., 2017).

1.3.6. Protein sensing

Protein has a satiating effect greater than that of other macronutrients (Porrini et al., 1997, Reid and Hetherington, 1997, Trigazis et al., 1997, Bensaid et al., 2002, Anderson and Moore, 2004, Halton

and Hu, 2004). High protein diets reduce food intake, facilitate weight loss and improve body composition in animals and humans (Potier et al., 2009, Harper and Peters, 1989, Jean et al., 2001, Morens et al., 2000, Morens et al., 2001, Hannah et al., 1990, Kinzig et al., 2007). The protein sensing mechanisms discussed below are summarised in figure 1.5.

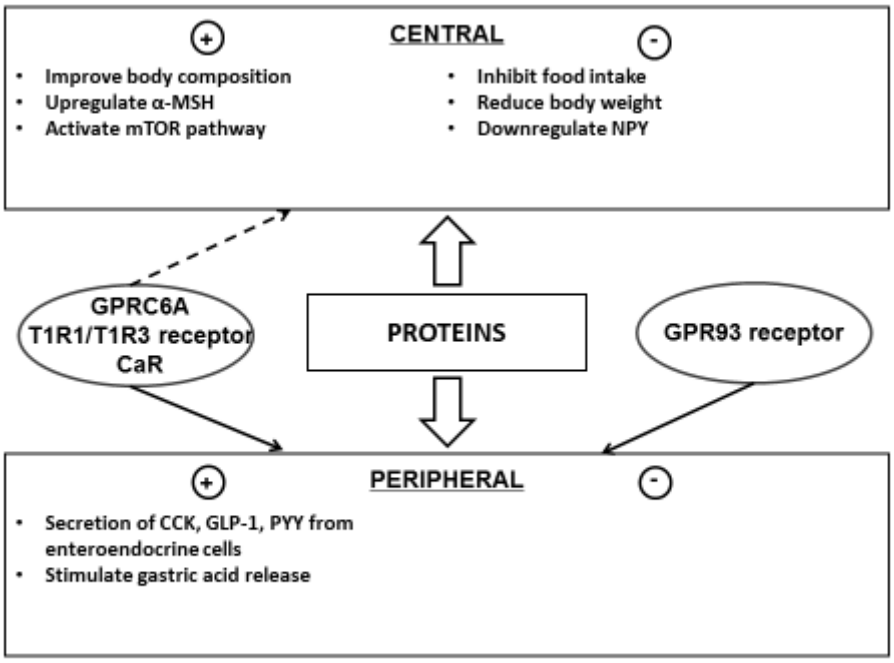


Figure 1.5. Central and peripheral effects of dietary protein on stimulatory (+) and inhibitory (-) pathways involved in appetite regulation.
Adapted from (Amin and Murphy, 2012)

1.3.6.1. **Central protein sensing**

Amino acids can access the CNS through areas with an incomplete blood-brain barrier such as the AP in the brainstem. Specific amino acids are precursors for neurotransmitters. Tryptophan and tyrosine are the precursor molecules for the synthesis of serotonin and dopamine respectively. Histidine is also a precursor molecule for histamine synthesis (Fromentin et al., 2012). These neurotransmitter products have all been implicated in appetite regulation (Volkow et al., 2011, Bassil et al., 2007, Goto et al., 2007). Glutamate and aspartate are excitatory amino acids that stimulate the N-methyl-D-aspartate receptor. Glycine also has partial activity at this receptor (Fromentin et al., 2012).

High protein diets in animals are associated with the upregulation of hypothalamic α-melanocyte-stimulating hormone (α-MSH), a peptide product of POMC with inhibitory effects on appetite, and

with the downregulation of hypothalamic NPY. Amino acids such as alanine, arginine, leucine, lysine, serine, tryptophan, valine, and monosodium glutamate stimulate afferent sympathetic and parasympathetic pathways in the NTS and stimulate efferent neural pathways to the gut and liver in animal models (Tome et al., 2009), which may represent a mechanism by which amino acids can regulate food intake and metabolism.

The mammalian Target of Rapamycin (mTOR) protein is a serine-threonine kinase, which controls cell-cycle progression and growth by detecting changes in energy status. Central amino acid sensing is thought to regulate energy homeostasis by acting on mTOR (Cota et al., 2006) (also refer to Section 1.3.9.1). Leucine is one of the three branched-chain amino acids, which may act as a physiological signal of hypothalamic amino acid availability. Leucine appears to be a signal of amino acid excess, regulating processes such as protein synthesis (Nair et al., 1992), gene transcription and translation (Kimball and Jefferson, 2006) and insulin secretion (Henquin et al., 2006). Ingested leucine mostly bypasses first-pass metabolism, (Brosnan and Brosnan, 2006), thus reaching the central circulation more efficiently than other amino acids in rodents and resulting in a rapid rise in plasma leucine levels after a meal (Kanamori et al., 1998). Leucine is also the most potent activator of the mTOR pathway (Proud, 2004). Central administration of low doses of leucine in rats activates the signalling pathway and reduces food intake and body weight (Cota et al., 2006). This effect is blocked by co-administration of an mTOR inhibitor such as rapamycin (Cota et al., 2006).

Various G-protein coupled receptors respond promiscuously to amino acids. These receptors, discussed in greater detail in section 1.3.7 below, may sense the amino acids produced from the breakdown of dietary protein to regulate energy homeostasis. The exact role of these receptors in the brain remains poorly understood.

Dietary protein may also play a role in food-related reward, but the evidence mostly comes from animal studies rather than from human studies (Peuhkuri et al., 2011). A number of postprandial mechanisms have been proposed, including both neural and endocrine signals from the gut to the brain. In order to stimulate reward pathways, proteins have to be absorbed through the gut and reach target cells in adequate concentrations, or act via luminal receptors in the gastrointestinal tract. The complex mechanisms behind protein-related reward remain unclear, although the anterior piriform complex (APC) of the brain is thought to be important in adaptive eating behaviour related to amino acid availability with the general control nonrepressed 2 (GCN2) protein being an important regulator in sensing changes in amino acid availability (Section 1.3.9.3).

1.3.6.2. **Peripheral protein sensing**

There is evidence that dietary protein influences gastrointestinal hormones to alter satiety. Animal and human studies suggest that dietary protein stimulates anorectic gut hormone release to a greater degree than other macronutrients (Hall et al., 2003, Calbet and Holst, 2004, Nilsson et al., 2004, Batterham et al., 2006b). A high protein meal increases circulating concentrations of both PYY and GLP-1 in normal weight humans (van der Klaauw et al., 2012). High protein meals are associated with significantly higher PYY responses in both lean and obese humans when compared to high-fat and high-carbohydrate diets (Batterham et al., 2006b).

It is possible that rather than sensing protein directly, the gastrointestinal tract senses the amino acid products of protein digestion (Section 1.3.7). However, G-protein receptor 93 (GPR93) is a family A G-protein receptor which responds to peptone, a mixture of peptides similar to gut proteolytic degradation products. This receptor is highly expressed within the small intestine (Choi et al., 2007a) and is involved in protein hydrolysate-mediated rises in CCK expression in, and release from, intestinal cells (Nemoz-Gaillard et al., 1998, Nishi et al., 2001). *In* vitro work suggests peptone stimulation of GPR93 in enteroendocrine cells activates G-protein signalling cascades, which promote CCK gene transcription and CCK release (Choi et al., 2007a, Choi et al., 2007b). However, the exact ligand or ligands stimulating this receptor are currently unknown.

1.3.7. Amino acid sensing receptors

There are three members of the G-protein coupled receptor (GPCR) family class C known to promiscuously bind a number of L-amino acid ligands, and thus suited to detecting the varied products of protein digestion. These receptors are the calcium sensing receptor (CaSR), the T1R1/T1R3 heterodimeric receptor and the G-protein coupled receptor group C member 6A (GPRC6A) receptor (Figure 1.6). The Class C receptors have large extracellular domains consisting of an N- terminal Venus Flytrap (VFT) domain that contains a dimerization interface and an orthosteric binding site for the endogenous agonist. VFTs have a cysteine rich domain (CRD), which connects the VFT to the seven transmembrane (7TM) domain, and can bind neurotransmitters or nutrient molecules such as amino acids. The 7TM domain is coupled to a G-protein via a C- terminal intracellular signalling domain (Smajilovic et al., 2014).

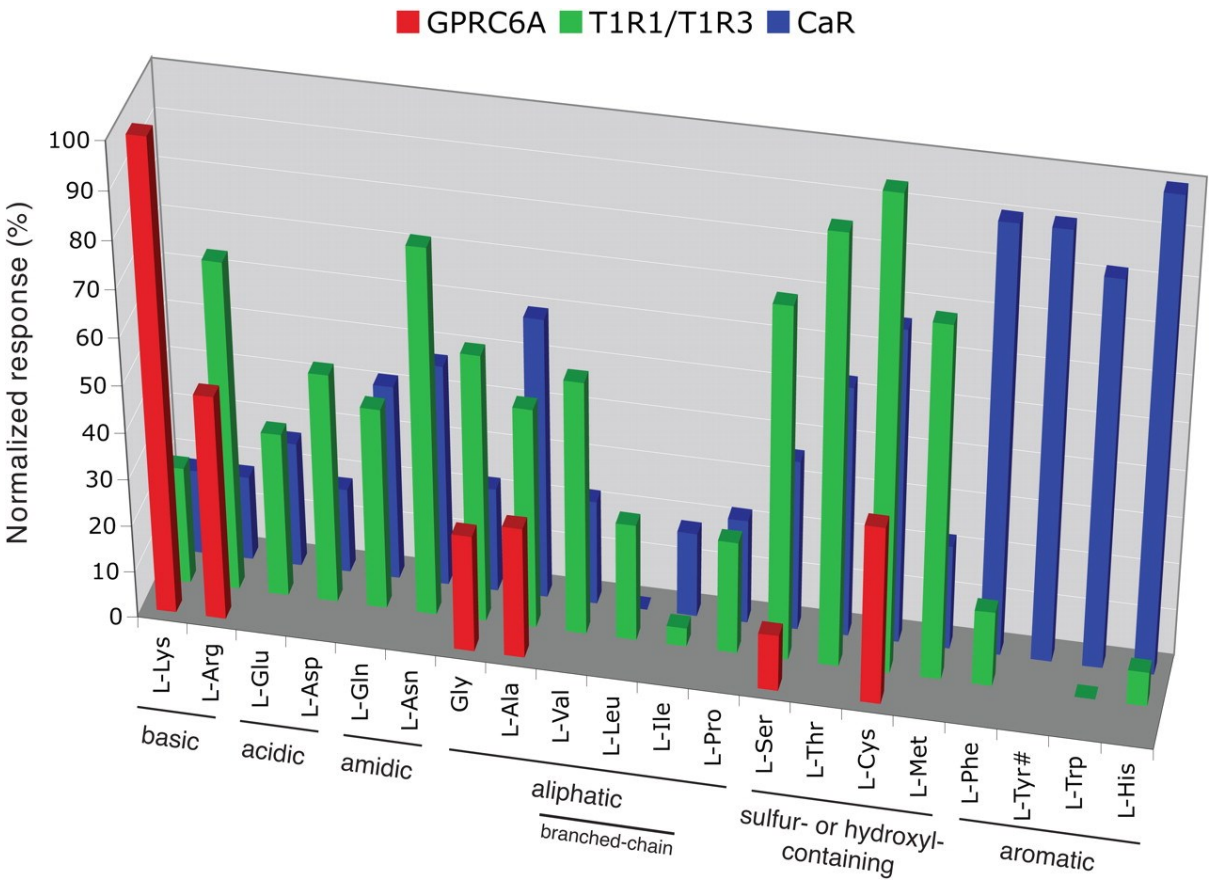


Figure 1.6. **L-amino acid selectivity for L-specific amino acid receptors CaSR, GPRC6A and T1R1-T1R3.**
Effect of the 20 proteinogenic amino acids at 10mM on the mT1R1/T1R3 response in the presence of 2.5mM IMP expressed as a percentage normalized to L-cysteine response. CaSR percentage response is normalised to L-histidine. mGPRC6A values are based on EC50 values of the amino acids in the presence of 1mM Ca2+ and 1mM Mg2+ relative to L- lysine (Wellendorph et al., 2009a).

1.3.7.1. **The calcium sensing receptor**

The CaSR is expressed in organs involved in calcium homeostasis and in other tissues including the stomach, the lower gastrointestinal tract, the liver and the pancreas (Ray et al., 1997, Sheinin et al., 2000, Canaff et al., 2001, Squires et al., 2000, Bandyopadhyay et al., 2010). It has a major role in calcium homeostasis, and is also involved in the regulation of processes including cell proliferation and differentiation, gene expression, ion channel function, gastric acid secretion, hepatic bile secretion, digestive enzyme release and glucagon and insulin release (Dufner et al., 2005, Canaff et al., 2001, Squires et al., 2000, Bruce et al., 1999, Rasschaert and Malaisse, 1999) . The CaSR is also expressed in the taste tissue of the tongue, where it may mediate the sensing of the taste of calcium and amino acids (San Gabriel et al., 2009). At physiological levels of Ca^{2+}, the CaSR responds to pH, other divalent cations, L-amino acids (aromatic>aliphatic> polar) and di- and tri-peptides, in an

allosteric manner (Brown and MacLeod, 2001, Conigrave et al., 2002, Conigrave et al., 2000). The receptor is usually expressed at the cell surface as a homodimer (Bai et al., 1998) and is thought to signal mainly through $G\alpha_q$ but also through $G\alpha_{i/o}$ or $G\alpha_s$ in a cell-specific manner (Chen et al., 1989, Chang et al., 1998, Handlogten et al., 2001).

The CaSR is highly expressed in the gastrointestinal tract, where it has been suggested it may play a role in energy homeostasis. Within the gut, it is found in enteroendocrine, gastrin-producing G-cells (Ray et al., 1997), CCK-producing I-cells (Liou et al., 2011), somatostatin-producing D-cells (Haid et al., 2012) and GLP-1-producing L-cells (Diakogiannaki et al., 2013). The expression of the CaSR on hormone releasing enteroendocrine cells suggests involvement of this receptor in the release of gut hormones from these cells. The CaSR has also been shown to respond to L-amino acids and is especially sensitive to aromatic amino acids such as L-tryptophan and L-phenylalanine (Conigrave et al., 2000). Whereas other G-protein family C receptors are directly activated by amino acids and are positively modulated by Ca^{2+}, the CaSR is directly activated by Ca^{2+} and is positively modulated by amino acids (Conigrave et al., 2000). The CaSR mediates CCK release from duodenal enteroendocrine cell lines and isolated CCK-cells (Hira et al., 2008, Wang et al., 2011). L-phenylalanine, L-tryptophan, L-asparagine, L-arginine and L-glutamine have also been shown to stimulate GIP, GLP-1 and PYY secretion from isolated rat small intestine through a CaSR-dependent mechanism (Mace et al., 2012), and L-phenylalanine, L-tryptophan and L-leucine stimulate secretion of gastric acid from *ex vivo* stomach parietal cells via allosteric modulation of the CaSR (Busque et al., 2005). Collectively these studies identify the CaSR as a possible mechanism for amino acid sensing in the gut that may be involved in appetite regulation. *In vivo* studies are necessary to determine the physiological role of the CaSR in gut hormone release and appetite regulation. However, the CaSR knockout mouse has a severe phenotype, which includes severely dysfunctional calcium homeostasis, retarded growth and premature death (Ho et al., 1995), thus making it difficult to determine whether the CaSR has a physiological role in appetite.

1.3.7.2. **The T1R1/T1R3 receptor**

There are three known taste receptor subunits: T1R1, T1R2 and T1R3. The T1R receptors act as heterodimers, forming receptor complexes that respond to sweet, bitter or umami tastants. The T1R2/T1R3 receptor acts as a sweet taste receptor and is activated by a number of different sweet molecules. The T1R1/T1R3 receptor is thought of as the umami taste receptor, which is activated by monosodium glutamate (especially the human receptor) as well as a range of other L-amino acids (Wellendorph et al., 2010). The T1R1/T1R3 receptor responds to L-amino acids including cysteine, alanine, glutamine, serine, methionine, asparagine, glycine and threonine (Nelson et al., 2002).

Additionally the T1R3 subunit is thought to be involved in calcium sensing and calcium taste (Tordoff et al., 2012).

The T1R1/T1R3 receptor signals via a transient receptor potential cation channel subfamily M member 5 (TRPM5)-dependent mechanism through the heterotrimer G-protein gustducin (Zhang et al., 2003b, Ruiz et al., 2003). There are differences in T1R1/T1R3 receptor agonist selectivity between species, which is probably a result of the relatively low homology (approximately 70%) between human and rodent T1R receptors: L-glutamate is the most potent agonist at the hT1R1/T1R3 receptor, while L-cysteine is the most potent at the mT1R1/T1R3 (Li et al., 2002, Nelson et al., 2002). Interestingly, the mouse T1R1/T1R3 receptor appears more promiscuous, responding to a greater number of L-amino acids than the human receptor (Li et al., 2002, Nelson et al., 2002). It is known that T1R1 knockout mice show complete loss in preference for umami tastants (Zhao et al., 2003), which include the L-amino acids known to activate the T1R1/T1R3 receptor.

The T1R1/T1R3 heterodimer was initially identified in taste tissue in the tongue, but was subsequently found to be expressed in a number of other tissues including the brain, skeletal muscle, pancreas and gut, with higher expression in the distal gut (Dyer et al., 2005, Bezencon et al., 2007, Wauson et al., 2012). The exact role of the receptor in non-taste tissue remains unclear. Interestingly, *in vitro* work has shown that many of the same amino acids that activate the T1R1/T1R3 receptor also stimulate GLP-1 release (Reimann et al., 2004), and thus it is possible this receptor is involved in the regulation of appetite and glucose via GLP-1. The T1R1/T1R3 receptor is thought to regulate CCK secretion in the immortalized intestinal enteroendocrine STC-1 cell line, with T1R1 knockdown attenuating the stimulatory effect of L-phenylalanine, L-leucine and L-glutamate on CCK release; positive and negative allosteric modulators of the receptor also modulated CCK secretion in response to these amino acids from mouse small intestine tissue explants (Daly et al., 2013). The T1R1/T1R3 receptor is thought to play a role in L-glutamate and L-arginine-induced insulin release from the MIN-6 cells (an immortalized mouse pancreatic beta-cell line) (Oya et al., 2011). The suggestion is that amino acids signal via the T1R1/T1R3 to activate extracellular signal-regulated kinases (ERK1/2) and Mammalian target of rapamycin complex 1 (mTORC1), initiating mRNA translation via mechanisms dependent on extracellular amino acid availability, and that this signalling cascade ultimately results in cell growth, autophagy and apoptosis (Wauson et al., 2012).

1.3.7.3. **The GPRC6A receptor**

The human GPRC6A receptor was first cloned in 2004 (Wellendorph and Brauner-Osborne, 2004) although its function at the time was unknown. It was later identified as receptor for L-amino acids (Wellendorph et al., 2005). The mouse and the rat receptors were subsequently cloned and also characterised as L-amino acid receptors (Wellendorph et al., 2005). There are three GPRC6A isoforms. Isoform 1 has the longest structural sequence, is the most abundant and is expressed in a number of different tissues. Isoform 2 is mainly expressed in the kidney, and isoform 3 is expressed at much lower levels than isoforms 1 and 2, and is primarily in the brain, kidney and skeletal muscle (Wellendorph and Brauner-Osborne, 2004). The GPRC6A receptor is expressed in a number of different tissues including the gut, brain, pancreas and kidney, suggesting a wide range of physiological roles (Wellendorph and Brauner-Osborne, 2004), although the exact nature of these roles remains unclear. The mouse and the human receptor have 80% homology, but only one rat isoform has been identified to date (Wellendorph et al., 2007). The GPRC6A receptor preferentially binds basic amino acids and, to a lesser extent, aliphatic amino acids. L-ornithine, a non-proteinogenic amino acid, is the most potent amino acid agonist of the mGPRC6A and rGPRC6A, whereas L-arginine is the most potent agonist of the hGPRC6A (Pi et al., 2005). Agonists and allosteric modulators of the GPRC6A include calcium and other divalent cations, calcimimetics, osteocalcin and L-amino acids (Pi et al., 2005). The GPRC6A signals by coupling to Gαq and subsequently activating phospholipase C (Kuang et al., 2005).

Three knockout models have been created to further investigate the role of the GPRC6a. One knockout was created by selective deletion of exon 2 ($GPRC6A_{exon2(-/-)}$), which encodes the VFT domain of the receptor (Pi et al., 2008) and had a number of metabolic and reproductive abnormalities compared to the wild-type (WT) mice, including defective osteoblast-mediated bone mineralization, abnormal renal handling of calcium and phosphorus, fatty liver, glucose intolerance and disordered steroidogenesis (Pi et al., 2008). This model also suggested a role for the GPRC6A in L-arginine stimulated insulin release from primary cultured beta cells (Pi et al., 2012). The second knockout model was created by selective deletion of exon 6 ($GPRC6A_{exon6(-/-)}$) which encodes the entire 7TM and C-terminal domains (Wellendorph et al., 2009b). Unlike the first knockout model, this model has been reported to have normal bone mineralisation and normal L-arginine-induced insulin secretion (Smajilovic et al., 2013). Further studies into the $GPRC6A_{exon6(-/-)}$ knockout model show these mice are more susceptible to diet-induced obesity, have higher fat mass, lower lean mass and decreased insulin sensitivity when placed on a high fat diet (Clemmensen et al., 2013a), similar to the metabolic phenotype seen with the $GPRC6A_{exon2(-/-)}$ model. The reasons for the

discrepancies between these knockout models are unclear. It may be that the 7TM region is not necessary for all the activities of the receptor and that the GPRC6A functions as a heterodimer (Wellendorph et al., 2005). The most recent knockout model featured an entire deletion of the GPRC6A locus with no resulting metabolic phenotype. Acute and chronic high protein diet suppressed food intake and body weight in these animals similar to WT animals, suggesting GPRC6A does not mediate protein-induced satiety (Kinsey-Jones et al., 2015). *In vitro* work has suggested a role for the GPRC6A receptor in L-ornithine-induced GLP-1 secretion from GLUTag cells, an immortalized murine cell line that expresses the proglucagon gene and secretes glucagon-like peptides (Oya et al., 2013). However, these effects were not replicated in primary murine small intestinal cultures, casting doubt on their physiological relevance.

1.3.8. Amino acid transporters

Amino acid transporters facilitate the absorption of amino acids from the gut lumen and their transfer to the bloodstream, and the subsequent supply of amino acids to tissues. These transporters are present on both the apical and basolateral membranes of gastrointestinal epithelial cells. They are organized into families that form part of the solute-linked carrier (SLC) group of membrane transport proteins. These transporters are promiscuous, with each system transporting a variety of different amino acids. Despite their promiscuity, specific transporters are known to have physiological relevance, as a defective system can be associated with particular diseases and disorders (Broer, 2008). Recent evidence suggests that in addition to their transport role, amino acid transporters may also have receptor like functions, allowing cells to sense changes in extracellular amino acid availability and modulating nutrient-induced signals. The sodium-coupled neutral amino acid transporter 2 (SNAT2) has recently been shown to have a role in nutrient sensing and gut hormone release. SNAT2 acts as a secondary active transporter, coupling the transfer of amino acids against their concentration gradient with the simultaneous inward movement of Na^+ down its electrochemical gradient. The SNAT2 transporter is thought to be involved in the stimulation of gut hormone release through consequential increases in intracellular Ca^{2+} (Young et al., 2010), specifically in glutamine-stimulated GLP-1 release from intestinal L-cells (Tolhurst et al., 2011).

1.3.9. Cellular amino acid sensing

Amino acids are essential nutrients for protein synthesis and cell growth. Mammalian cells integrate nutrient and growth factor availability with anabolic responses to support cell growth and proliferation. There are a number of key signalling hubs responding to amino acid supply, which are outlined below.

1.3.9.1. **Mammalian target of rapamycin complex 1 (mTORC1)**

mTORC1 is a serine/threonine protein kinase that acts as a crucial regulatory molecule in metabolism, protein synthesis and growth. It acts as a central hub for a vast signalling network that reflects an organism's physiological state, including its nutritional status. Its activity is controlled by insulin, growth factors, oxidative stress and amino acids. Amino acids have been shown to be important in mTORC1 activation but the mechanism by which they modulate mTORC1 activity remains poorly understood. Branched chain amino acids such as leucine are particularly implicated in mTORC1 signalling (Blouet et al., 2009). Administration of leucine into the mediobasal hypothalamus (MBH) acutely suppress food intake by reducing meal size. This effect is mediated by activation of mTORC1 within ARC POMC neurons through a MBH-PVN-NTS circuit (Blouet et al., 2009). Similarly L-leucine administration into the caudomedial NTS also reduces food intake and meal size via a mTORC1-dependent mechanism (Blouet and Schwartz, 2012). The mechanisms by which this occurs remain unclear. In its guanosine triphosphate (GTP) bound state, the GTPase Rheb is a direct activator of mTORC1, and is essential for mTORC1 activation (Stocker et al., 2003). The activity of Rheb is in turn regulated by the tuberous sclerosis (TSC) complex. The TSC complex includes TSC2, which activates the GTPase enzyme, and regulates mTORC1 activity by hydrolysing the guanosine triphosphate (GTP) of the active Rheb-GTP to the inactive guanosine diphosphate (GDP) bound form (Inoki et al., 2003, Carrasquer et al., 1993, Zhang et al., 2003a). The activity of the TSC complex itself is controlled through phosphorylation, which leads to its increased or decreased GTPase activating protein activity. mTORC1 activation is also dependent on Rag GTPases. This mechanism is sensitive to amino acid availability within the cell. Amino acid availability promotes the activity of a guanine nucleotide exchange factor named Ragulator. The Ragulator complex interacts with Rag GTPases which leads to the accumulation of a RagA/BGTP-RagC/DGDP complex. The presence of amino acids activates the Ragulator-Rags complex, which interacts with the lysosomal membrane protein v-ATPase, a protein pump. This complex recruits mTORC1 to the lysosomal surface where it can ultimately be activated by the Rheb (Efeyan et al., 2015). There may be other mechanisms by which amino acids regulate basal mTORC1 activity, but what is clear is that amino acids are essential signals in mTORC1 activation by growth factors and are therefore themselves key regulators of metabolism and growth.

1.3.9.2. **Fat mass and obesity associated protein (FTO)**

Fat mass and obesity associated protein (FTO) is an mRNA demethylase enzyme encoded by the fto gene on human chromosome 16 (Gerken et al., 2007). Single-nucleotide polymorphisms (SNPs) in this gene are strongly associated with BMI and obesity (Frayling et al., 2007) related to increased

energy intake (Cecil et al., 2008, Haupt et al., 2009). FTO is expressed within the ARC, with expression regulated according to energy availability: fasting decreases expression and chronic high fat feeding increases expression (Tung et al., 2010). This regulation is controlled by amino acid availability, with essential amino acid deprivation causing downregulation of FTO mRNA and protein levels (Cheung et al., 2013). Mouse embryonic fibroblasts (MEFs) derived from FTO knockout mice show decreased mTOR1 activity and increased autophagy compared to cells expressing FTO (Gulati et al., 2013). It is likely that FTO plays a role in amino acid sensing via the regulation of mTORC1 activity, with possible effects on body composition.

1.3.9.3. **General control nonrepressed 2 protein (GCN2)**

The essential amino acid content of a diet can influence feeding behaviour in animals. Animals can acutely detect and reject a diet lacking specific essential amino acids and preferentially choose diets containing the limiting essential amino acids (Koehnle et al., 2003). The APC region of the brain is thought to be important in regulating this adaptive eating behaviour (Leung and Rogers, 1971). GCN2 protein is an important regulator in sensing changes in amino acid availability. It is a serine/threonine protein kinase that binds to uncharged transfer ribonucleic acids (tRNAs) that are accumulated subsequent to amino acid deficiency within the APC. GCN2 phosphorylates eukaryotic initiation factor 2 alpha (elf2α), resulting in down-regulation of protein synthesis. In contrast, activating transcription factor 4 (ATF4) increases concentrations of available intracellular amino acids (Hao et al., 2005). ATF4 is a transcriptional factor involved in the activation of amino acid biosynthesis and transport by activating transporters systems such as SNAT2 (Blais et al., 2003). The sensing of essential amino acid deficiency is thought to be relayed to feeding centres including the hypothalamus, particularly the LHA and amygdala, via a number of possible mechanisms including: the rheogenic activity of SNAT1, ERK1/2 signalling (Franchi-Gazzola et al., 1999), increased glutamatergic activity and AMPA receptor activation (Sharp et al., 2004), and loss of gamma-aminobutyric acid (GABA)-ergic inhibitory neurotransmission (Truong et al., 2002).

1.3.10. Protein digestion

When food is ingested, there are three main phases to the digestion of protein: the gastric phase, the pancreatic phase and the intestinal phase. The gastric phase commences in the stomach and is facilitated by pepsin, an endopeptidase that cleaves peptide bonds between hydrophobic and aromatic amino acids. This results in the formation of peptides of different lengths including proteoses, peptones, polypeptides and some free amino acids. Further protein digestion takes place in the duodenum and jejunum. During this pancreatic phase the peptides resulting from the gastric

phase trigger the release of hormones such as CCK and secretin from the first part of the small intestine. This stimulates the release of pancreatic enzymes including protease precursors made in the pancreatic acinar cells (Meyer and Kelly, 1976). Once these precursors enter the duodenum, enterokinase produced by the gut epithelium cleaves inactive trypsinogen resulting in the formation of active trypsin, which subsequently autocatalyses this reaction and converts other protease precursors into their active forms. The subsequent activation of enteropeptidase enzymes such as trypsin, chymotrypsin and elastase results in the cleaving of polypeptides and oligopeptides (Freeman and Kim, 1978). The last stage of protein digestion is the intestinal phase, which takes place at both the brush border and within the enterocytes of the intestinal mucosa. Amino acid absorption takes place predominantly in the jejunum. This occurs following the breakdown of di- and tripeptides to free amino acids, which is catalysed by membrane bound proteases. Amino- and carboxy-specific peptidases within the small intestine ensure the full cleavage of amide- and carboxyl terminal amino acids from any remaining oligo- or polypeptides. A number of di- and tripeptides are absorbed, although most are then broken down to free amino acids within the enterocytes. A small minority of oligopeptides to manage to enter the portal system (Freeman and Kim, 1978).

1.3.11. Amino acids

Amino acids are the final product of protein digestion within the gut. Subsequent to intestinal absorption, they enter the portal circulation and are delivered to tissues, where they can serve as building blocks for protein synthesis, precursors for a number of bioactive molecules or as an energy source.

Normal human diet consists of a range of different amino acids. They can be classified in different ways, although broadly speaking they fall into two categories: proteinogenic and non-proteinogenic amino acids. There are only 20 proteinogenic amino acids that can be used for protein synthesis. Proteinogenic amino acids can be considered as essential or non-essential (Table 1.1). Non-essential amino acids can be made within the body, whilst essential amino acids cannot and must therefore be obtained through diet. There is a subset of non-essential amino acids which is known as conditionally-essential, as at certain stages of life *de novo* synthesis cannot meet the body's requirements. These conditionally-essential amino acids are L- arginine, L-cysteine and L-glutamine (Wu, 2009). Amino acids can also be divided according to the chemical properties of their side chain (Table 1.2).

Essential amino acids	Non-essential amino acids
Histidine (His, H)	Alanine (Ala, A)
Isoleucine (Iso, I)	Arginine (Arg, R)*
Leucine (Leu, L)	Asparagine (Asn, N)
Lysine (Lys, K)	Aspartic acid (Asp, D)
Methionine (Met, M)	Cysteine (Cys, C)*
Phenylalanine (Phe, P)	Glutamic acid (Glu, E)
Threonine (Thr, T)	Glutamine (Gln, Q)*
Tryptophan (Trp, W)	Glycine (Gly, G)
Valine (Val, V)	Proline (Pro, P)
	Serine (Ser, S)
	Tyrosine (Tyr, Y)

Table 1.1. Essential and non-essential amino acids.
Essential and non-essential amino acids with their three letter and one letter codes. *Conditionally-essential amino acids

Aliphatic	Basic	Acidic	Amidic	-S or –OH containing	Aromatic
Alanine	Arginine	Aspartic acid	Asparagine	Cysteine	Histidine
Glycine	Lysine	Glutamic acid	Glutamine	Methionine	Phenylalanine
Isoleucine				Serine	Tryptophan
Leucine				Threonine	Tyrosine
Proline					
Valine					

Table 1.2. Amino acid classification according to their side-chain.

1.3.12. High protein diets

A standard Western human diet usually derives 55% of its energy from carbohydrates, 30% from fats and 15% from proteins. A high protein diet (typically in the region of 25-30% of energy intake) has been shown to increase satiety, improve body composition and facilitate weight loss in animal models and humans (Potier et al., 2009, Harper and Peters, 1989, Jean et al., 2001, Morens et al., 2000, Morens et al., 2001, Hannah et al., 1990, Kinzig et al., 2007). These effects are not secondary to changes in palatability; studies have demonstrated that high protein diets do not cause conditioned taste aversion and that protein ingestion leads to typical behavioural satiety sequences (Harper and Peters, 1989, Bensaid et al., 2003). Protein has a satiating effect superior to that achieved with other macronutrients (Porrini et al., 1997, Reid and Hetherington, 1997, Trigazis et al., 1997, Bensaid et al., 2002, Anderson and Moore, 2004, Halton and Hu, 2004). High protein diets are typically associated with a reduction in calorie intake compared to other macronutrient rich diets (Weigle et al., 2005) . Subjects presented with *ad libitum* access to high protein diets consistently consume fewer calories compared to control individuals with *ad libitum* access to isocaloric normal protein diets, but still report similar levels of fullness (Weigle et al., 2005). Several other studies have demonstrated the satiating power of protein by using a macronutrient preload (Poppitt et al., 1998), comparing high or low protein preloads (Veldhorst et al., 2009c, Veldhorst et al., 2009e), or comparing the effects of different types of protein on appetite (Veldhorst et al., 2009a, Veldhorst et al., 2009b) (Veldhorst et al., 2009a, Veldhorst et al., 2009b). However, high protein diets are difficult to adhere to in the longer-term (Sargrad et al., 2005), and the exact mechanisms by which a high protein diet regulates food intake remain largely unknown. Several mechanisms have been suggested and these include the thermic effects of protein, the stimulation of gluconeogenesis, and changes in plasma gut hormone levels.

1.3.12.1. **Thermic effects of protein**

In order to process ingested macronutrients, the body requires energy. Dietary induced thermogenesis (DIT) is the the increase in energy expenditure above basal fasting level divided by the energy content of the food ingested (Westerterp, 2004).. The DIT for protein is higher than that of other macronutrients, equating to approximately 20-30% of energy consumed, compared to 5-10% for carbohydrates and 0-3% for fats (Westerterp-Plantenga et al., 1999, Tappy, 1996). Unlike carbohydrate and fat, protein cannot be stored and therefore requires rapid metabolic processing. Subsequent to digestion, protein can be 'disposed' of in a number of ways depending on the metabolic requirements. These include protein synthesis, urea production and gluconeogenesis, all of which require high amounts of energy. Multiple studies have shown an increase in dietary protein

content results in increased energy expenditure (Luscombe et al., 2003, Luscombe et al., 2002). The type of protein can affect the degree of protein-induced thermogenesis. Consumption of "complete' proteins, which are proteins containing all the essential amino acids, results in a higher thermogenic response than vegetable proteins such as soy which are often 'incomplete' proteins (Mikkelsen et al., 2000). Increasing energy expenditure whilst maintaining the same energy intake would result in a negative energy balance, which could be promising in terms of weight loss. However, despite increased dietary protein resulting in a significant rise in DIT, these changes are quantitatively small and therefore unlikely to account for the magnitude of weight loss seen with high protein diets (Eisenstein et al., 2002).

1.3.12.2. **Gluconeogenesis**

High protein diets increase gluconeogenesis. Increased intestinal gluconeogenesis is thought to contribute to the beneficial effects of high protein diets (Veldhorst et al., 2012). Induction of gluconeogenic genes in the small intestine results in a portal release of glucose, lasting throughout the post-absorptive period in rats fed a high-protein diet (Penhoat et al., 2011). The satiating effects of high protein diets may be partly related to modulation of glucose homeostasis via regulation of hepatic gluconeogenesis (Veldhorst et al., 2009f, Veldhorst et al., 2012, Mithieux et al., 2005, Penhoat et al., 2011). However, not all amino acids are preferentially used for gluconeogenesis, and hence protein composition will influence the magnitude of the resulting gluconeogenesis.

1.3.12.3. **Protein-induced satiety**

Protein has been consistently shown to be the most satiating of macronutrients when compared to carbohydrate and fat. This effect has been studied in a number of different ways: comparison of different macronutrient preloads (Poppitt et al., 1998, Stubbs et al., 1999), comparing high or low protein preloads (Veldhorst et al., 2009c, Veldhorst et al., 2009e, Porrini et al., 1997, Porrini et al., 1995), or comparing the effects of different types of protein on appetite (Veldhorst et al., 2009a, Veldhorst et al., 2009b). It has been shown that the satiating power of a protein load correlates with the timing of systemic amino acid appearance following intake (Veldhorst et al., 2009d, Luhovyy et al., 2007), which suggests specific amino acids may be responsible for inducing satiety. There is long-standing data to show a possible link between plasma amino acid levels and satiety (Mellinkoff et al., 1956). More recently, a study looking at the effect of protein on appetite showed that satiety directly correlated with the appearance of systemic amino acid levels (Veldhorst et al., 2009e). In addition, the type of protein is associated with the appearance of different amino acids: soy-protein mediated satiety is associated with the presence of taurine (Veldhorst et al., 2009e), whey protein

with serine, threonine, isoleucine and alanine (Veldhorst et al., 2009a) and casein with branched-chain amino acids (Veldhorst et al., 2009c). This suggests an important role for amino acids in protein-induced satiety. However, the presence of these amino acids in circulation is also likely to be related to other events in the gut,

There is evidence that protein influences gastrointestinal hormones to alter satiety. Protein has been reported to increase levels of anorectic gut hormones (Hall et al., 2003, Calbet and Holst, 2004, Nilsson et al., 2004, Batterham et al., 2006b). A high protein meal increases circulating concentrations of both PYY and GLP-1 in normal weight humans compared to isocaloric meals rich in fats or carbohydrates (van der Klaauw et al., 2012). High protein meals are associated with significantly higher PYY responses in both lean and obese humans when compared to high-fat and high-carbohydrate diets (Batterham et al., 2006b). Protein can suppress post-prandial circulating ghrelin levels more potently and/or for longer than carbohydrates (Al Awar et al., 2005, Blom et al., 2006, Tannous dit El Khoury et al., 2006, Bowen et al., 2006a, Bowen et al., 2006b, Leidy et al., 2007, Foster-Schubert et al., 2008). The role of GLP-1 in protein-mediated satiety is less clear, and seems to be related to the length of exposure. GLP-1 levels are higher in people on a chronic high protein diet compared to those on a normal protein diet (Lejeune et al., 2006). However, evidence does suggest that carbohydrate stimulates GLP-1 release to a higher degree than protein (Smeets et al., 2008). Interestingly different protein sources have varying effects on anorectic gut hormones (Diepvens et al., 2008, Abou-Samra et al., 2011), which may be related to the digestion kinetics of each protein, and hence it may be the appearance of specific amino acids in the gut or in the blood that regulate gut hormone release and satiety (Anderson et al., 2004).

High protein diets can also have effects in the CNS, in particular in areas involved in appetite regulation. High protein diets increase the activation of noradrenergic neurons in the NTS and increase the activation of POMC neurons in the ARC compared to normal protein diets (Faipoux et al., 2008). In addition, high protein diets reduce activity in orexin neurons in the lateral hypothalamus when compared to a normal protein diet (Journel et al., 2012). They are also said to inhibit the activation of opioid and GABAergic neurons in the nucleus accumbens (NAcc), reducing the hedonic response to food (Fromentin et al., 2012). However, the exact mechanisms by which high protein diets influence neuronal signalling remain poorly understood.

Protein has been shown consistently to reduce satiety, however it is thought that it is the actual amino acid products of protein digestion that are being sensed which drive changes in food intake and gut hormone release.

1.3.12.4. **Amino acids and central appetite regulation**

Amino acids enter the brain through areas where there is an incomplete blood-brain barrier, such as the brainstem. Specific amino acids are precursors for neurotransmitters. For example, tryptophan and tyrosine are precursors for serotonin and dopamine respectively. Histidine is a precursor for histamine synthesis (Fromentin et al., 2012). These neurotransmitters are thought to play a role in appetite regulation (Volkow et al., 2011, Bassil et al., 2007, Goto et al., 2007). Glutamate, aspartate and glycine are all active at the N-methyl-D-asparate (NMDA) receptor (Fromentin et al., 2012). Branched-chain amino acids such as leucine and isoleucine have effects on appetite thought to occur via the mTOR pathway (Section 1.3.9.1) (Blouet et al., 2009).

1.3.12.5. **Amino acids and peripheral appetite regulation**

Individual amino acids are thought to be involved in the peripheral regulation of appetite through effects on gut hormone release. Cell studies using the enteroendocrine cell line NCI-H716 showed that a mixture of essential amino acids was better at stimulating GLP-1 release than a mixture of non-essential amino acids (Reimer, 2006). In addition, branched-chain amino acids can stimulate GLP-1 release in a dose-dependent manner from the same cell line (Chen and Reimer, 2009). The GLUTag cell line is another GLP-1 secreting cell line, which releases GLP-1 in response to a variety of amino acids including L-glutamine (Reimann et al., 2004). There is also evidence from murine primary colonic cultures showing that L-glutamine, L-phenylalanine and L-asparagine stimulate GLP-1 secretion (Tolhurst et al., 2011). Amino acids can also stimulate the release of other hormones involved in appetite regulation. Aromatic amino acids such as L-phenylalanine and L-tryptophan stimulate the release of CCK from isolated CCK-cells (Wang et al., 2011). In addition, specific L-amino acids such as L-phenylalanine, L-tryptophan, L-arginine, L-glutamine and L-asparagine stimulate the release of the gut hormones PYY, GLP-1 and GIP in isolated rat small intestine preparations (Mace et al., 2012).

1.4. **Preliminary work**

There is currently an urgent need for effective treatments for obesity. High protein diets have been shown to be effective in weight loss management but are difficult to adhere to in the long-term, and the exact mechanisms by which a high protein diet regulates food intake remain poorly understood. There is evidence that protein influences gastrointestinal hormones to alter satiety. Interestingly, these hormones rise within 10-15 minutes after eating, which is before the digested food comes in

to contact with the majority of the L cells of the distal gut. It is still not well understood what triggers this early hormonal release or indeed how it can be potentiated in order to maximise satiation.

1.5. **Hypotheses and general aims**

Hypotheses:

- L-arginine acutely suppresses appetite and food intake in humans following oral administration.
- Meals of altered macronutrient intake, including a high protein meal, undergo differential digestive and absorptive processing to yield digesta of varied metabolomic profiles. These metabolomic profiles vary in their ability to stimulate gastrointestinal hormone release .

General aims:

- The effect on gut hormone release and *ad libitum* food intake following oral ingestion of L-arginine compared to a control amino acid.
- The effect of a high protein meal on gastric and duodenal nutrients and metabolites, gastric emptying times and hormonal response profiles and subjective measures of appetite and future energy consumption.

Chapter 2:

The effect of L-arginine on appetite

2.1. **Introduction**

2.1.1. L-arginine

L-arginine is an amino acid necessary for the biosynthesis of proteins. It was initially identified from lupin and pumpkin seedlings in the late 19th century, at which time its structure was also confirmed. It was not until the 1930s, when the urea cycle was characterised, that its role in metabolism and physiology was confirmed. It is a conditionally essential amino acid found in high protein foods, including meat, chicken, fish, dairy products and nuts. Arginine levels are higher in plant based protein compared to animal protein (Reyes et al., 1994). In healthy human adults, plasma arginine levels range between 80-120µmol/L (100-200 µM) (Tangphao et al., 1999). L-arginine contains a 3-carbon aliphatic side chain (Figure 2.1). It also has a positively charged guanidinium group which renders L-arginine basic. The L-arginine cation actively binds negatively charged phosphate anions and it is therefore often present in the centre of proteins which bind phosphorylated substrates, where it may be essential in maintaining the overall charge and therefore the stability of a protein molecule (Toi et al., 1967).

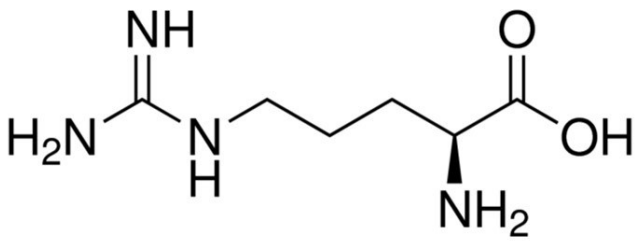


Figure 2.1. Chemical structure of L-arginine.
L-arginine consists of a 3-carbon aliphatic side chain and a guanidinium group.

2.1.2. L-arginine synthesis

L-arginine is obtained from diet, endogenous synthesis and protein turnover. In mammals, L-arginine is a conditionally essential amino acid. Levels of endogenous synthesis are usually adequate to meet body requirements. However, in times of infection or inflammation, endogenous synthesis may not be sufficient, and hence the required levels must be derived from another source.

Most of the endogenous synthesis results from a series of biochemical reactions, which occur mainly within the intestinal epithelium, and the proximal tubule of the kidney. The biosynthesis of L-arginine can be divided into three main steps which include the synthesis of L-ornithine, L-citrulline and finally of L-arginine (Figure 2.2) (Reyes et al., 1994).

L-ornithine is synthesised from glutamine or proline, via reactions catalysed by the enzymes L-Δ1-pyrroline-5-carboxylate (P5C) synthetase (P5CS) and proline dehydrogenase (PROHD), respectively. This process only takes place in the small intestine; both P5CS and PROHD are exclusively expressed in the intestinal mucosa (Windmueller, 1982, Wakabayashi et al., 1994).

The synthesis of L-citrulline from L-ornithine is mediated by the action of the enzymes ornithine carbamoyltransferase (OCT) and carbamoylphosphate synthetase 1 (CPS1). Both enzymes are expressed in hepatocytes and epithelial cells in the small intestine, and to a lesser degree in the large intestine (Raijman, 1974). The reaction in the liver is thought to be part of the urea cycle. The L-citrulline produced in the small intestine enters systemic circulation, from where it is taken up by the cells of the proximal tubule of the kidney and converted into L-arginine (Windmueller and Spaeth, 1981, Rabier and Kamoun, 1995).

Synthesis of L-arginine from L-citrulline is regulated by the enzymes argininosuccinate synthase 1 (ASS1) and argininosuccinate lyase (ASL). Unlike OTC and CPS1, ASS1 and ASL are expressed in many different cell types, albeit at lower levels than in the kidney, facilitating the synthesis of L-arginine from L-citrulline in multiple tissues (Wu and Morris, 1998).

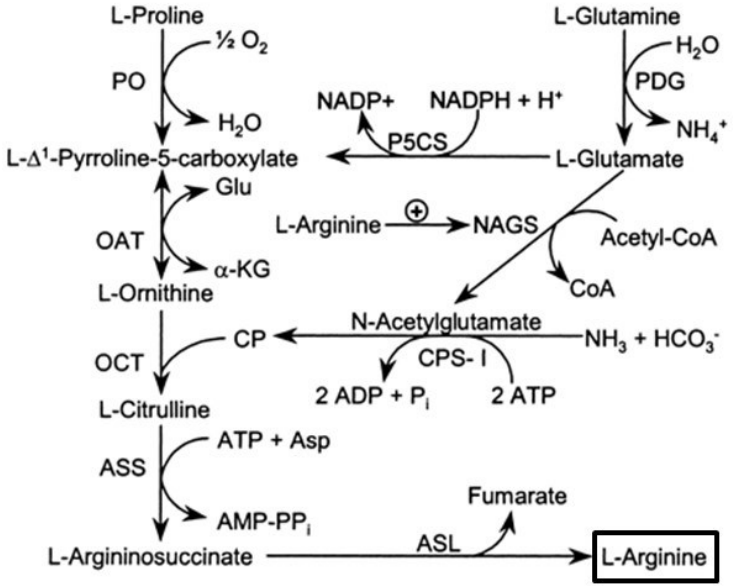


Figure 2.2. Biosynthesis of L-arginine.
Pathways of intestinal citrulline and arginine synthesis from glutamine and proline. ASL, argininosuccinate lyase; Asp, aspartate; ASS, argininosuccinate synthase; CP, carbamoylphosphate; Glu, glutamate; {alpha}-KG, {alpha}-ketoglutarate; OAT, ornithine aminotransferase; OCT, ornithine carbamoyltransferase; PDG, phosphate-dependent glutaminase; P5C: L-Δ1-pyrroline-5-carboxylate; and PO, proline oxidase. Adapted from (Wu et al., 2004).

2.1.3. L-arginine metabolism

L-arginine is metabolised via a number of different pathways. L-arginine is a vital intermediate in the urea cycle, a source of amidino groups for creatine synthesis, a substrate for nitric oxide synthase (NOS), and a source of ornithine for polyamine synthesis. L-arginine is the source of the nitrogen atom in nitric oxide (NO), which is vital in various physiological processes, which include the regulation of vascular tone (Palmer et al., 1988), neurotransmission (Liu and Barajas, 1993) and immune system function (Albina et al., 1989). L-arginine is converted to NO by the enzyme nitric oxide synthase. L-arginine can also be converted to ornithine and urea by the enzyme arginase. This process is part of a pathway that eliminates nitrogen-containing molecules from the body (Reyes et al., 1994). Ornithine is also part of a proliferative pathway implicated in cell division and tissue regeneration. In addition to these pathways, the synthesis of creatine and creatinine involves the metabolism of L-arginine in the kidney. L-arginine is a substrate that is converted to guanidine acetic acid by the addition of glycine. The guanidine acetic acid is then used by the liver rendering creatine, which is ultimately taken up by muscle. Creatine undergoes chemical modification steps within muscle to produce creatinine (Walker, 1979).

2.1.4. L-arginine cellular transport

A number of different amino acid transporter systems are involved in the uptake of extracellular amino acids. L-arginine shares one of the same transport systems as other cationic amino acids such as L-lysine and L-ornithine (White, 1985). In most cells, arginine transport through the plasma membrane is not energized by coupling to the Na^+ gradient. A single Na^+-independent transport system called system y^+ is thought to be the major entry route for most cationic amino acids into most cells (Closs et al., 2006). System y^+ is made up of the cationic amino acid transporter (CAT) proteins CAT1, CAT-2A, CAT-2B and CAT3, in which CAT-2A and CAT-2B are splice variants of the same gene. CAT-1 is expressed in most tissue except the liver (Aulak et al., 1996). CAT-2A is mainly expressed in the liver (Closs et al., 2006) and CAT-3 is only expressed in the brain (Hosokawa et al., 1999). L-arginine can also be transported by broad spectrum amino acid transporter systems including system y^+L, b^{0+} and B^{0+} via sodium-dependent mechanisms (Broer, 2008). As well as the transport systems themselves, modulators of the transporter activity may also indirectly control the uptake of L-arginine. L-arginine is competitively inhibited by other cationic amino acids such as L-lysine or L-ornithine. In addition, a number of NOS inhibitors are thought to inhibit the cellular uptake of L-arginine (Bogle et al., 1992).

2.1.5. L-arginine and amino acid sensing receptors

Evidence suggests that the amino acid products of protein digestion may be sensed both peripherally and centrally to regulate energy intake (Fromentin et al., 2012). Individual amino acids have been shown to influence gastrointestinal hormone release and appetite (McGavigan et al., 2015, Meek et al., 2015). GPCR family class C amino acid sensing receptors are present in the gastrointestinal tract, where some are known to be expressed on enteroendocrine L cells. Arginine activates the GPRC6A, T1R1/T1R3 and the CaSR (Christiansen et al., 2007, Nelson et al., 2002, Conigrave et al., 2000). Although arginine activation of the CaSR can cause downstream signalling effects, there is no evidence that it acts via this receptor to modulate gastrointestinal hormone release. The absorption of arginine in the gastrointestinal tract is relatively inefficient (Stoll et al., 1998), suggesting that it may reach the distal intestine following oral administration. Amino acid sensing receptors are located along the entire length of the gastrointestinal tract, where they are thought to play a role in energy homeostasis (Symonds et al., 2015).

The GPRC6A in preference binds basic amino acids which include L-arginine (Wellendorph et al., 2005). At the human GPRC6A receptor, L-arginine is the most potent amino acid activator (Pi et al., 2005). L-arginine potently stimulates insulin secretion (Floyd et al., 1966). Dietary supplementation

with L-arginine improves glycaemic control in rodents and humans (McKnight et al., 2010). It has been reported that L-arginine induced insulin secretion is mediated by GPRC6A activity (Pi et al., 2012). In addition, L-arginine has been shown to stimulate insulin secretion by promoting GLP-1 release and improving glucose tolerance in mice (Clemmensen et al., 2013b). GPRC6A is expressed on the surface of an intestinal cell line (Oya et al., 2013). The anorectic effects of L-arginine do not appear to be dependent on GPRC6A or on vagal signalling and although L-arginine can stimulate GLP-1 and PYY release acutely *in vitro* and *in vivo*, its anorectic effects do not seem to be mediated by changes in these gut hormones (Alamshah et al., 2016b). L-arginine can also stimulate insulin release directly from pancreatic β-cells through membrane depolarization (Smith et al., 1997, Thams and Capito, 1999).

The T1R1/T1R3 heterodimer has been identified in the gut, where it can act as a luminal sensor for certain L-amino acids, resulting in release of gut hormones (Daly et al., 2013). Interestingly unlike GPRC6A, the T1R1/T1R3 receptor has been shown to play a role in L-arginine-induced insulin secretion (Oya et al., 2011) but seems not to have effects on anorectic gut hormone release.

There is good evidence that L-arginine plays a role in appetite regulation *in vivo* however the effects in humans are less clear.

2.2. **Hypotheses and Aims**

Hypothesis:

- L-arginine acutely suppresses appetite and food intake in humans following oral administration.

Aims:

- To determine the effect on gut hormone release following a dose of 17.2mmol of L-arginine compared to a control amino acid (glycine).
- To determine the effect on *ad libitum* food intake following a dose of 17.2mmol of L-arginine compared to a control amino acid (glycine).

2.3. **Methods**

2.3.1. Amino acids

Amino acids were purchased from SynPharma International Ltd (Horsham, UK) and encased in size 00 white vegetarian capsules. Vehicle controls were empty capsules. L-arginine has a guanidinium cationic side chain rendering it basic in solution (pH 12). To avoid any effects of pH in these studies, the salt L-arginine hydrochloride was used as it is pH neutral. A dose of 3g arginine (17.2mMol) was used, as this is the highest daily dose not considered an investigational medicinal product by the Medicines and Healthcare products Regulatory (MHRA). A dose of 17.2mMol glycine was used as a control. Glycine was chosen as a control amino acid as this amino acid had previously been shown to have no effect on food intake in rodent studies (unpublished data). D-arginine which does not activate the amino acid sensing G-protein coupled receptors and seems to have no effect on food intake and appetite in rodents was the obvious choice for a control amino acid, however it was considered to be a drug (rather than a foodstuff) which is why glycine was used instead as an alternate amino acid with little effect on appetite.

2.3.2. Randomization

Treatment order was randomly determined prior to the start of the study using opaque envelopes by a member of the laboratory who was not involved in the study.

2.3.3. Participants

2.3.3.1. **Participant recruitment**

All participants were recruited by advertisement either from posters at the Imperial College campus sites (Hammersmith Hospital, Charing Cross Hospital, South Kensington) or Metro and Evening Standard newspapers. Potential participants were screened to ensure they were healthy and exclude those with disordered eating using Sick Control One Fat Food (SCOFF) questionnaire, Dutch Eating Behaviour Questionnaire (DEBQ) and Eating Attitudes Test (EAT) (see Section 1.3.3.2 and 1.3.3.3 for formal inclusion and exclusion criteria). After providing written informed consent, participants underwent a health screening consisting of questions on their medical history and a physical examination. Female participants were required to provide a urine sample for a pregnancy. Twenty mL of venous blood was taken to measure full blood count, urea and electrolytes, liver and thyroid function and glucose. All screening blood analysis was conducted by the Department of Chemical Pathology at Imperial College Healthcare NHS Trust. During the screening visit for Part B,

they also tasted a meal, which was served as the *ad libitum* meal and asked to rate it on a 9-point hedonic scale. If the score was between 6 and 8, then that meal would be served as their *ad* libitum meal, else an alternative meal was offered until an appropriate meal was found. A three day food diary was also completed to determine their food habits after the study visit. The research was approved by the West London Research Ethics Committee (reference number 11/H0707/7). All participants gave written informed consent, and the research was planned and performed in accordance with the Declaration of Helsinki.

2.3.3.2. **Inclusion criteria**

Inclusion criteria were: male or female, aged 18 to 60 years (inclusive), normal and overweight/obese people as classified by a BMI 18.5-30 kg/m2 (inclusive) who were otherwise healthy (based on a pre-study assessment) and people willing and able to give written informed consent and to understand, participate and comply with the study requirements.

2.3.3.3. **Exclusion criteria**

Exclusion criteria were: treatment with any medication that might have affected the study outcome (e.g. medication affecting appetite regulation and/or blood flow), haemorrhagic disorders and anticoagulant treatment, inborn errors of metabolism, hepatic impairment as defined by screening visit liver function tests (aspartate aminotransferase (AST) or alanine aminotransferase (ALT) or gamma-glutamyl transpeptidase (GGT) of three times the upper normal reference limit), current pregnancy or breast feeding, delivery within the last year, significant intercurrent disease or history of clinically significant disease of any type, in particular liver, kidney, or heart disease, any form of diabetes mellitus or psychiatric illness, history of cancer (excluding skin cancer), history of severe or multiple allergies, severe adverse drug reaction or leucopaenia, smokers, regular drinkers of more than three units of alcohol daily, participants who had taken amino acid supplements either continuously or intermittently in the preceding 12 months, history of, or current evidence of, abuse of alcohol or any drug substance, licit or illicit, regular intake of over-the-counter medication (other than the occasional paracetamol/aspirin), poor compliers or participants unlikely to attend, and blood donation within the 12 week period before the initial study dose.

2.3.3.4. **Withdrawal criteria**

Withdrawal criteria were: significant deviation from the approved protocol, the participant no longer satisfying the inclusion and exclusion criteria set out by the approved protocol, a positive pregnancy test result, and the participant withdrawing consent.

2.3.4. Study design

2.3.4.1. **Tolerability study**

Participants attended four study visits each separated by at least one week. The evening before each study visit, participants were requested to consume an identical meal, supplied by the experimenters, at approximately 2000 hours. Participants then reported to the clinical research facility at 0830 hours the following day having fasted from 2100 hours the night before. On arrival, participants had an intravenous cannula inserted in the antecubital fossa for serial blood sampling. They were asked to consume hypromellose capsules containing a total of 17.2mmol of L-arginine hydrochloride or 17.2mmol of glycine (Euro-OTC-Pharma, Horsham, UK) or empty capsules in a double-blind randomised order. This dose of L-arginine was chosen as it was similar to levels of L-arginine found in a high-protein meal. Hypromellose capsules, which are relatively inert and break down in the stomach, were used to deliver the amino acids. The first visit was an acclimatisation visit, the results of which were not included in the final analysis.

Blood samples were taken at 15 minute intervals commencing at t=-15 minutes for 2.5 hours after dosing. Blood for gut hormones was transferred to lithium heparin-coated tubes (International Scientific Supplies Ltd, Bradford, UK) containing 6000 kallikrein inhibitor units (60µL) aprotinin (Trasylol, Bayer Schering Pharma, Berlin, Germany), a protease inhibitor to prevent the degradation of gut hormones. Blood for glucose analysis was transferred to fluoride oxalate tubes. Blood for insulin analysis was transferred to serum-separating tubes and allowed to clot before centrifugation. Samples underwent centrifugation at 3000g for 10 minutes at 4°C (Hettich Zentrifugen Rotina 420R). The separated plasma and serum was aliquoted into eppendorf tubes, with 300 µL plasma aliquoted separately into an eppendorf with 1M hydrochloric acid to a concentration of 0.05N. All the eppendorfs were stored at -20°C until analysis.

Participants were asked to complete visual analogue scales (VAS) at the corresponding time-points to the bloods. Participants rated subjective feelings of hunger ('How hungry do you feel right now?'), pleasantness to eat ('How pleasant would it be to eat right now?'), prospective food intake ('How much could you eat right now?'), fullness ('How full do you feel right now?') and sickness ('How sick do you feel right now?') using 100 mm horizontal visual analogue scales (VAS) at -15, 0, 15, 30, 45, 60, 75, 90, 105, 120,135 and 150 min following ingestion of L-arginine. Participants were also asked to report any additional side-effects that occurred during the visit or after the study visit was completed. A typical study day is summarized in figure 2.3.

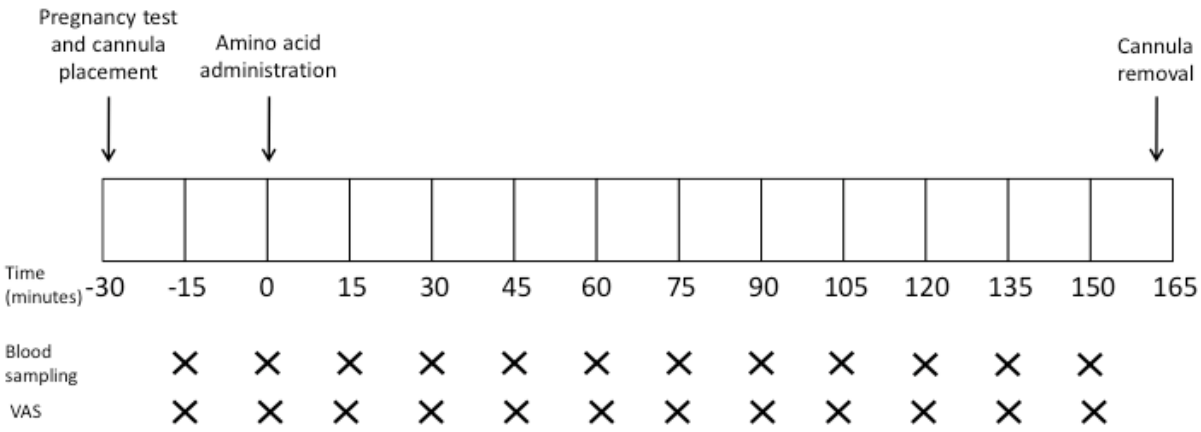


Figure 2.3. Timeline for a typical study day for the tolerability study.
× represents time points at which blood samples and visual analogue scales (VAS) were collected.

2.3.4.2. **Appetite and gut hormone release study**

The study protocol was identical to the tolerability study above, except at t=60 minutes, participants received an *ad libitum* meal and had 30 minutes to consume it (so had no blood taken or VAS to complete at t=75 minutes). During the screening visit, samples of the *ad libitum* meal were provided, and an assessment of palatability was made using a nine-point hedonic scale. Participants who disliked the study meals or rated the study meals as 'like extremely' were excluded. Participants were asked to eat until they were comfortably full. Water was freely available during the mealtime. The meal was served with the curtains drawn around each participant for 30 minutes, and consisted of either a tomato mozzarella pasta bake (768kJ/100g), macaroni cheese (844kJ/100g) or chilli con carne (594kJ/100g). Each participant was served the same meal according to their preference during each of their study visits. Food was weighed pre- and post-consumption. Energy intake was calculated from the weight of food consumed and the energy density of the food. Participants were asked to complete food diaries for the rest of the day and the subsequent day following their departure from the clinical research facility. Food diaries were analysed using Dietplan 6 nutritional analysis software (Forestfield Software, Horsham, UK).

A typical study day is summarized in figure 2.4.

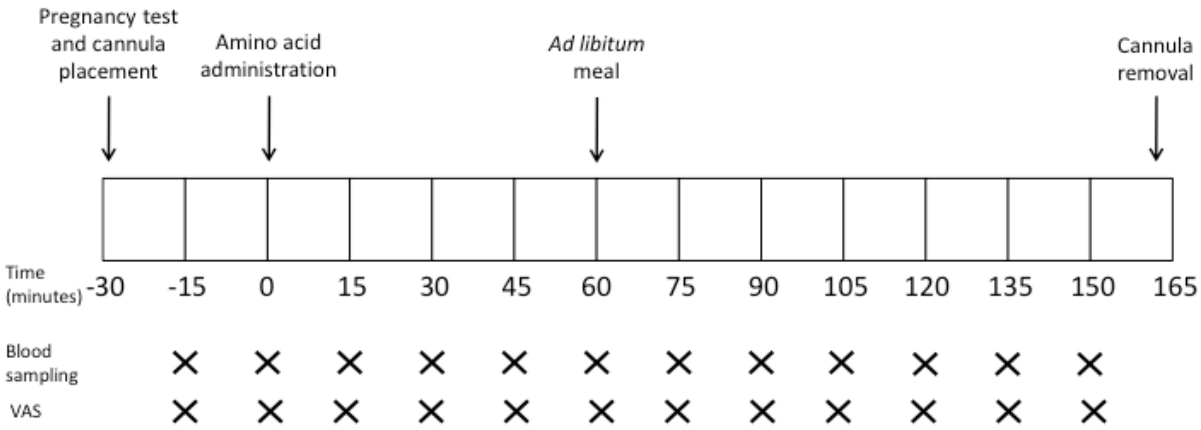


Figure 2.4. Timeline for a typical study day for the appetite and gut hormone release study.
× represents time points at which blood samples and visual analogue scales (VAS) were collected.

2.3.5. Plasma hormone assays

2.3.5.1. **PYY**

PYY-like immunoreactivity was measured with a previously established specific and sensitive in-house radioimmunoassay (Adrian et al., 1985a). The antiserum (Y21) was produced in rabbits against synthetic porcine PYY coupled to bovine serum albumin by glutaraldehyde and used at a final dilution of 1:50,000. This antibody cross-reacts fully with both PYY_{3-36} and PYY_{1-36}, but not with pancreatic polypeptide, neuropeptide Y, or other known gastrointestinal hormones. The ^{125}I PYY was prepared by the Iodogen method (Wood et al., 1981) and purified by reverse phase high pressure liquid chromatography (RP-HPLC). The specific activity of the ^{125}I PYY label was 54 Bq/fmol. The assay was performed in total volume of 0.7 ml of phosphate buffer (0.05M $Na_2HPO_4.2H_2O$, 0.006M KH_2PO_4, 0.01M $C_{10}H_{14}H_2O_8Na_2.2H_2O$, 0.008M NaN_3) (pH 7.3±0.1) containing 0.3% bovine serum albumin. The assay was incubated for three days at 4ºC before separation of the free and antibody bound label by goat anti-rabbit antibody. Samples were pre-incubated for at least 60 minutes with 100µl of goat anti-rabbit solid-phase secondary antibody (Pharmacia Diagnostics AB, Uppsala, Sweden). Immediately prior to centrifugation, 500µl of 0.01% Triton-X-100 solution and 100µl polyethylene glycol (PEG) (1:1) was added to each tube. The samples were then centrifuged at 1,500*g* at 4°C for 20 minutes. Bound and free label were separated and both the pellet and supernatant counted for 180 seconds in a γ-counter (model NE1600 (Thermo Electron Corporation,

San Jose, California, U.S.A.)). PYY concentrations in the samples were calculated using a non-linear plot (RIA software, Thermo Electron Corporation) and the results calculated with reference to the standard. The detection limit of the assay was 2.5 pmol/l, with an intra-assay coefficient of variation of 5.0 %. All samples were assayed in one assay to avoid inter-assay variation.

2.3.5.2. **GLP-1**

GLP-1- like immunoreactivity was measured by a specific and sensitive in-house radioimmunoassay, previously established (Kreymann et al., 1987). The antibody was produced in rabbits against GLP-1 coupled to bovine serum albumin. The antibody cross-reacted 100% with all amidated forms of GLP-1 but did not cross react with glycine extended forms [GLP-$1_{1\text{-}37}$ and GLP-$1_{7\text{-}37}$] or any other known pancreatic or gastrointestinal peptide. ^{125}I-GLP-1 was prepared by the Iodogen method (Wood et al., 1981) and purified by RP-HPLC. The specific activity of the ^{125}I GLP-1 label was 48 Bq/fmol. The assay was performed in a total volume of 0.7 ml of sodium barbitone buffer (0.05M $C_8H_{11}N_2O_3Na$, 0.005M NaN_3) (pH 8) containing 0.3 % bovine serum albumin (BSA). The assay was incubated for three days at 4ºC before separation of the free and antibody bound label by charcoal absorption. The free fraction was separated using 4mg of charcoal per tube suspended in phosphate buffer with gelatine (0.0625mM gelatine, 0.05M $Na_2HPO_4.2H_2O$, 0.006M KH_2PO_4, 0.01M $C_{10}H_{14}H_2O_8Na_2.2H_2O$, 0.008M NaN_3), added immediately prior to centrifugation. The samples were then centrifuged at 1,500*g* at 4ºC for 20 minutes. Bound and free label were separated, counted and analysed as described above. The limit of detection was 7.5 pmol/l with an intra-assay variation of 5.6%. All samples were assayed in one assay to avoid inter-assay variation.

2.3.5.3. **Ghrelin**

Human plasma acylated ghrelin concentrations were measured using a specific and sensitive enzyme-linked immunosorbent assay (ELISA) (Merck Millipore, Billerica, MA, USA). Plasma was separated by centrifugation as described above, acidified with HCl to a final concentration of 1N (Hosoda et al., 2004), frozen and stored at -20C. The assay was carried out as per manufacturer's instructions.

2.3.5.4. **CCK**

Human plasma CCK concentrations were measured using a specific and sensitive radioimmunoassay (Eurodiagnostica, Malmö, Sweden). Plasma was separated by centrifugation as described above, frozen and stored at -20C. The assay was carried out as per manufacturer's instructions.

2.3.6. Statistical analysis

The tolerability study was a pilot study so did not require a power calculation. For the gut hormone and appetite study, it was predicted that 9 participants would be required for this study, based on a difference in gut hormone release of 30% and power of 80%. This is the approximate magnitude of increase in PYY release observed following a high protein meal compared to a high carbohydrate meal (Batterham et al., 2006b, van der Klaauw et al., 2012).

Data are presented as the mean ± standard error of the mean (SEM). Gut hormones and VAS data were analysed using statistical software (SPSS Statistics Version 22 (IBM, USA)). Normality of data was verified with the Kolmogorov-Smirnov test. Comparisons of VAS scores and gut hormones were by a two-way repeated measures analysis of variance (ANOVA), followed by a post hoc Bonferroni's multiple comparison test. Area under the curve (AUC) was generated for all data sets, with a one-way ANOVA used to detect statistical significance followed by a post hoc Bonferroni's multiple comparison test. Acute food intake and nutrient intake from food diaries was analysed using a one-way ANOVA to detect statistical significance followed by a post hoc Bonferroni's multiple comparison test. All graphs were created using statistical software, Prism 6 (GraphPad, USA). The threshold for statistical significance in each case was set at $p<0.05$.

2.4. **Results**

2.4.1. Tolerability study

2.4.1.1. **Baseline characteristics**

Healthy male (n=1) and female (n=6) participants with a mean (± s.d.) age of 39.4 ± 11.4 years and BMI of 24.6 ± 4.7 kg/m^2 who had been weight stable for three months prior to study enrolment were recruited. The clinical characteristics of the recruited participants are summarized in Table 2.1.

Characteristic	All subjects
Age (yr)	39.4 ± 11.4
Female:Male	6:1
Weight (kg)	68.9 ± 17.3
Height (m)	1.67 ± 0.08
BMI (kg/m^2)	24.6 ± 4.7
Blood pressure	112/68 ± 13/8
Ethnic group	
Caucasian	5
Chinese	1
Afro-Caribbean	1

Table 2.1. Baseline characteristics of the 5 participants recruited for tolerability study.
Data represent the mean ± SD. BMI: body mass index.

2.4.1.2. **Tolerability of L-arginine**

L-arginine was well-tolerated, with no self-reported side-effects following administration. In addition, there were no significant changes on the visual analogue scales with regards to nausea.

2.4.1.3. **The effect of L-arginine on subjective measures of appetite using visual analogue scales**

There was no significant difference in subjective measures of appetite using visual analogue scales following ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (Figures 2.5 and 2.6)

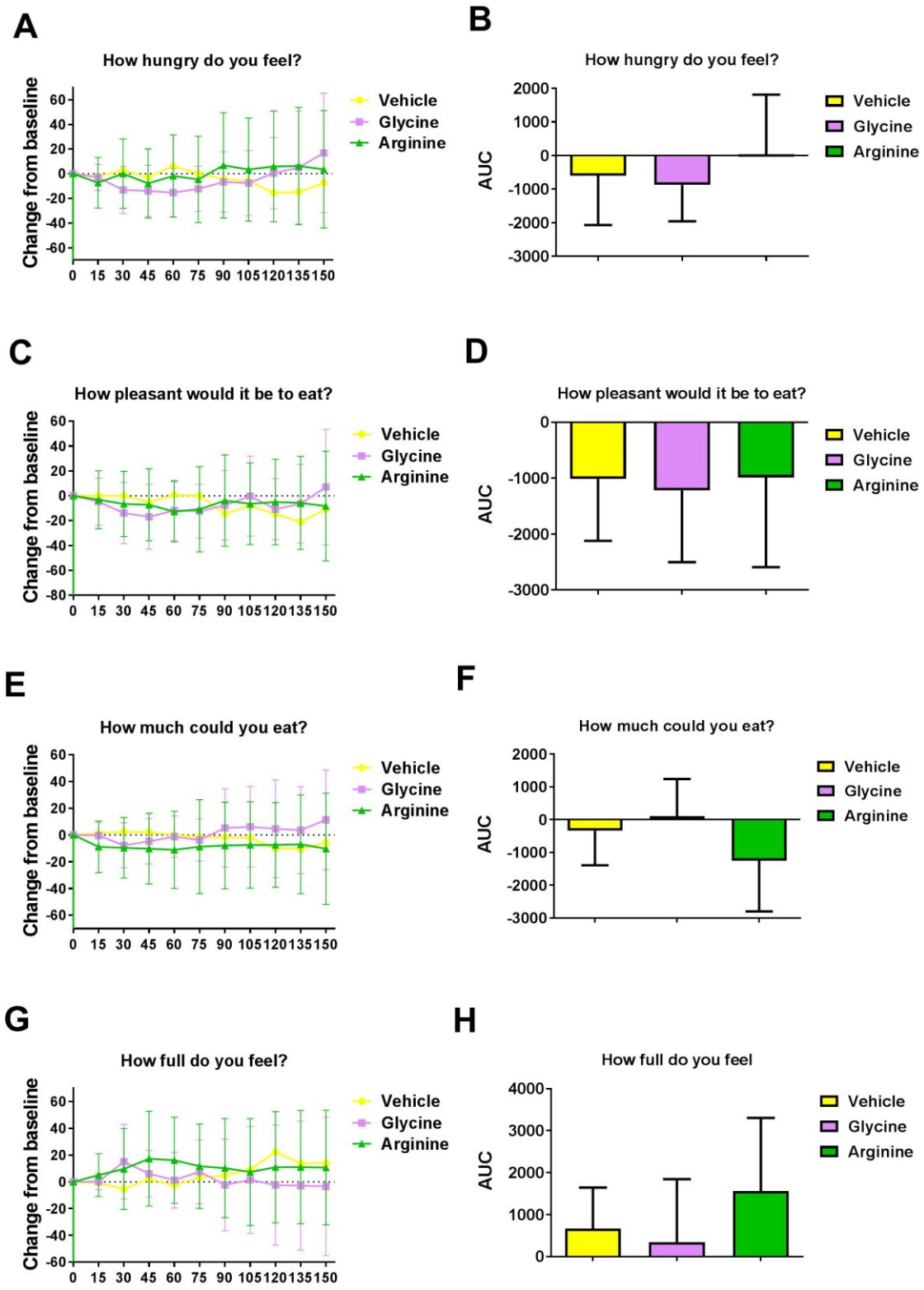


Figure 2.5. **Analysis of VAS ratings of 7 subjects following administration of vehicle, glycine or arginine.**

Subjects consumed vehicle, glycine or arginine at t=0, following an overnight fast. During the study, subjects completed 100 mm visual analogue scale questionnaires relating to appetite. A, How hungry do you feel? B, AUC for How hungry do you feel? C, How pleasant would it be to eat? D, AUC for How pleasant would it be to eat? E, How much could you eat? F, AUC for How much could you eat? G, How full do you feel? H, AUC for How full do you feel? $AUC_{0\text{-}150}$ displayed as mm·min. Data are expressed as mean + SEM.

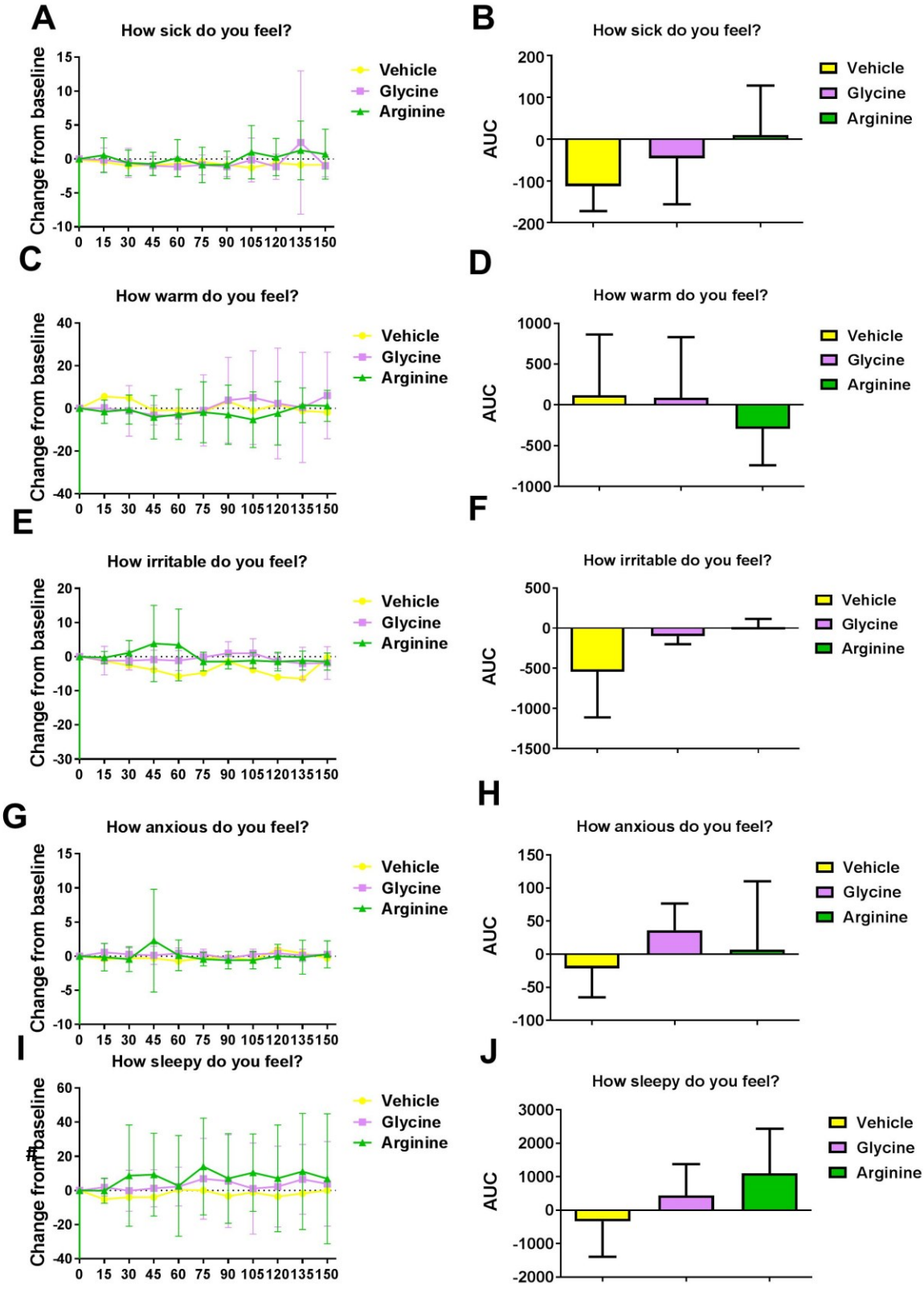


Figure 2.6. **Analysis of VAS ratings of 7 subjects following administration of vehicle, glycine or arginine.**

Subjects consumed vehicle, glycine or arginine at t=0, following an overnight fast. During the study, subjects completed 100 mm visual analogue scale questionnaires relating to appetite. A, How sick do you feel? B, AUC for How sick do you feel? C, How warm do you feel? D, AUC for How warm do you feel? E, How irritable do you feel? F, AUC for How irritable do you feel? G, How anxious do you feel? H, AUC for How anxious do you feel? I, How sleepy do you feel? J, AUC for How sleepy do you feel? $AUC_{0\text{-}150}$ displayed as mm·min. Data are expressed as mean ± SEM.

2.4.1.4. **The effect of L-arginine on plasma gut hormones**

L-arginine had no significant effect on plasma ghrelin (Figure 2.7), which was in accord with animal studies performed within our group.

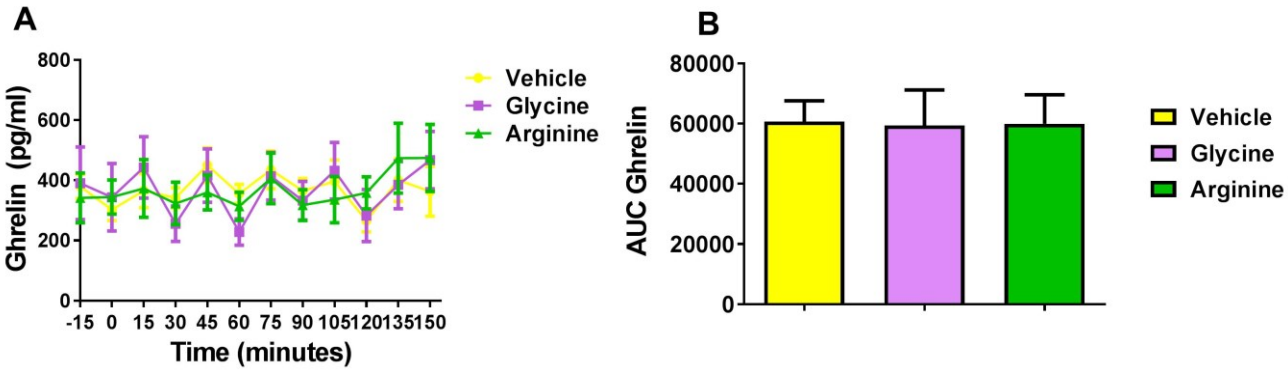


Figure 2.7. The effect of L-arginine on ghrelin release in healthy volunteers.
The effect of oral ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (*n*=7) on (A) plasma acylated ghrelin (B) AUC of plasma acylated ghrelin. $AUC_{0\text{-}150}$ displayed as pg/ml·min. Data are expressed as mean ± SEM.

There was a trend for an increase in plasma GLP-1 following ingestion of L-arginine, though this effect did not achieve statistical significance (Figure 2.8).

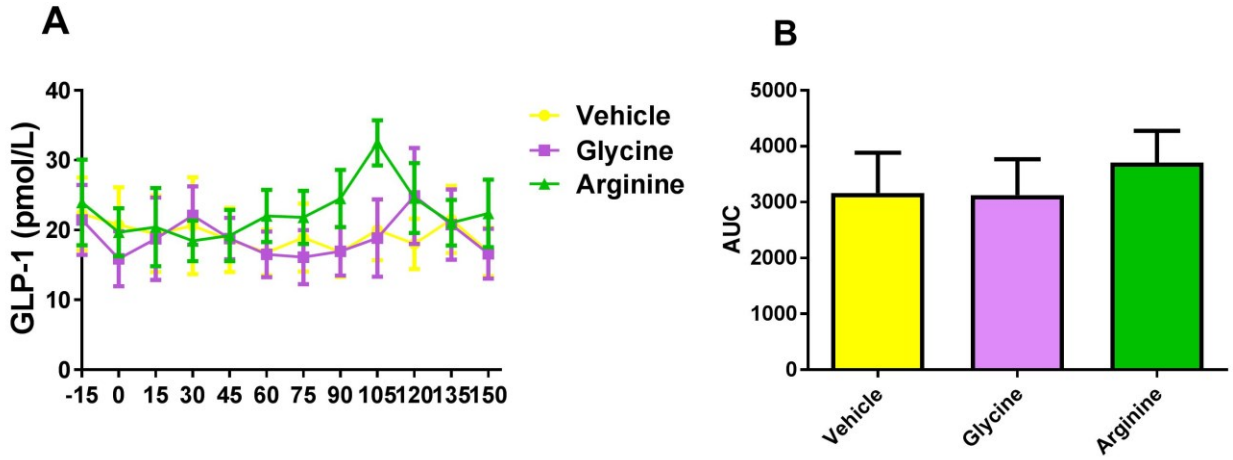


Figure 2.8. The effect of L-arginine on GLP-1 release in healthy volunteers.
The effect of oral ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (*n*=7) on (A) total GLP-1 (B) AUC of total GLP-1. $AUC_{0\text{-}150}$ displayed as pmol/l·min. Data are expressed as mean ± SEM.

There was no significant increase in plasma PYY following ingestion of L-arginine (Figure 2.9).

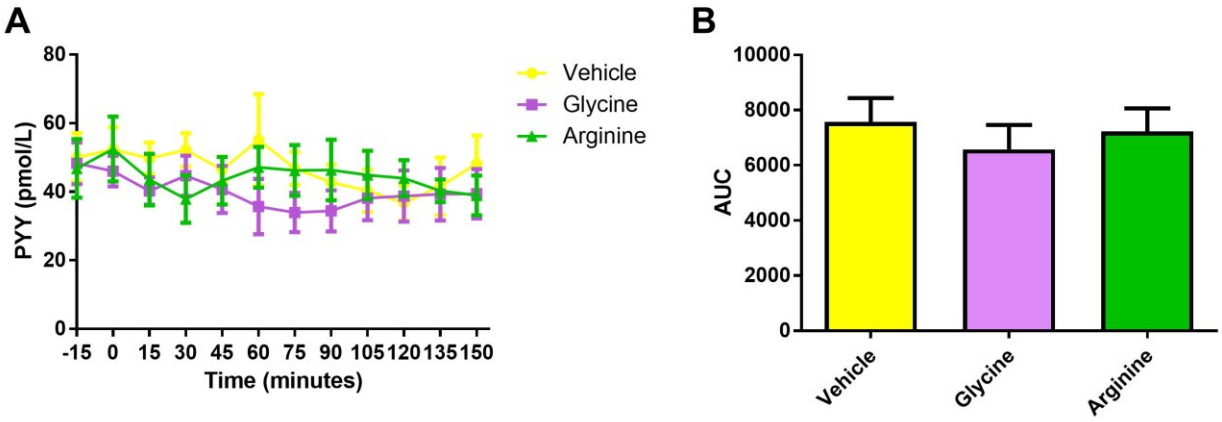


Figure 2.9. The effect of L-arginine on PYY release in healthy volunteers.
The effect of oral ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (*n*=7) on (A) PYY (B) AUC of PYY. $AUC_{0\text{-}150}$ displayed as pmol/l·min. Data are expressed as mean ± SEM.

2.4.2. Gut hormone and appetite study

2.4.2.1. **Baseline characteristics**

Healthy male (n=1) and female (n=8) participants with a mean (± s.d.) age of 36.0 ± 10.8 years and BMI of 25.1 ± 3.5 kg/m^2 who had been weight stable for three months prior to study enrolment were recruited. The clinical characteristics of the recruited participants are summarized in Table 2.2.

Characteristic	All subjects
Age (yr)	36.0 ± 10.8
Female:Male	8:1
Weight (kg)	69.4 ± 16.4
Height (m)	1.65 ± 0.09
BMI (kg/m^2)	25.1 ± 3.5
Blood pressure	113/74 ± 6/5
Ethnic group	
Caucasian	6
Chinese	1
Mixed race	2

Table 2.2. Baseline characteristics of the 5 participants recruited for the gut hormone and appetite.
Data represent the mean ± SD. BMI: body mass index.

2.4.2.2. **Tolerability of L-arginine**

L-arginine was well-tolerated, with no self-reported side-effects following administration. In addition, there were no significant changes on the visual analogue scales with regards to nausea.

2.4.2.3. **The effect of L-arginine on subjective measures of appetite using visual analogue scales**

There was no significant difference in subjective measures of appetite using visual analogue scales following ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (Figures 2.10 and 2.11)

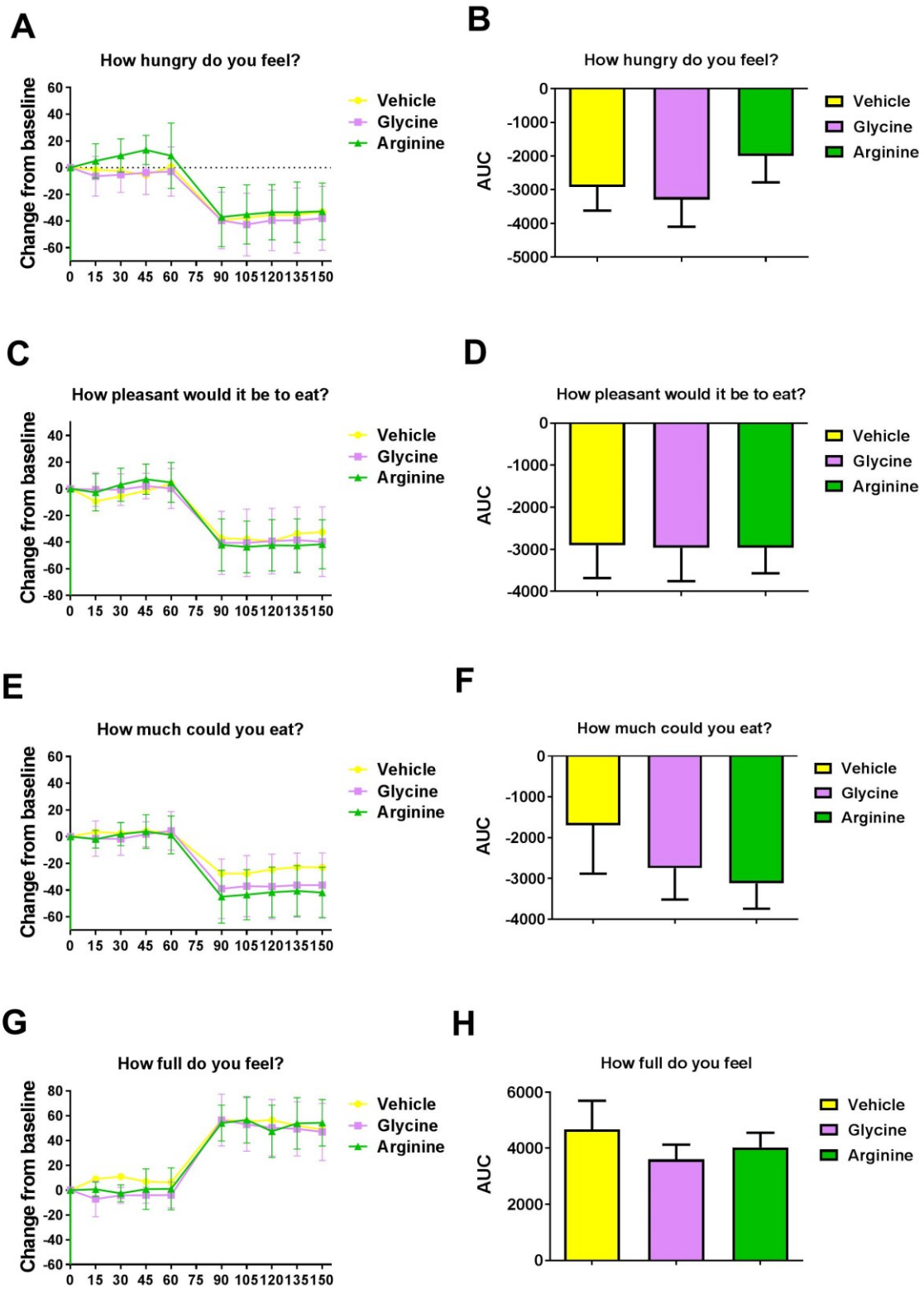


Figure 2.10. Analysis of VAS ratings of 9 subjects following administration of vehicle, glycine or arginine.

Subjects consumed vehicle, glycine or arginine at t=0, following an overnight fast with an a*d libitum* meal at t=60. During the study, subjects completed 100 mm visual analogue scale questionnaires relating to appetite. A, How hungry do you feel? B, AUC for How hungry do you feel? C, How pleasant would it be to eat? D, AUC for How pleasant would it be to eat? E, How much could you eat? F, AUC for How much could you eat? G, How full do you feel? H, AUC for How full do you feel? $AUC_{0\text{-}150}$ displayed as mm·min. Data are expressed as mean + SEM.

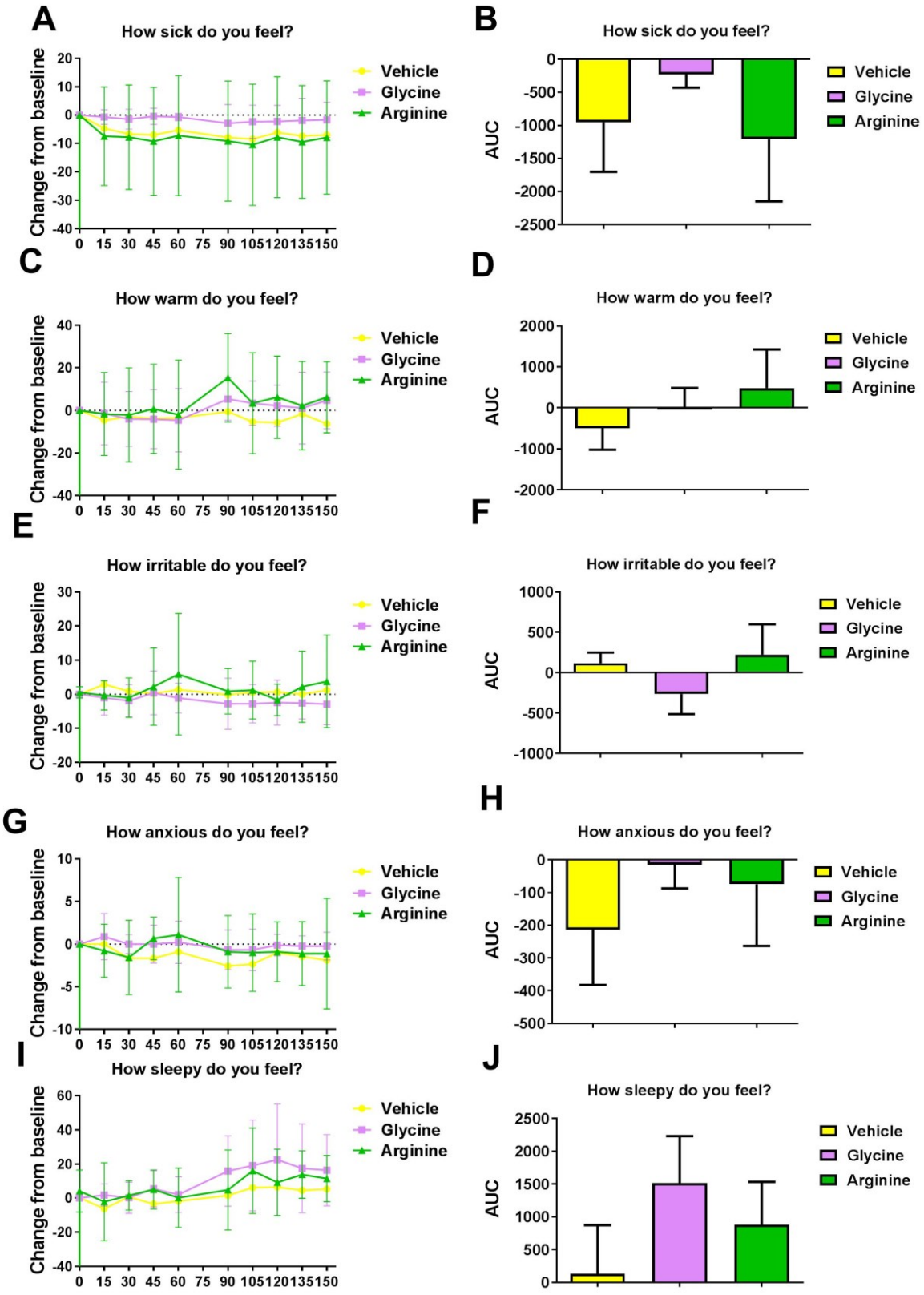


Figure 2.11. Analysis of VAS ratings of 9 subjects following administration of vehicle, glycine or arginine.

Subjects consumed vehicle, glycine or arginine at t=0, following an overnight fast with an a*d libitum* meal at t=60. During the study, subjects completed 100 mm visual analogue scale questionnaires relating to appetite. A, How sick do you feel? B, AUC for How sick do you feel? C, How warm do you feel? D, AUC for How warm do you feel? E, How irritable do you feel? F, AUC for How irritable do you feel? G, How anxious do you feel? H, AUC for How anxious do you feel? I, How sleepy do you feel? J, AUC for How sleepy do you feel? $AUC_{0\text{-}150}$ displayed as mm·min. Data are expressed as mean ± SEM.

2.4.2.4. **The effect of L-arginine on plasma gut hormones**

L-arginine had no significant effect on plasma CCK (Figure 2.12).

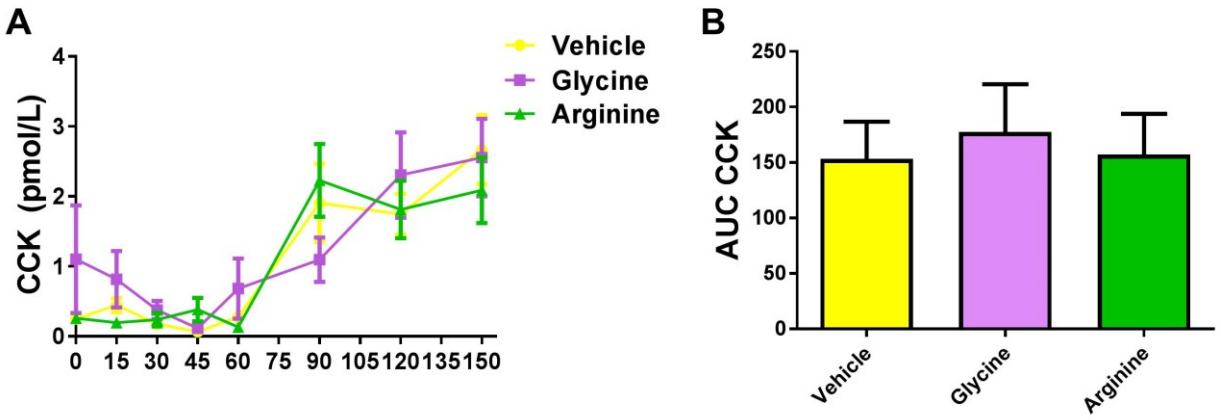


Figure 2.12. The effect of L-arginine on CCK release in healthy volunteers.
The effect of oral ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (*n*=9) on (A) CCK (B) AUC of CCK. Subjects consumed vehicle, glycine or arginine at t=0, following an overnight fast with an a*d libitum* meal at t=60. $AUC_{0\text{-}150}$ displayed as pmol/l·min. Data are expressed as mean ± SEM.

L-arginine significantly increased plasma GLP-1 levels following ingestion of the *ad libitum* meal compared with levels following vehicle treatment (*P*<0.05) (Figure 2.13).

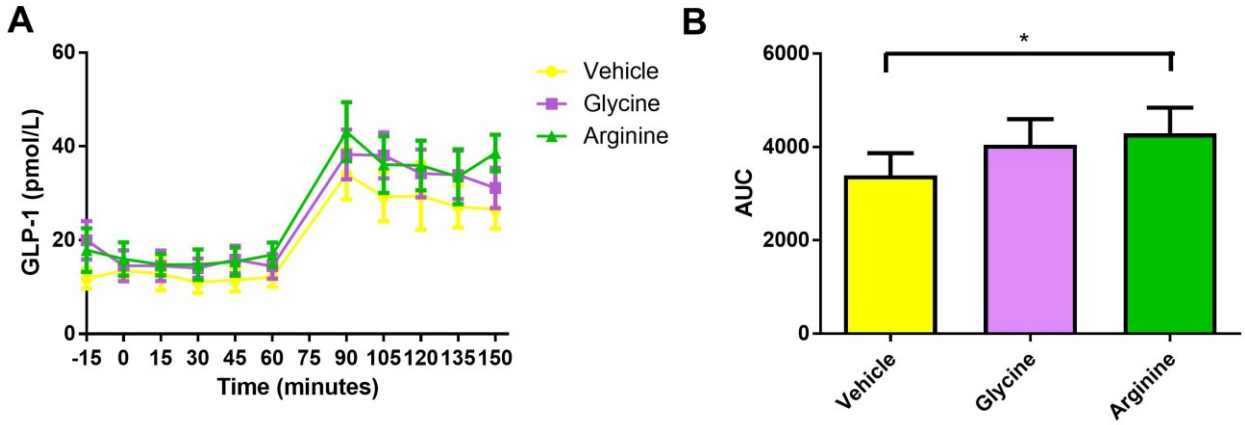


Figure 2.13. The effect of L-arginine on total GLP-1 release in healthy volunteers.
The effect of oral ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (*n*=9) on (A) total GLP-1 (B) AUC of total GLP-1. Subjects consumed vehicle, glycine or arginine at t=0, following an overnight fast with an a*d libitum* meal at t=60. $AUC_{0\text{-}150}$ displayed as pmol/l·min. Data are expressed as mean ± SEM. *p<0.05

L-arginine significantly increased plasma PYY levels following administration of the *ad libitum* meal compared with levels following vehicle treatment (P<0.001) (Figure 2.14).

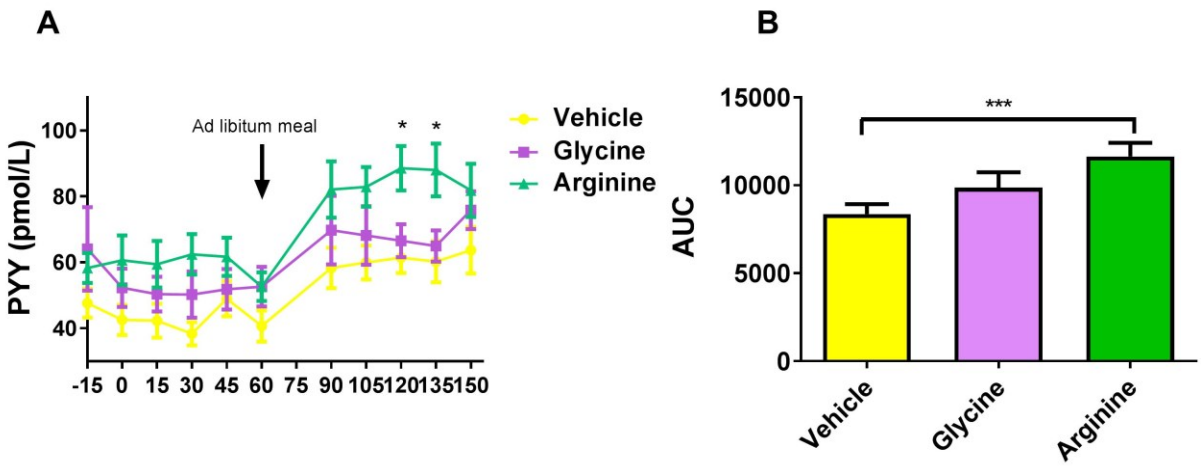


Figure 2.14. The effect of L-arginine on PYY release in healthy volunteers.
The effect of oral ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (n=9) on (A) PYY (B) AUC of PYY. Subjects consumed vehicle, glycine or arginine at t=0, following an overnight fast with an a*d libitum* meal at t=60. $AUC_{0\text{-}150}$ displayed as pmol/l·min. Data are expressed as mean ± SEM. *p<0.05 ***p<0.001.

2.4.2.5. **The effect of L-arginine on acute food intake**

There was no difference in energy intake or subjective measures of appetite using visual analogue scales following ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (Figure 2.15).

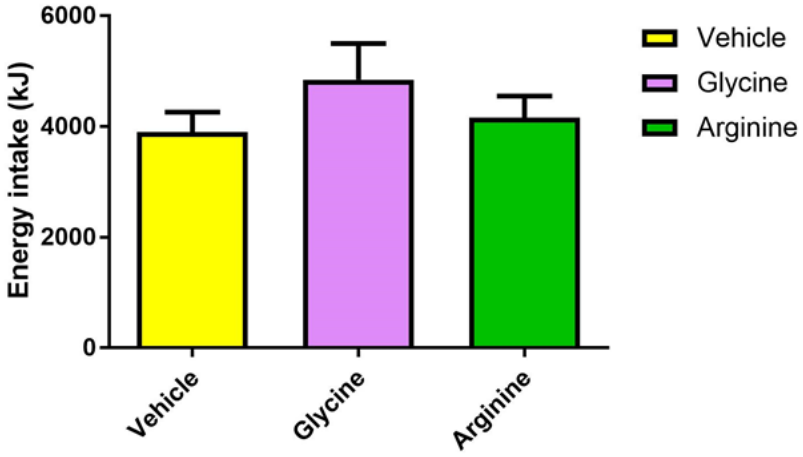


Figure 2.15. The effect of L-arginine on energy intake in healthy volunteers.
The effect of oral ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (n=9) on acute energy intake following an *ad libitum* meal. Data are expressed as mean ± SEM.

2.4.2.6. **The effect of L-arginine on subsequent food intake using food diaries**

Assessment of subsequent energy intake by self-reported food diaries showed a trend towards lower energy intake and particularly fat intake (Figure 2.16), though these effects did not achieve statistical significance.

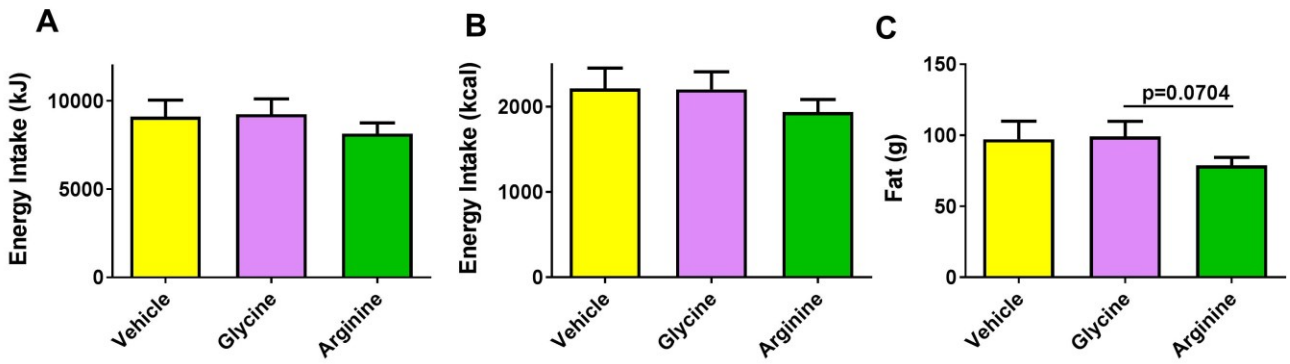


Figure 2.16. The effect of L-arginine on 12 hour food intake in healthy volunteers.
The effect of oral ingestion of vehicle, 17.2mmol arginine or 17.2mmol glycine (*n*=9) on energy intake kJ following study visit (A), 12 hour energy intake in kcal following study visit (B) and 12 hour fat intake following study visit (C) as reported by food diaries. Data are expressed as mean ± SEM.

2.5. **Discussion**

High protein diets induce satiety and reduce weight in both humans and animal models. It is thought that the anorectic effects of high protein diets may be related to the amino acid composition of the protein ingested (Veldhorst et al., 2008). The studies in this chapter investigated the effects of L-arginine on appetite in humans. L-arginine does have effects on other endocrine systems, which include its role in the stimulation of GnRH/LH secretion (Recabarren et al., 1996).

Supplementing foods with specific amino acids selected for their appetite-reducing effects may represent a novel approach for the prevention of weight gain or the treatment of obesity. The eventual goal would be to design foods or dietary regimens that cause an increased sense of fullness and encourage the individual to stop eating sooner, thus reducing total energy intake (Hill and Peters, 2002, Halford and Harrold, 2012). The aim in these studies was thus to investigate the effects of an amino acid administered at physiological levels (i.e. similar to the amount present in a high protein meal) on anorectic gut hormone release and the regulation of appetite.

L-arginine appeared a good candidate for an appetite-reducing amino acid. L-arginine is a conditionally essential amino acid which can activate three of the known promiscuous amino acid sensing receptors, and in particular the T1R1/T1R3 receptor in rodents (Wellendorph et al., 2009a). Previous work has shown that L-arginine reduces food intake and elevates circulating levels of GLP-1 and PYY in rodents (Jordi et al., 2013a, Clemmensen et al., 2013b, Alamshah et al., 2016a), whilst other L-amino acids, including glycine, had no effect (McGavigan et al., 2015). There is evidence that oral L-arginine acts as a GLP-1 secretagogue both *in vitro* (Mace et al., 2012) and *in vivo* in rodents (Clemmensen et al., 2013b). However, to date there is no evidence of this effect in humans. The effect of L-arginine on circulating levels of appetite-modulating gastrointestinal hormones in humans, and the consequent effects on appetite was thus investigated in these two human studies.

Following observations that L-arginine was well tolerated with no reported side-effects, and showed a trend for an increase in plasma GLP-1 release, the effect of L-arginine on gut hormone release and food intake was investigated. The second study showed that in combination with an *ad libitum* meal, L-arginine significantly elevates GLP-1 and PYY in healthy human volunteers compared to vehicle treatment. However, the baselines for both the vehicle and the L-arginine study days are not identical (mean PYY at t=-15 minutes of 58.3 pmol/L on the L-arginine visit versus mean PYY of 47.6pmol/L on the vehicle visit). It is thus possible that the significant post-prandial effects may not be as pronounced as they seem. Similar to the tolerability study, L-arginine, at the doses administered, did not result in any side-effects – either self-reported or from analysis of visual analogue scales.

The effects observed suggest L-arginine may augment post-prandial release of anorectic gut hormones. The mechanisms by which L-arginine stimulates release of plasma GLP-1 and PYY remain unclear (Clemmensen et al., 2013b). Interestingly, there may be delayed effects of L-arginine on appetite as suggested by the trends in the self-reported food diaries. L-arginine appears to have effects on energy intake and fat intake subsequent to the study period. This may be a result of the post prandial effects on gut hormone release seen in the second study. L-arginine has been shown to have a delayed and sustained anorectic effect in animals, with a reduction in food intake when food was returned 8 hours after administration in mice (Alamshah et al., 2016a).

A high protein meal consisting of 200g of cooked chicken would be expected to contain 3-5 grams of arginine, and hence doses of L-arginine used (approximately 3 grams) were comparable to those that might be ingested at a single high protein meal. However, it is possible that the release profile of similar amounts of L-arginine within the gut might be quite different from hypromellose capsules compared to from whole protein ingestion. At these physiological doses, administration of L-

arginine along with other macronutrients (from the meal) results in the release of anorectic gut hormones. It is likely that the release of L-arginine is a gradual process once it is in the small intestine, and it is difficult to know how far down the gastrointestinal tract significant concentrations of L-arginine would reach before being absorbed. From these studies, it is possible that L-arginine is exerting its effects on gut hormone release at around 120 minutes; this may reflect the point at which it comes in direct contact with amino acid sensing L cells in the distal small intestine. There is a higher concentration of L cells in the distal small intestine (Habib et al., 2012, Eissele et al., 1992), and the elevation in PYY levels observed following a high protein meal is sustained for several hours (Batterham et al., 2006b, van der Klaauw et al., 2012). It may be this delayed effect is a result of direct sensing of amino acids in the distal small intestine, perhaps several hours after ingestion. The lack of suppression of ghrelin is in line with work carried out in our group looking at the effect of L-arginine in rodents (unpublished data).

Further studies are required to investigate the effects of L-arginine in combination with other macronutrients to determine whether this can result in a reduction in appetite. These studies had a small number of participants, as it was initially a pilot study to determine tolerability, with the second study being powered to detect a 30% change in gut hormone release. The dose of L-arginine was chosen as it corresponded with levels found in a high-protein meal, and therefore allowed a physiological response to be observed. It is possible that higher doses of L-arginine may have a greater effect on gut hormone release that would be sufficient to drive changes in appetite. The doses of L-arginine administered in the human studies were relatively small compared to those administered to rodents from work in our laboratory. It is possible that L-arginine may exert effects at higher doses in humans. However, 3g L-arginine a day is also the maximum dose considered to be a foodstuff rather than a drug by the Medicines and Healthcare products Regulatory Agency (MHRA), and effectiveness at this dose would facilitate the design of a supplement subject to food law rather than drug law. It is also possible that co-administering L-arginine with other specific macronutrients may result in changes in gut hormone release sufficient to influence food consumption and satiety. Further work is thus required to determine the effect of L-arginine when combined with other nutrients on gut hormone release and appetite.

Chapter 3:

Identifying the metabolites responsible for mediating the effects of macronutrients on gut hormone release and glucose homeostasis

3.1. **Introduction**

3.1.1. Diets of altered macronutrient intake

Standard diet in UK adults provides an average intake of 2470±635 kcal/day for men and 1827±419kcal/day for women. These calories, which are representative of the average Western diet, are mainly derived from the major macronutrients: 15.6% from protein, 46.6% from carbohydrate and 33.0% from fat (Zhou et al., 2003). Many studies have investigated the effects of diets rich in specific macronutrients, but are complicated by difficulties in varying dietary protein, carbohydrate, and fat content independently of each other and energy content.

Meals rich in specific macronutrients have specific effects on the release of appetite-regulating hormones, glucose homeostasis, and subjective feelings of appetite. The coordinated release of these hormones plays a role in satiation, i.e. the immediate suppression of appetite during a meal, and satiety, i.e. the persistent reduction in appetite that delays subsequent meals. The post-prandial release of the gastrointestinal hormones GLP-1 and PYY is thought to be associated with satiation and satiety. The release of these hormones is triggered from enteroendocrine L cells. Once released, these gut hormones suppress appetite through mechanisms that involve signalling either indirectly via the vagus nerve or directly to appetite centres of the brain. The gut detects these nutrients via complex nutrient sensing mechanisms, as described in Chapter 2. L cells are found throughout the intestine, though they are more common in the distal gut. Interestingly, circulating levels of GLP-1 and PYY rise relatively early following a meal (usually within 10-15 minutes after eating), which is when the digesta is present in the upper intestine (mainly within the stomach and duodenum) and well before it comes into contact with the majority of the L cells of the distal gut (Holst, 2007b). It is still not well understood what triggers this early hormonal release or how such mechanisms might be targeted to maximise satiation.

Recently, several studies have suggested that changes in the macronutrient composition of meals can influence gut hormone release independently of energy content, with differences in the time course (including this early rise) and the peak concentrations achieved (Lomenick et al., 2009, Batterham et al., 2006a). Understanding what happens to macronutrients in the early stages of digestion (in the stomach and upper small intestine), and what metabolites they yield, is critical in understanding the stimulus for early gut hormone release and satiety. Determining the mechanisms of macronutrient-induced satiety could provide a basis for dietary manipulation aimed at providing long-term health benefits, for example reducing appetite and improving glucose homeostasis.

Additive or synergistic effects also may be seen with combinations of the most satiating constituents.

3.1.2. High protein diets

The effects of high protein meals and diets have been described in detail in chapter 2, and include increased satiation and satiety, increased release of anorectic gut hormones, and in the long term, weight loss.

3.1.3. Functional foods

Functional foods are bioactive compounds that can exert biological effects and deliver additional or enhanced physiological benefits over and above their basic nutritional value (Ghanbari et al., 2012, Lobo et al., 2010). The term 'functional foods' encompasses a very wide range of products. Some functional foods are designed around a particular functional ingredient, whilst other functional foods or drinks can be foods fortified with a nutrient that would not usually be present in high concentrations.

Particular bioactive food components can promote health and prevent diseases (Liu, 2003, Hasler, 2000). Some of these products are known to increase satiety, however by identifying more of these, combinations could be developed which may increase or potentiate the effects on satiety. The aim of designing such foods or dietary regimens is to increase the sense of fullness and encourage the individual to stop eating sooner, thus reducing total energy intake (Hill and Peters, 2002). In part, this may be due to the effects of such foods on gut hormone release. Although exogenous gut hormone administration can reduce food intake in humans, often this is linked to side effects such as nausea and requires intravenous or at least subcutaneous administration. Altering the diet to manipulate endogenous gut hormone release may reduce the occurrence of side effects and would be easier to adhere to (Chaudhri et al., 2008b). Better understanding the physiological regulation of appetite may identify novel targets for pharmacological or nutraceutical agents to prevent or treat obesity.

3.1.4. Metabolomics

"Metabolomics" studies metabolites in biological fluids. Following digestion, absorption and metabolism, foods give rise to thousands of different metabolites and the appearance of certain metabolites in particular fluids is characteristic of particular foods (Garcia-Perez et al., 2017, Esko et al., 2017).

Metabolomic approaches allow a comprehensive profiling of the metabolome or "library of metabolites" that provides chemical signatures of metabolic activity. Metabolic profiling of biofluids using spectroscopic technologies can detect thousands of compounds simultaneously, generating a profile that can be related to specific states of health or disease (Posma et al., 2017). Such comprehensive studies of metabolic alterations in disease conditions may facilitate the development of targeted treatments.

Metabolomics studies investigating digestive processes may allow the ingestion of particular macronutrients to be linked to the production of specific metabolites and small molecules that in turn modulate gut hormone release and appetite. Such studies could provide a timeline of the occurrence of particular metabolites within the gastrointestinal tract following meals of varying macronutrient content, and their potential effect on early gut hormone release and satiety.

Using such an approach might allow us to understand the processes by which high protein meals suppress appetite, and suggest the combination of agents required to replicate this effect.

3.2. **Hypothesis and Aims**

Hypothesis:

- A high protein meal will yield digesta with a specific metabolomic profile, elements of which will correlate with gastrointestinal hormone release and satiety.

Aims:

- To determine the effects of meals of varying macronutrient content, including a high protein meal, on gastric and duodenal nutrients and metabolites, gastric emptying times, hormonal response profiles, and subjective measures of appetite and future energy consumption.

3.3. **Methods**

3.3.1. Test meals

The meals used in the study were isocaloric, all containing 500kcal. The dominant nutrient in each meal made up 70% of the total energy content (Table 3.1). Study meals were prepared in the meal

kitchen at NIHR/Wellcome Trust Imperial CRF by a researcher. All study meals were constituted from nutritional food supplements and in the form of a liquid shake. The nutritional supplements pure whey isolate™ 90 (Bulk Powder, Essex, UK) powder, Maxijul super soluble (Nutricia, Wiltshire, UK) and fresh double cream were used as the main source of protein, carbohydrate or fat, respectively in amounts described in Table 3.2.

% of Energy Intake	Protein	Carbohydrate	Fat
High protein	70	15	15
High carbohydrate	15	70	15
High fat	15	15	70

Table 3.1. Macronutrient composition of study meals.
Meals were isocaloric and contained approximately 500kcal of energy.

Drink 1 - High Protein	Grams	Kcal	CHO(g)	PR(g)	Fat(g)
Cream fresh, double	13	64	0.2	0.2	7
Sugar White	6	24	6.3	0	0
Crusha syrup	5	6	1.3	0.1	0
MAXIJUL glucose polymer	9	34	9	0	0
Whey protein	98	363	2.1	88.2	1
Water	269	0	0	0	0
Sum	400	491	18.9	88.5	8

Drink 2 - High Carbohydrate	Grams	Kcal	CHO(g)	PR(g)	Fat(g)
Cream fresh, double	15	74	0.7	0.2	8.1
Sugar White	6	24	6.3	0	0
Crusha syrup	5	6	1.3	0.1	0
MAXIJUL glucose polymer	80	304	79.8	0	0
Whey protein	21	78	0.4	18.9	0.2
Water	273	0	0	0	0
Sum	400	486	88.5	19.2	8.3

Drink 3 - High Fat	Grams	Kcal	CHO(g)	PR(g)	Fat(g)
Cream fresh, double	72	357	1.2	1.2	38.7
Sugar White	6	24	6.3	0	0
Crusha syrup	5	6	1.3	0.1	0
MAXIJUL glucose polymer	9	34	9	0	0
Whey protein	20	74	0.4	18	0.2
Water	288	0	0	0	0
Sum	400	495	18.2	19.3	38.9

Table 3.2. Composition of test meals
Meals were isocaloric and made up to approximately 500kcal. Protein (PR), carbohydrate (CHO) and fat content varied according to meal being tested.

3.3.2. Enteral tubes and CORTRAK system

This study used 12F 92cm CORTRAK nasogastric and 10F 140cm CORTRAK nasoduodenal tubes (Halyard Health, Georgia, USA) inserted using the CORTRAK system, which uses electromagnetic sensing technology to track and display the path of the feeding tube during placement. The CORTRAK system consists of three major parts: a portable, battery powered monitor unit with an LCD display and touchscreen interface, a single-use polyurethane CORTRAK radiopaque tube and tip, and a receiver unit. Each tube is supplied with a pre-inserted, single use braided stainless steel stylet with a small electromagnetic coil (transmitter) located at the tip. A cable connects the stylet to the monitor unit. Centimetre markings are printed on the tube to aid placement and check for migration during use. The receiver unit is used to track the electromagnetic signal from the transmitting stylet throughout the placement procedure and is attached by a cable to the monitor

unit, which then provides a graphical display of the feeding tube tip location relative to the receiver unit.

3.3.3. Randomization

Randomisation was done by an investigator not directly involved in the study, with the use of opaque, sealed, sequentially numbered envelopes that each contained all three dietary interventions in a random order. The envelopes were stored securely, away from the research site, and opened in sequence by an investigator as each participant was enrolled.

3.3.4. Participants

3.3.4.1. **Participant recruitment**

All participants were recruited by advertisement either from posters at the Imperial College campus sites (Hammersmith Hospital, Charing Cross Hospital, South Kensington) or through the Imperial Clinical Research Facility (ICRF) healthy volunteers database. Potential participants were screened to ensure they were healthy and to exclude those with disordered eating using SCOFF, DEBQ and EAT questionnaires using the formal inclusion and exclusion criteria described in Sections 1.3.3.2 and 1.3.3.3. After providing written informed consent, participants underwent a health screening consisting of questions on their medical history and a physical examination. Height, weight and body fat measurements were also carried out using a segmental body composition analyser (BC-418MA, Tanita). Female participants were required to provide a urine sample to ensure they were not pregnant. Twenty mL of venous blood was taken to measure full blood count, urea and electrolytes, liver and thyroid function and HbA1c. All screening blood analysis was conducted by the Department of Chemical Pathology at Imperial College Healthcare NHS Trust. The research was granted approval by the London Bloomsbury Ethics Committee (reference number 15/LO/1060). All participants gave written informed consent, and the research was planned and performed in accordance with the Declaration of Helsinki.

3.3.4.2. **Inclusion criteria**

Inclusion criteria were: male or female, aged 18 to 60 years (inclusive), normal and overweight/obese people as classified by a BMI 18.5-30 kg/m2 (inclusive) who were otherwise healthy (based on a pre-study assessment) and people willing and able to give written informed consent and to understand, participate and comply with the study requirements.

3.3.4.3. **Exclusion criteria**

Exclusion criteria were: treatment with any medication that might have affected the study outcome (e.g. medication affecting appetite regulation and/or blood flow), haemorrhagic disorders and anticoagulant treatment, inborn errors of metabolism, hepatic impairment as defined by screening visit liver function tests (aspartate aminotransferase (AST) or alanine aminotransferase (ALT) or gamma-glutamyl transpeptidase (GGT) of three times the upper normal reference limit), current pregnancy or breast feeding, delivery within the last year, significant intercurrent disease or history of clinically significant disease of any type, in particular liver, kidney, or heart disease, any form of diabetes mellitus or psychiatric illness, history of cancer (excluding skin cancer), history of severe or multiple allergies, severe adverse drug reaction or leucopaenia, smokers, regular drinkers of more than three units of alcohol daily, history of, or current evidence of, abuse of alcohol or any drug substance, licit or illicit, use of dietary supplements (e.g. omega 3/amino acids) either continuously or intermittently in the preceding 12 months, lactose or milk protein intolerance, oesophageal stricture, regular intake of over-the-counter medication (other than the occasional paracetamol/aspirin), poor compliers or participants unlikely to attend, and blood donation within the 12 week period before the initial study dose.

3.3.4.4. **Withdrawal criteria**

Withdrawal criteria were: significant deviation from the approved protocol, the participant no longer satisfying the inclusion and exclusion criteria set out by the approved protocol, a positive pregnancy test result, severe anxiety during the enteral tube placement and the participant withdrawing consent.

3.3.5. Study design

3.3.5.1. **Main study**

Participants attended for a single four-day study visit, the outline of which is summarised in Figure 3.1.

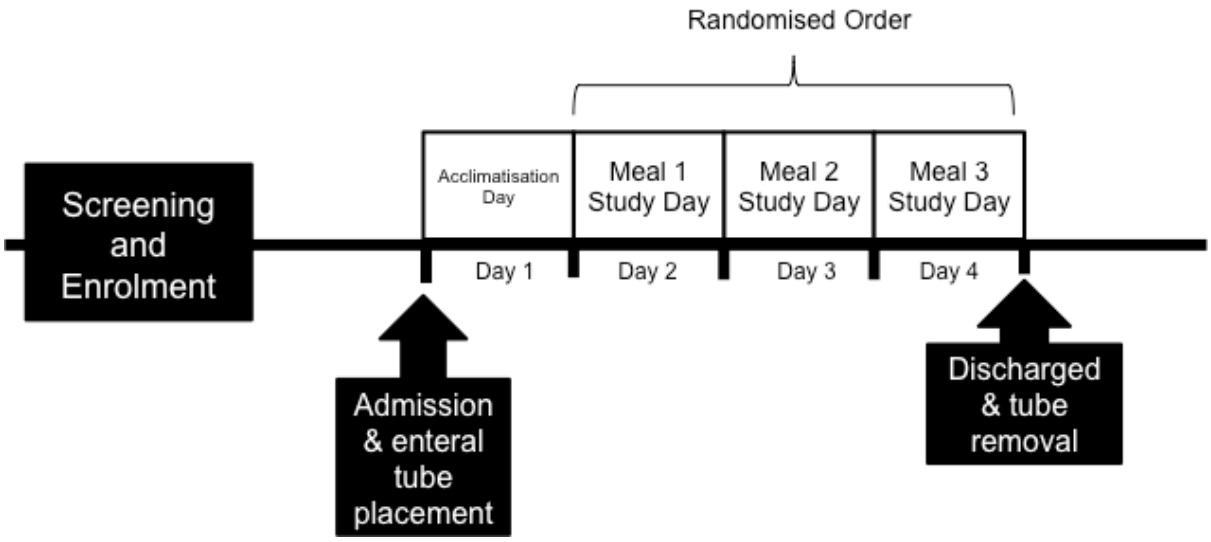


Figure 3.1. Timeline for a typical study day

Participants reported to the clinical research facility at 0830 hours (day 1) having fasted from 2100 hours the night before. They were asked to refrain from alcohol and strenuous exercise for 24 hours prior to arriving at the research facility. On arrival, participants had a 12F 92cm CORTRAK nasogastric tube inserted into one nostril and a 10F 140cm CORTRAK nasoduodenal tube inserted into the other nostril. The ends of each tube were in the stomach and D2/D3 of the duodenum respectively. Participants were free to eat and drink after insertion of the tubes. Day 1 was considered to be an acclimatisation day so that participants could get used to the sensation of having the enteral tubes in. The evening of day 1, participants were requested to consume a meal of their choice, supplied by the ICRF, at approximately 2000 hours. They would then eat the identical meal at the same time every evening that they took part in the study. Following this evening meal, they would be requested to fast from 9pm until the next morning. On the morning of day 2 at 0830 hours, an intravenous cannula was inserted in the antecubital fossa for serial blood sampling. Participants were asked to consume a liquid meal (high-protein, high-fat or high carbohydrate) at the beginning of each study morning in a single-blinded randomised order. Blood samples were taken at the following time-points: -15, 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150 and 180 minutes. Blood for gut hormone measurement (3mL) was transferred to lithium heparin-coated tubes (International Scientific Supplies Ltd, Bradford, UK) containing 6000 kallikrein inhibitor units (60µL) of aprotinin (Trasylol, Bayer Schering Pharma, Berlin, Germany), a protease inhibitor to prevent the degradation of gut hormones. Blood for metabolomics was transferred to lithium heparin-coated tubes. Blood for glucose analysis was transferred to fluoride oxalate tubes. Blood for insulin analysis was transferred to serum-separating tubes and allowed to clot before centrifugation. Samples underwent centrifugation at 3000g for 10 minutes at 4°C (Hettich Zentrifugen Rotina 420R).

The separated plasma and serum was aliquoted into eppendorf tubes which were stored at -80°C until analysis.

Prior to the study starting, the position of both the gastric and duodenal tubes were checked using the CORTRAK machine, and adjusted if necessary to ensure the ends were in the stomach and D2/D3 of the duodenum respectively. At the same time points as the blood samples were taken, gastric and duodenal samples were also taken using syringes from the nasogastric and nasoduodenal tubes and aliquoted into eppendorfs. All the eppendorfs were stored at -80°C until analysis.

Participants were asked to complete visual analogue scales (VAS) at the same time-points at which blood and gut content were sampled. Participants rated subjective feelings of hunger ('How hungry do you feel right now?'), pleasantness to eat ('How pleasant would it be to eat right now?'), prospective food intake ('How much could you eat right now?'), fullness ('How full do you feel right now?') and sickness ('How sick do you feel right now?') using 100 mm horizontal visual analogue scales (VAS) following ingestion of the test meals. Participants were also asked to report any additional side effects that occurred during the visit or after the study visit was completed. A typical study day is summarized in figure 3.2.

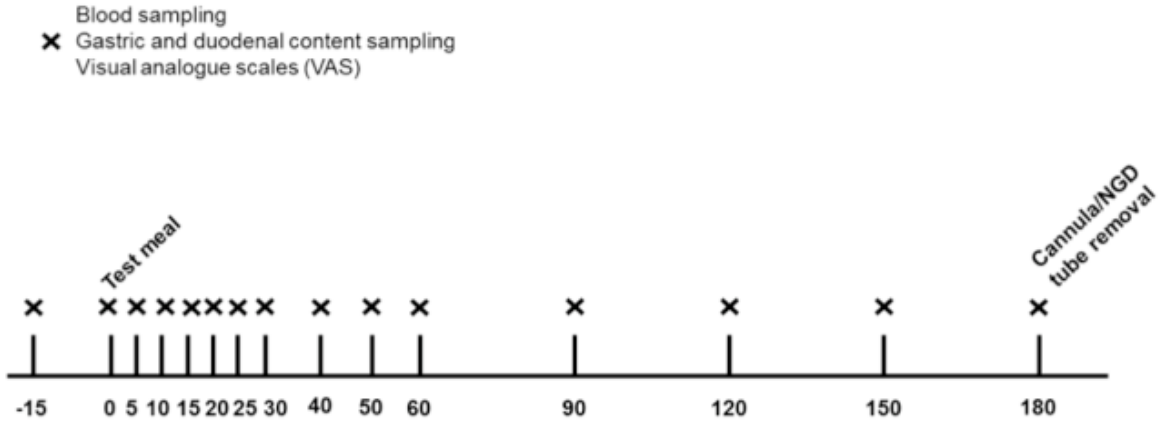


Figure 3.2. Time course of a typical study day.

3.3.5.2. **Gastric emptying study**

Paracetamol was used as a marker for gastric emptying. As there were concerns that paracetamol at the dose to be used could interfere with the metabolomics analysis, a supplementary study was performed on a separate occasion. Five of the original participants attended extra study days to evaluate gastric emptying in response to the three meals. The composition of meals was identical to

the main study, with the exception of 1500mg of Paracetamol (Zentiva, UK) being added to each test meal. Participants were given a different meal on each study day in a randomized order with blood samples being taken at identical time points to the main study (-15, 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150 and 180 minutes). This supplementary study did not require insertion of the enteral tubes. Plasma samples were analysed using an Acetaminophen L3K Assay (Sekure Chemistry, UK) by the Department of Chemical Pathology at Imperial College Healthcare NHS Trust.

3.3.6. Blood assays

3.3.6.1. **Total GLP-1 and PYY**

These were measured as described in Chapter 2. The limit of detection for GLP-1 was 7.5 pmol/l with an intra-assay variation of 5.3%. The limit of detection for PYY was 2.5 pmol/l, with an intra-assay coefficient of variation of 8.5 %. All samples were assayed in one assay to avoid inter-assay variation.

3.3.6.2. **CCK**

Human plasma CCK-8 concentrations were measured using a commercial radioimmunoassay (Eurodiagnostica, Malmö, Sweden) with a detection limit of 0.3pmol/L. Plasma was separated by centrifugation as described above, frozen and stored at -80C. The assay was carried out as per manufacturer's instructions.

3.3.6.3. **Active ghrelin**

Human plasma active ghrelin concentrations were measured using a commercial enzyme-linked immunosorbent assay (ELISA) (Merck Millipore, Billerica, MA, USA) with a detection limit of 15pg/mL. Plasma was separated by centrifugation as described above, acidified with HCl to a final concentration of 1N (Hosoda et al., 2004), frozen and stored at -80C. The assay was carried out as per manufacturer's instructions.

3.3.6.4. **Neurotensin**

Human plasma neurotensin concentrations were measured using a commercial enzyme immunoassay (EIA) (Ray Biotech, Norcross, GA, USA) with a detection limit of 0.2 ng/mL. Plasma was separated by centrifugation as described above, frozen and stored at -80C. The assay was carried out as per manufacturer's instructions.

3.3.6.5. **Somatostatin**

Human plasma somatostatin concentrations were measured using a commercial enzyme immunoassay (EIA) (Ray Biotech, Norcross, GA, USA) with a detection limit of 10.9 ng/mL. Plasma was separated by centrifugation as described above, frozen and stored at -80C. The assay was carried out as per manufacturer's instructions.

3.3.6.6. **Secretin**

Human plasma secretin concentrations were measured using a specific and sensitive enzyme immunoassay (EIA) (Ray Biotech, Norcross, GA, USA) with a detection limit of 0.3 ng/mL. Plasma was separated by centrifugation as described above, frozen and stored at -80C. The assay was carried out as per manufacturer's instructions.

3.3.6.7. **GIP, Insulin and Glucagon**

Human plasma GIP, insulin and glucagon concentrations were measured using the Milliplex MAP magnetic bead-based multi-analyte, metabolic panel, 4-plex immunoassay (Millipore, St. Charles, MO, USA) and measured using Luminex Magpix with xPONENT software (Luminex Corporation, Austin, TX, USA). The minimum detectable concentration for GIP was 0.6pg/mL, for insulin was 87 pg/mL, and for glucagon was 13 pg/mL. The kit is a capture sandwich immunoassay which uses a capture antibody attached to a microsphere and a detection antibody that incorporates a fluorescent label.

3.3.6.8. **Glucose**

Glucose analysis was performed in the Dept. of Biochemistry, Hammersmith Hospital using a ci8200 Analyser enzymatic method (Abbott Diagnostics, USA). In summary, glucose is phosphorylated by hexokinase. In this reaction one micromole of NADH is produced for every micromole of glucose consumed. NADH absorbs light at 340 nm and can be detected by spectrophotometry. The analyser then calculates the glucose concentration of a sample based on the absorbance.

3.3.7. Metabolomics analysis

This work was carried out in collaboration with the MRC–NIHR National Phenome Centre at Imperial College.

3.3.7.1. **Sample preparation**

Duodenal samples were spun at 17000g at 4°C for 20 minutes. Following centrifugation, 350µL of the supernatant were mixed with 350 µL of pH 7.4 phosphate buffer (0.075M Na_2HPO_4, 2mM NaN_3,

0.08% TSP solution, 20% D_2O). Following this, 550 µL of this solution were added into a 5mm NMR tube. Quality control samples (QCs) were prepared by pooling 50 µL of each of the duodenal samples.

Gastric samples were spun at 17000g at 4°C for 20 minutes. Following centrifugation, 350 µL of the supernatant were mixed with 350 µL of pH 7.4 phosphate buffer (0.4M Na_2HPO_4, 2mM NaN_3, 0.08% TSP solution, 20% D_2O). Following this, 600 µL of this solution were added into a 5mm NMR tube. QCs were prepared by pooling 50 µL of each of the gastric samples.

3.3.7.2. **Sample Analysis, data pre-processing and modelling**

^{1}H-NMR metabolic profiling analyses were performed using established methods (Dona et al., 2014). One QC was analysed every ten samples to evaluate the quality of the analysis. ^{1}H-NMR spectra were manually phased and digitised over the range δ0·5–9·5 and imported into MATLAB (release 2014a). The resulting data set were imported into SIMCA 14.1 (Umetrics) to conduct multivariate statistical analysis. Principal Component Analysis (PCA), followed by Partial Least Square Discriminant Analysis (PLS-DA) and Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) was performed to examine the data sets and to observe clustering in the results according to the predefined classes.

3.3.7.3. **Metabolite identification**

A combination of data-driven approaches such as SubseT Optimization by Reference Matching (STORM) and Statistical TOtal Correlation SpectroscopY (STOCSY) in combination with experimental structural elucidation techniques (1D 1H NMR and 2D 1H-1H NMR and 1H-13C NMR experiments) and spiking-in of chemical standards was used to aid structural identification of diet-discriminatory metabolites.

3.3.8. Statistical analysis

There are no published studies using a metabolomics approach to the analysis of gut contents. This study was thus a pilot study and so did not use a power calculation.

Data are presented as the mean ± standard error of the mean (SEM). Plasma hormones, glucose and VAS data were analysed using statistical software (SPSS Statistics Version 22 (IBM, USA)). Normality of data was verified with the Kolmogorov-Smirnov test. Comparisons of VAS scores, plasma hormones and glucose were by repeated measures ANOVA, followed by a post hoc Bonferroni's multiple comparison test. Area under the curve (AUC) was generated for all data sets, with a one-

way ANOVA used to detect statistical significance followed by a post hoc Bonferroni's multiple comparison test. All graphs were created using statistical software, Prism 6 (GraphPad, USA). The threshold for statistical significance in each case was set at $p<0.05$.

The statistical analyses used for the metabolite identification is outlined in Section 3.3.7.2.

3.4. **Results**

3.4.1. Baseline characteristics

Healthy male (n=6) and female (n=4) participants with a mean (± s.d.) age of 37.9 ± 15.9 years and BMI of 22.4 ± 3.0 kg/m^2 and body fat of 19.4 ± 12.5 %, who had been weight stable for three months prior to study enrolment were recruited. The clinical characteristics of the recruited participants are summarized in Table 3.3.

Characteristic	All subject
Female:Male	4:6
Height (m)	1.77 ± 0.11
% Body fat	19.4 ± 12.5
Ethnic group	
Caucasian	9
South Asian	1

Table 3.3. Baseline characteristics of the 10 recruited participants.
Data represent the mean ± SD. BMI: body mass index.

3.4.2. The effect of meals of altered macronutrient intake on subjective measures of appetite using visual analogue scales

There was no significant difference in subjective measures of appetite using visual analogue scales following ingestion of high carbohydrate, high fat and high protein meals (Figure 3.3). In addition, there were no significant changes on the visual analogue scales with regards to other factors such as nausea (Figure 3.4). Performing AUC analysis for the VAS results over the first 90 minutes following the meal, which might better detect acute and short-lived changes following the test meal, did not identify any significant differences between meals, though there was a strong trend for the high protein meal to reduce prospective food intake. These data are shown in appendix 3.

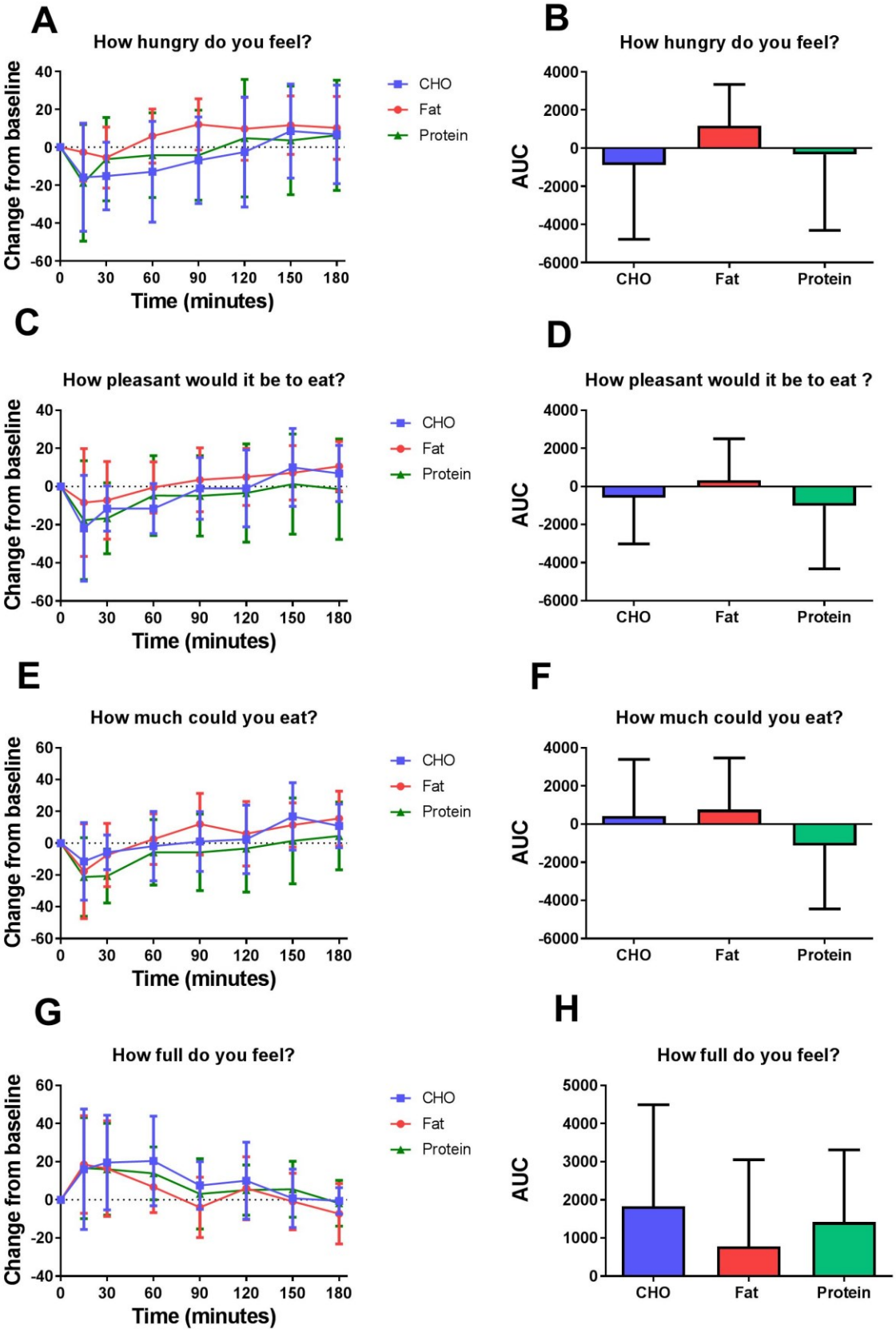


Figure. 3.3. **Analysis of VAS ratings of 10 subjects following administration of a high carbohydrate, high fat or high protein meal.**

Subjects consumed a high carbohydrate, high fat or high protein meal at t=0, following an overnight fast. During the study, subjects completed 100 mm visual analogue scale questionnaires relating to appetite. A, How hungry do you feel? B, $AUC_{0-180min}$ for How hungry do you feel? C, How pleasant would it be to eat? D, $AUC_{0-180min}$ for How pleasant would it be to eat? E, How much could you eat? F, $AUC_{0-180min}$ for How much could you eat? G, How full do you feel? H, $AUC_{0-180min}$ for How full do you feel? AUC_{0-180} displayed as mm·min. Data are expressed as mean + SEM.

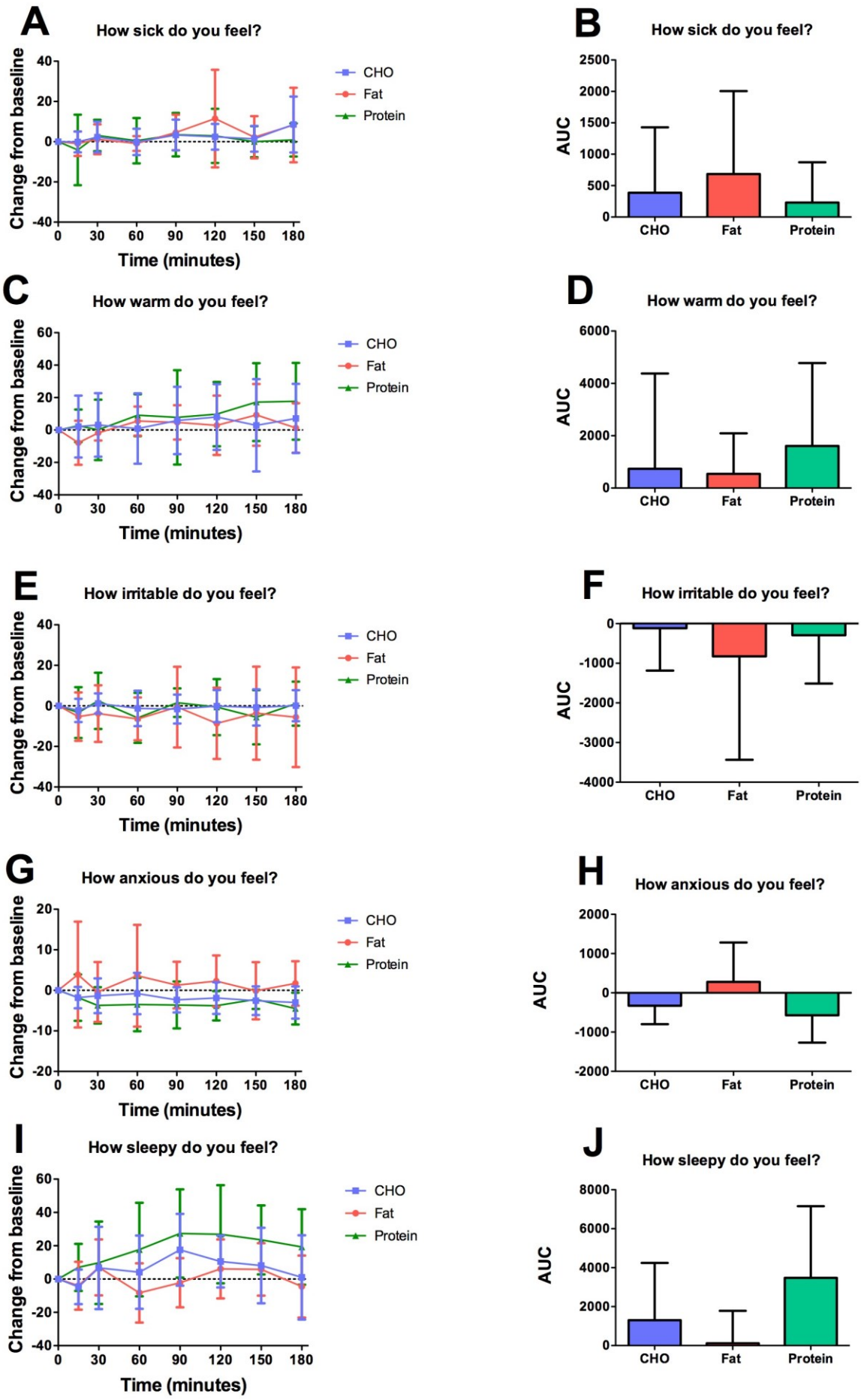


Figure 3.4. Analysis of VAS ratings of 10 subjects following administration of a high carbohydrate, high fat or high protein meal.

Subjects consumed a high carbohydrate, high fat or high protein meal at t=0, following an overnight fast. During the study, subjects completed 100 mm visual analogue scale questionnaires relating to appetite. A, How sick do you feel? B, $AUC_{0--180min}$ for How sick do you feel? C, How warm do you feel? D, $AUC_{0--180min}$ for How warm do you feel? E, How irritable do you feel? F, $AUC_{0--180min}$ for How irritable do you feel? G, How anxious do you feel? H, $AUC_{0--180min}$ for How anxious do you feel? I, How sleepy do you feel? J, $AUC_{0--180min}$ for How sleepy do you feel? AUC_{0-180} displayed as mm·min. Data are expressed as mean ± SEM.

3.4.3. The effect of meals of altered macronutrient intake on plasma gut hormone release

The high protein meal caused a greater increase in circulating levels of GLP-1 compared with a high carbohydrate and high fat meal (Figure 3.5A). This was reflected in AUC_{GLP-1}, which showed a significant difference (p=0.0490) between meals (Figure 3.5B). Further post-hoc analysis revealed a significant difference between the carbohydrate versus the protein meal (Figure 3.5B). In addition, repeated measures ANOVA analysis revealed statistically significant differences at specific time points between meals (Figure 3.5A).

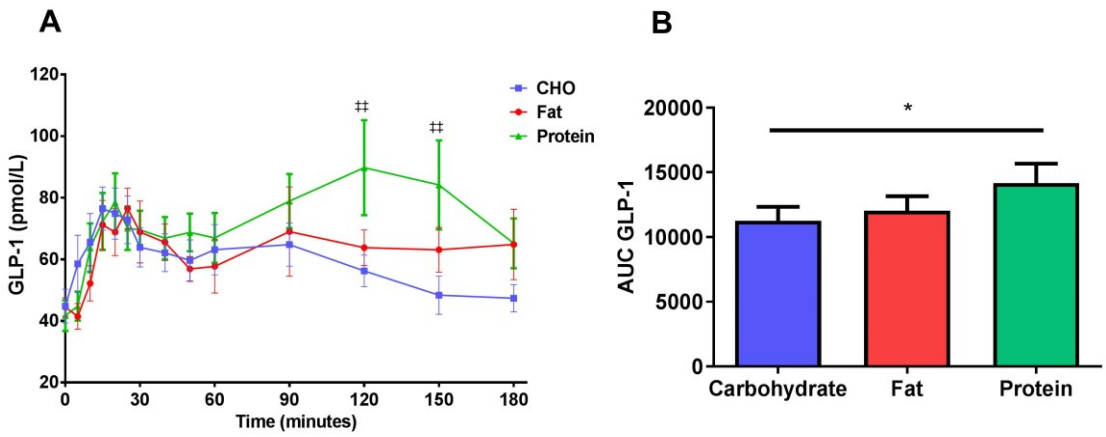


Figure 3.5. Plasma levels of the anorectic gut hormone glucagon- like peptide (GLP-1) in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in GLP-1 (Figure 3.5A) and $AUC_{0--180min}$ (Figure 3.5B) are displayed as mean ± SEM. Meals are provided at t=0 minutes. CHO vs. PRO ‡‡p<0.01. AUC_{0-180} displayed as pmol/l·min. A one-way ANOVA was used to determine statistical significance for $AUC_{0--180min}$ *p<0.05

There were no statistically significant differences observed in circulating PYY levels between meals based on the AUC_{PYY} (p=0.3181) (Figure 3.6B) or repeated measures ANOVA analysis.

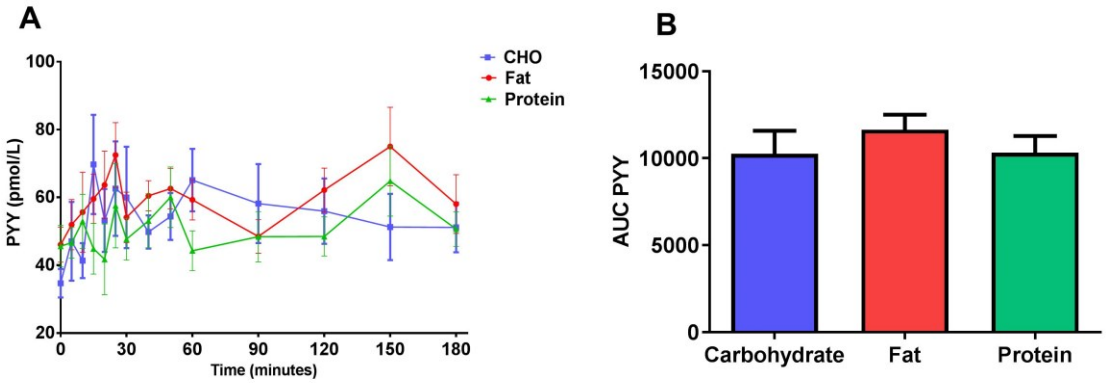


Figure 3.6. Plasma levels of the anorectic gut hormone peptide YY (PYY) in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in PYY (Figure 3.6A) and $AUC_{0-180min}$ (Figure 3.6B) are displayed as mean ± SEM. Meals are provided at t=0 minutes. AUC_{0-180} displayed as pmol/l·min. A one-way ANOVA was used to determine statistical significance for $AUC_{0-180min}$

Similarly, there were no statistically significant differences observed in circulating PP levels between meals based on the AUC_{PP} (p=0.4814) (Figure 3.7B) or repeated measures ANOVA analysis.

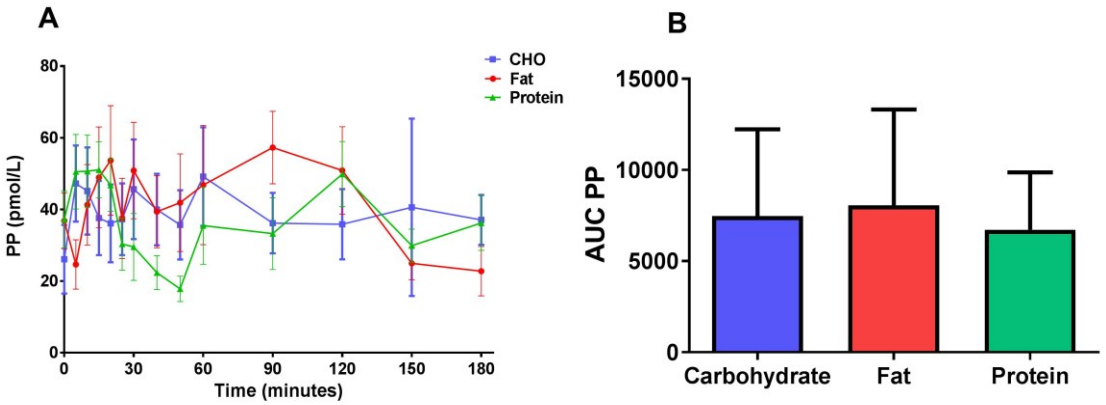


Figure 3.7. Plasma levels of the anorectic gut pancreatic polypeptide (PP) in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in PP (Figure 3.7A) and $AUC_{0-180min}$ (Figure 3.7B) are displayed as mean ± SEM. Meals are provided at t=0 minutes. AUC_{0-180} displayed as pmol/l·min. A one-way ANOVA was used to determine statistical significance for $AUC_{0-180min}$

Cholecystokinin (CCK) levels were found to be highest following a high carbohydrate meal, with AUC_{CCK} showing significant differences between groups (p=0.0307) (Figure 3.8B). Further post-hoc analysis revealed significant differences between the carbohydrate versus fat meals and fat versus protein meals. There were no significant differences at specific time points as assessed by repeated measures ANOVA analysis.

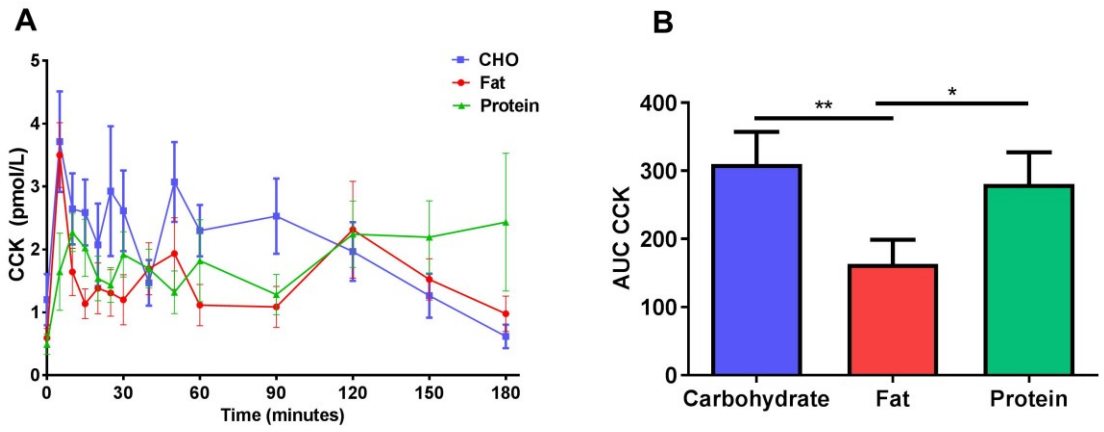


Figure 3.8. Plasma levels of the anorectic gut cholecystokinin (CCK) in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in CCK (Figure 3.8A) and $AUC_{0-180min}$ (Figure 3.8B) are displayed as mean ± SEM. Meals are provided at t=0 minutes. AUC_{0-180} displayed as pmol/l·min. A one-way ANOVA was used to determine statistical significance for $AUC_{0-180min}$ *p<0.05 **p<0.01

GIP levels were found to be highest following a high carbohydrate meal, with AUC_{GIP} showing significant differences between groups (P= 0.0322) (Figure 3.9B). Further post-hoc analysis revealed a significant difference between the carbohydrate versus protein meal. This was reflected in the repeated measures ANOVA, which showed statistically significant differences between the meals at specific time points (Figure 3.9A).

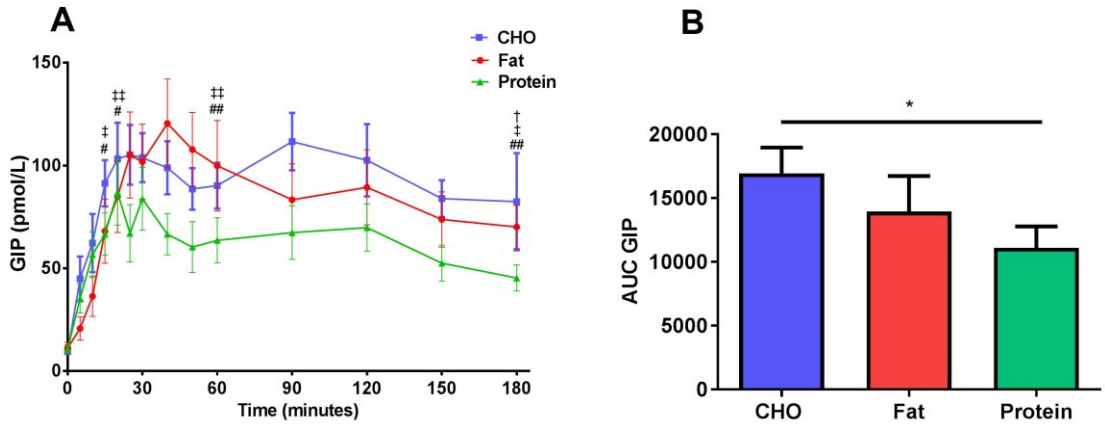


Figure 3.9. Plasma levels of the anorectic gut hormone glucose-dependent insulinotropic polypeptide (GIP) in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in CCK (Figure 3.9A) and $AUC_{0-180min}$ (Figure 3.9B) are displayed as mean ± SEM. Meals are provided at t=0 minutes. CHO vs. PRO ‡‡p<0.01, ‡p<0.05; FAT vs. PRO ##p<0.01, #p<0.05; CHO vs. FAT †p<0.05. AUC_{0-180} displayed as pmol/l·min. A one-way ANOVA was used to determine statistical significance for $AUC_{0-180min}$ *p<0.05

There were no statistically significant differences observed in circulating neurotensin, somatostatin or secretin between meals based on the AUC or repeated measures ANOVA analysis (Figure 3.10).

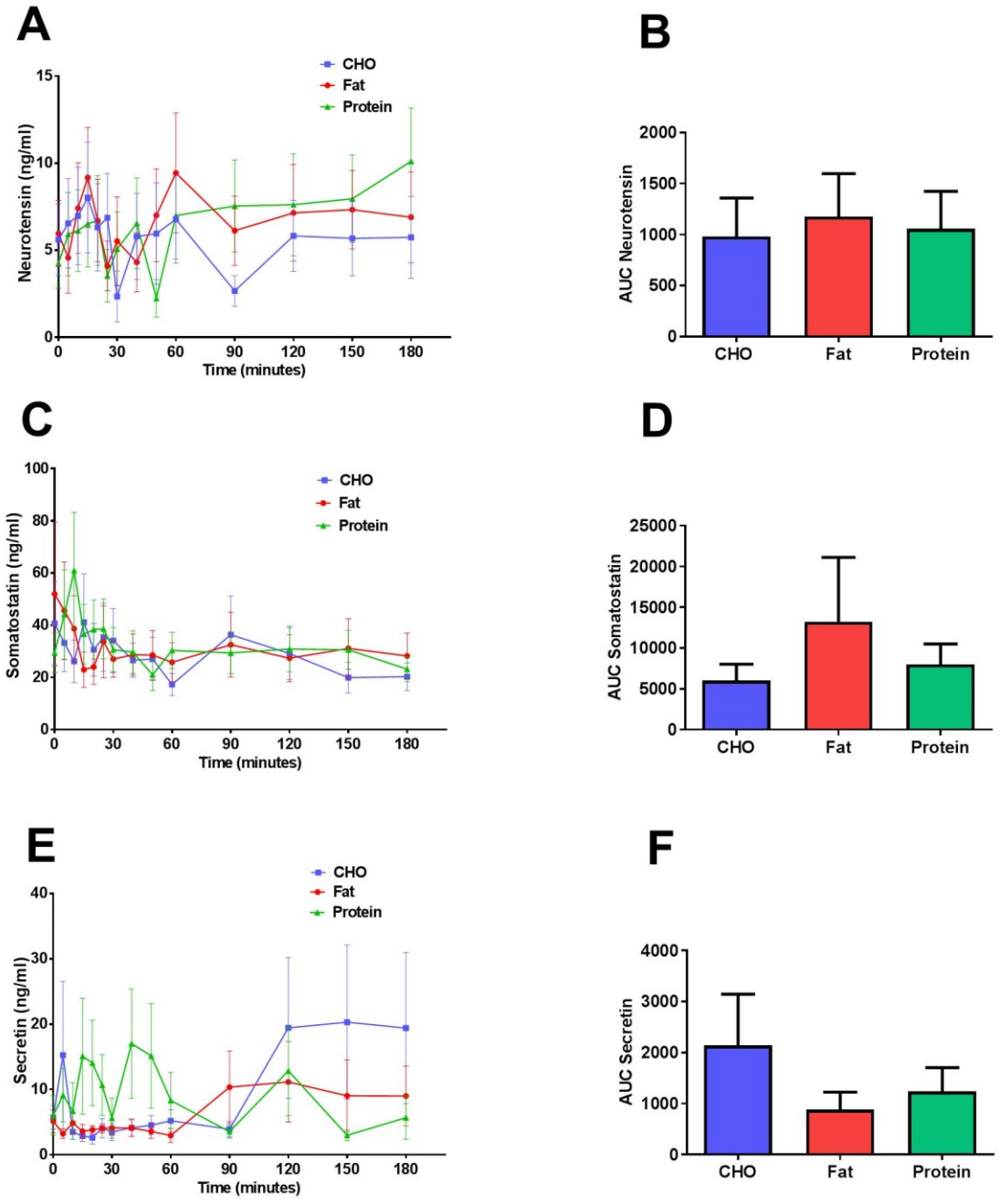


Figure 3.10. Plasma levels of neurotensin, somatostatin and secretin in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in neurotensin (Figure 3.10A) and $AUC_{0\text{--}180min}$ (Figure 3.10B), changes in somatostatin (Figure 3.10C) and $AUC_{0\text{--}180min}$ (Figure 3.10D), changes in secretin (Figure 3.10E) and $AUC_{0\text{--}180min}$ (Figure 3.10F) are displayed as mean ± SEM. Meals are provided at t=0 minutes. $AUC_{0\text{-}180}$ displayed as ng/ml·min.

There were no statistically significant differences observed in circulating ghrelin levels between meals overall based on the total $AUC_{Ghrelin}$ (0-180 minutes) (p=0.4182) (Figure 3.11B) or repeated

measures ANOVA analysis. However the $AUC_{90\text{-}180minutes}$ (Figure 3.11C) showed a statistically significant difference overall ($p<0.05$) however post-hoc analysis using Bonferroni's test for multiple comparisons showed no difference between pairs of meals..

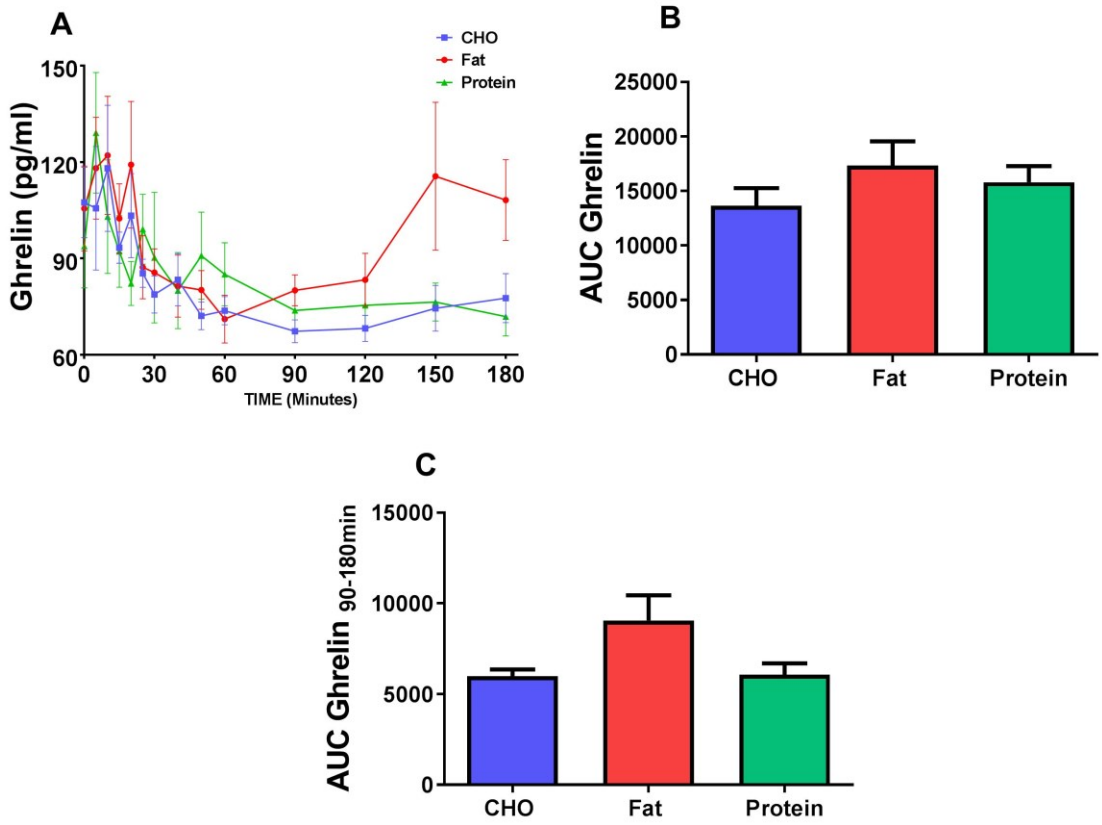


Figure 3.11. Plasma levels of the orexogenic hormone ghrelin in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in ghrelin (Figure 3.11A), $AUC_{0\text{--}180min}$ (Figure 3.11B) and $AUC_{90\text{--}180min}$ (Figure 3.11C) are displayed as mean ± SEM. Meals are provided at t=0 minutes. $AUC_{0\text{-}180}$ displayed as pg/ml·min. A one-way ANOVA was used to determine statistical significance for $AUC_{0\text{--}180min}$ and $AUC_{90\text{-}180min}$ $p<0.05$ overall. Post-hoc analysis using Bonferroni's test for multiple comparisons showed no significant differences.

3.4.4. The effect of meals of altered macronutrient intake on glucose homeostasis

The high carbohydrate meal resulted in a greater increase in serum glucose levels compared with the high fat and high protein meal (Figure 3.12). This was reflected in $AUC_{Glucose}$, showing significant differences ($P=0.0221$) between meals (Figure 3.12B). Further post-hoc analysis revealed significant differences between the carbohydrate versus fat meal and carbohydrate versus protein meal (Figure 3.12B). Supporting this, repeated measures ANOVA analysis revealed statistically significant differences at specific time points between meals (Figure 3.12A).

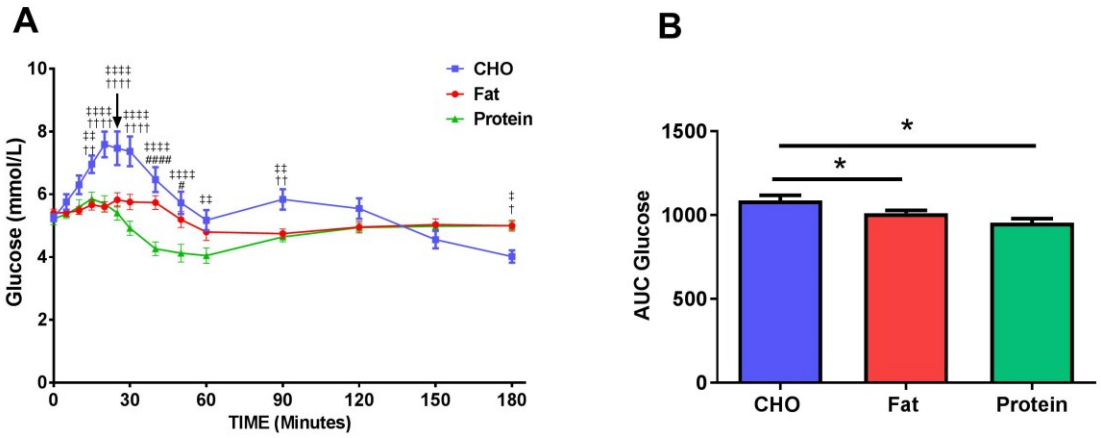


Figure 3.12. Plasma levels of glucose in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in glucose (Figure 3.12A) and $AUC_{0-180min}$ (Figure 3.12B) are displayed as mean ± SEM. Meals are provided at t=0 minutes. AUC_{0-180} displayed as pmol/l·min. CHO vs. PRO ‡‡‡‡p<0.0001, ‡‡p<0.01, ‡p<0.05; FAT vs. PRO ####p<0.0001, #p<0.05; CHO vs. FAT ††††p<0.0001, ††p<0.01, †p<0.05. A one-way ANOVA was used to determine statistical significance for $AUC_{0-180min}$ *p<0.05

Serum insulin levels were highest in response to the high carbohydrate meal (Figure 3.13). $AUC_{Insulin}$ showed significant differences between meals (P=0.0001) (Figure 3.13B). Further post-hoc analysis revealed statistically differences between the carbohydrate versus fat meal, the fat versus protein and the carbohydrate versus protein meal (Figure 3.13B). This was reflected in the repeated measures ANOVA, which showed statistically significant differences between meals at specific time points (Figure 3.13A).

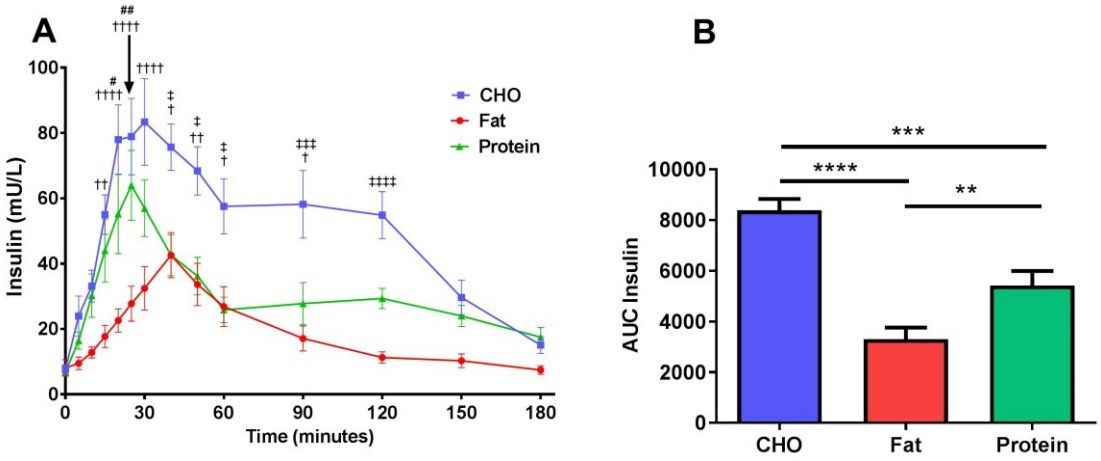


Figure 3.13. Plasma levels of insulin in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in insulin (Figure 3.13A) and $AUC_{0-180min}$ (Figure 3.13B) are displayed as mean ± SEM. Meals are provided at t=0 minutes. AUC_{0-180} displayed as mU/L·min. CHO vs. PRO ‡p<0.05; FAT vs. PRO ##p<0.01, #p<0.05; CHO vs. FAT ††††p<0.0001, †††p<0.001, ††p<0.01,

†p<0.05. A one-way ANOVA was used to determine statistical significance for $AUC_{0--180min}$ ****p<0.0001.

Conversely, the high protein meal resulted in higher glucagon levels compared with the high fat and high carbohydrate meal (Figure 3.14). $AUC_{Glucagon}$ showed significant differences between meals (P=0.0001) (Figure 3.14B). Further post-hoc analysis showed statistically significant differences between the fat versus protein and the carbohydrate versus protein meal (Figure 3.14B). This was reflected by the repeated measures ANOVA at specific time points (Figure 3.14A).

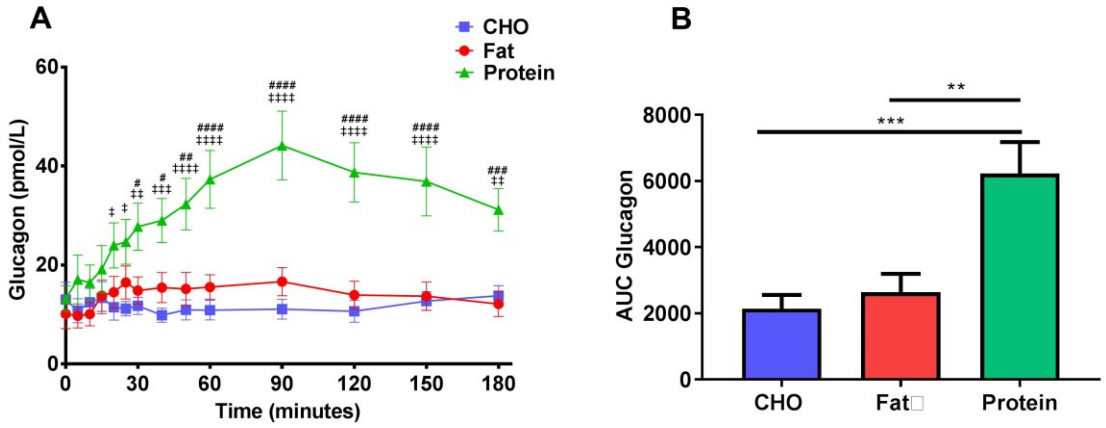


Figure 3.14. Plasma levels of glucagon in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=9). Changes in glucagon (Figure 3.14A) and $AUC_{0--180min}$ (Figure 3.14B) are displayed as mean ± SEM. Meals are provided at t=0 minutes. AUC_{0-180} displayed as pmol/l·min. CHO vs. PRO ‡‡‡‡p<0.0001, ‡‡‡p<0.001, ‡‡p<0.01, ‡p<0.05; FAT vs. PRO ####p<0.0001, ###p<0.001, ##p<0.01, #p<0.05. A one-way ANOVA was used to determine statistical significance for $AUC_{0--180min}$ ***p<0.001.

3.4.5. The effect of meals of altered macronutrient intake on gastric emptying

There was a statistically significant difference between the test meals over time (p<0.0001) however post-hoc analysis did not reveal these changes at specific time points between the meals. There was no significant difference in $AUC_{paracetamol}$ levels between the test meals over the three hour treatment period (P=0.1358) (Figure 3.15). High fat meals produced the greatest C_{max} compared to high protein and high carbohydrate meals (P=0.0194) (Figure 3.15C).

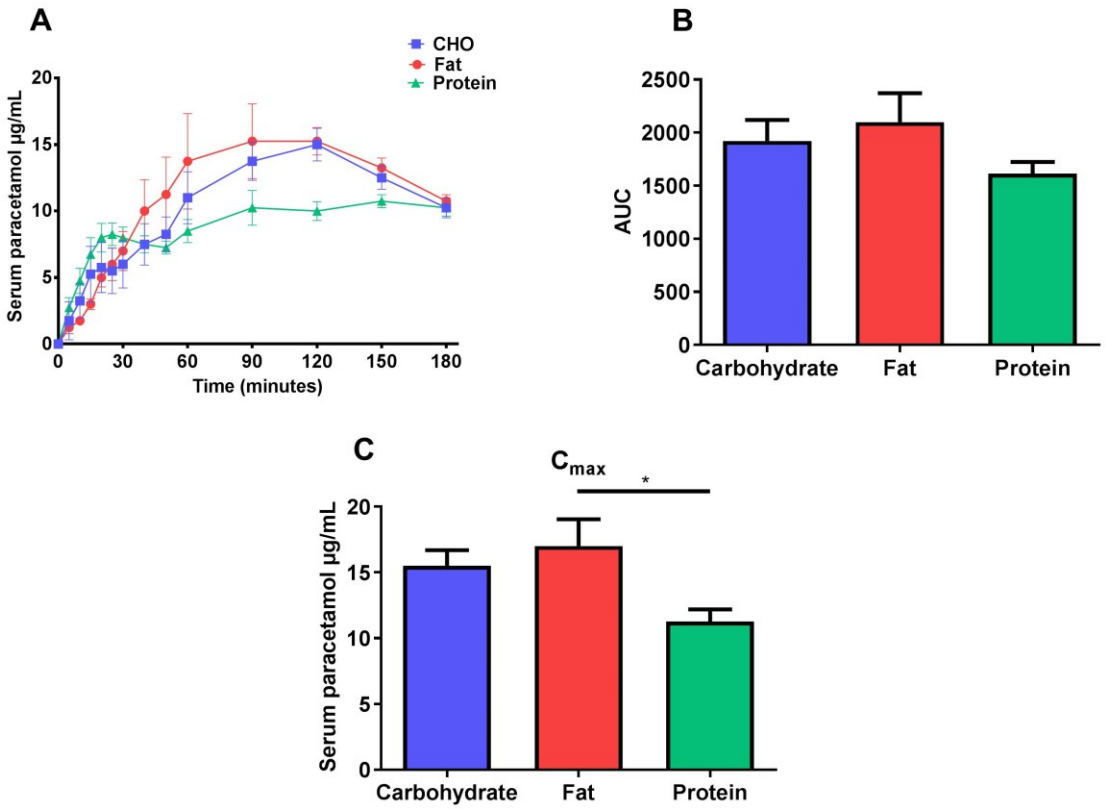


Figure 3.15. Serum levels of paracetamol in healthy volunteers in response to high carbohydrate, high fat and high protein meals (n=4). Changes in paracetamol (Figure 3.15A), $AUC_{0-180min}$ (Figure 3.15B) and C_{max} are displayed as mean ± SEM. Meals are provided at t=0 minutes. AUC_{0-180} displayed as µg/ml·min. A one-way ANOVA was used to determine statistical significance for $AUC_{0-180min}$ *p<0.05 and C_{max}.

3.4.6. Metabolite identification following meals of altered macronutrient intake

Two independent PCA models were built with the QCs and the gastric (figure 3.16A) and duodenal (figure 3.16B) in order to investigate the reproducibility and robustness of the analytical experiment. The QCs clustered in the center of both PCA scores plots, confirming the good analytical performance and the good quality of the data.

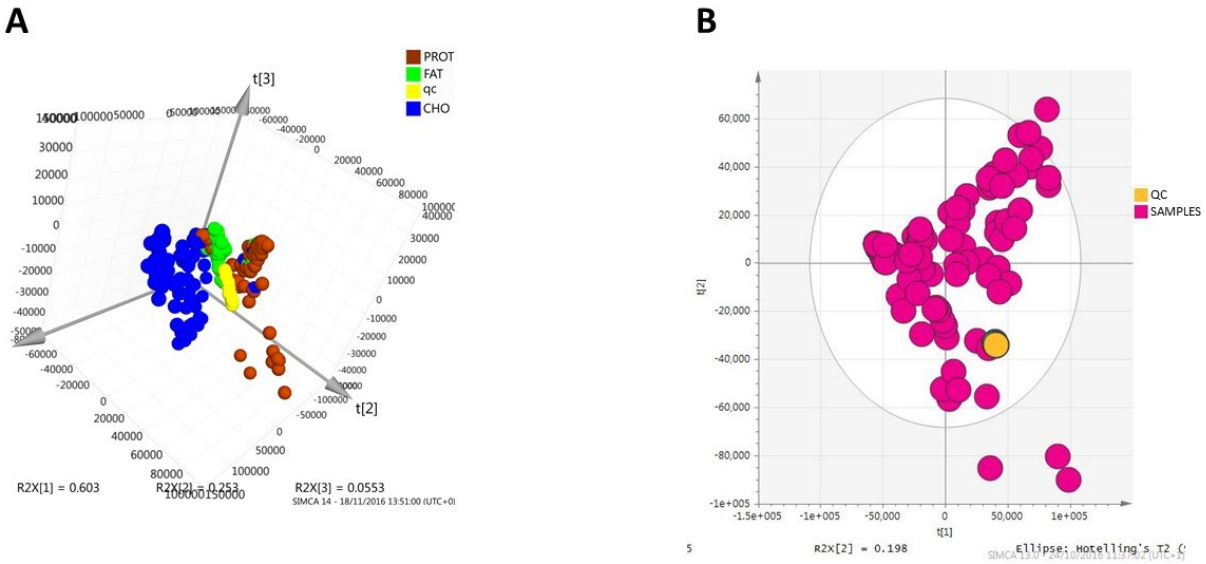


Figure 3.16. Principal component analysis (PCA) scores plot of all samples and all pooled QC samples for gastric (A) and duodenal (B) samples.
(A) Each sample is represented by a dot. Samples from the high carbohydrate meal are in blue, high fat meal in green and high protein meal in red (coloured for visual purposes only). There is a group clustering trend of the QCs (yellow). (B) Each sample is represented by a dot. All samples are represented by pink dots (coloured for visual purposed only. There is a group clustering trend of the QCs (yellow).

Data from the 3 different meals tested (high protein, high carbohydrate and high fat) in the study were examined for differences utilizing an unsupervised PCA model. Each sample is represented by a dot and they were colored according to class (meal) for visual purposes. Plots revealed clear class clustering of the different meals (Figure 3.17).

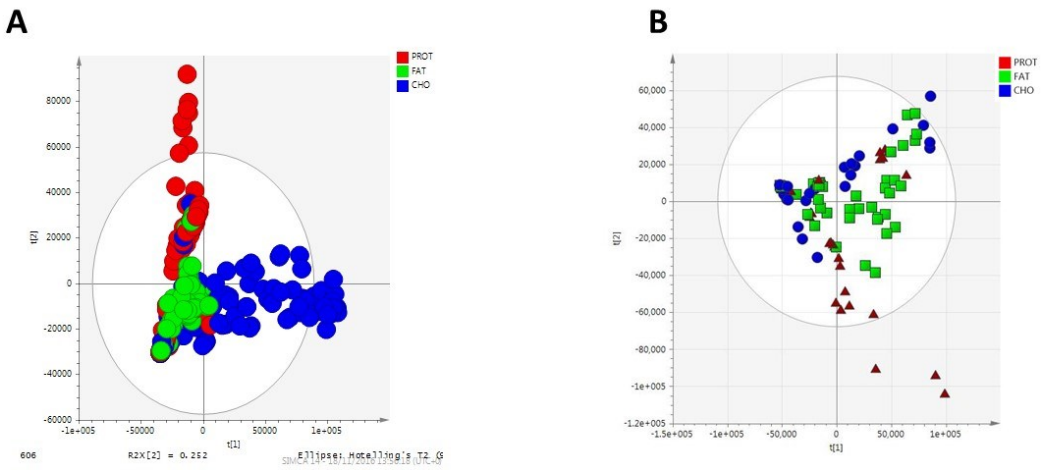


Figure 3.17. Principal component analysis (PCA) scores plot of all data for gastric (A) and duodenal (B) samples.
This shows a group clustering trend within the high protein meal (red dots), high fat meal (green dots) and high carbohydrate meal (blue dots).

Following this, metabolic trajectories were constructed from the PCA results for each meal over the study period (t=165 minutes) (Figure 3.18). There is a trend showing the trajectory for each sample, particularly for the gastric samples (Figure 3.18A).

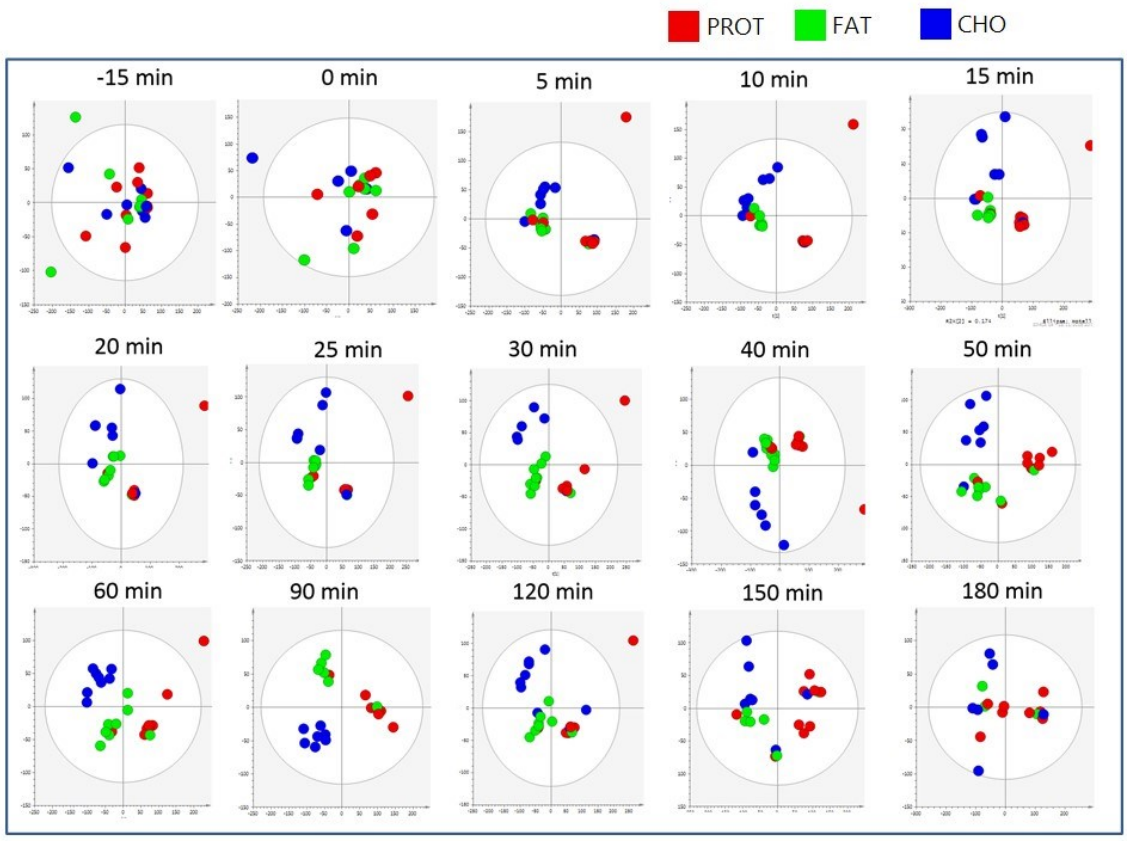


Figure 3.18. Time trajectory showing changes from -15 to 180 minutes in the high protein meal (red), high fat meal (green) and high carbohydrate meal (blue) for gastric samples.
Each point depicts a sequential time point plotted on a PCA map, representing the mean position for each meal over the course of the study period (t=165 minutes).

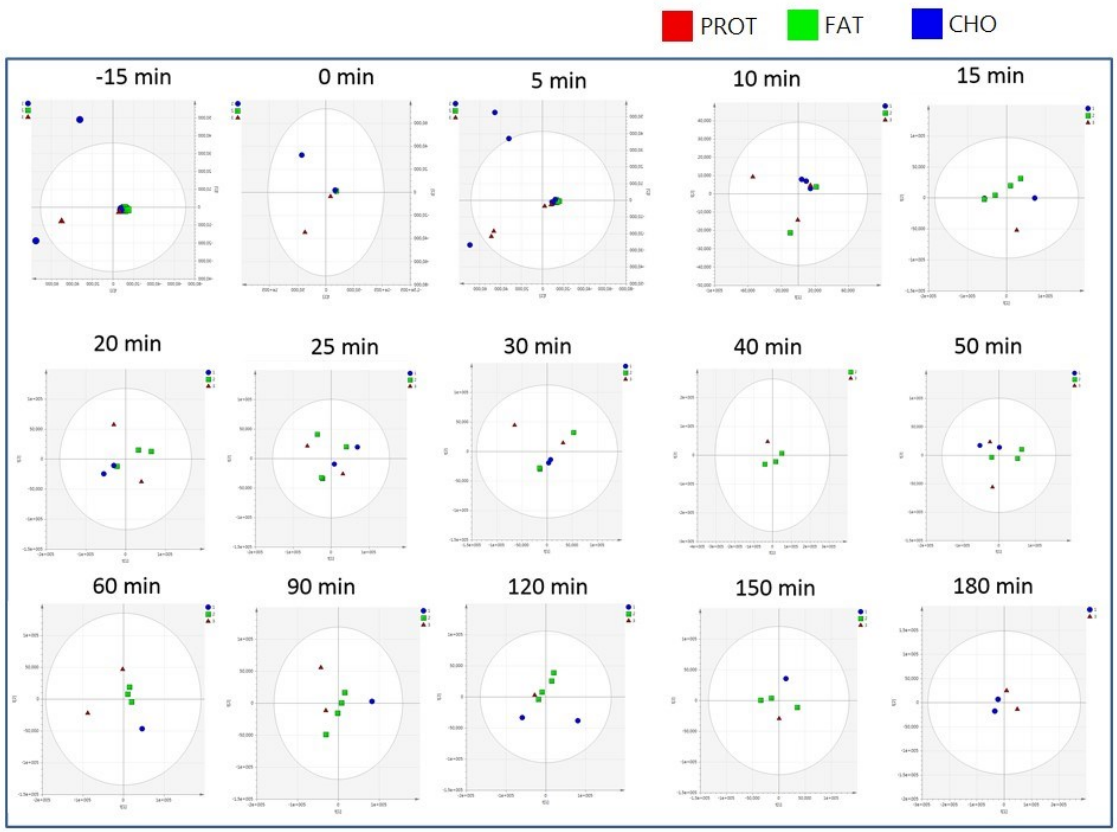


Figure 3.19. Time trajectory showing changes from -15 to 180 minutes in the high protein meal (red), high fat meal (green) and high carbohydrate meal (blue) for duodenal samples.
Each point depicts a sequential time point plotted on a PCA map, representing the mean position for each meal over the course of the study period (t=165 minutes).

The next step involved using a supervised strategy; partial least squares discriminant analysis (PLS-DA) constructed by including the sample class as part of the modelling process. As can be seen, the separation between the different meals was maximized. Loading plot corresponding to PLS-DA model from the gastric samples was investigated as shown in Figure 3.19. Following the high carbohydrate meal, there were clear increases in the sugars region post-meal.

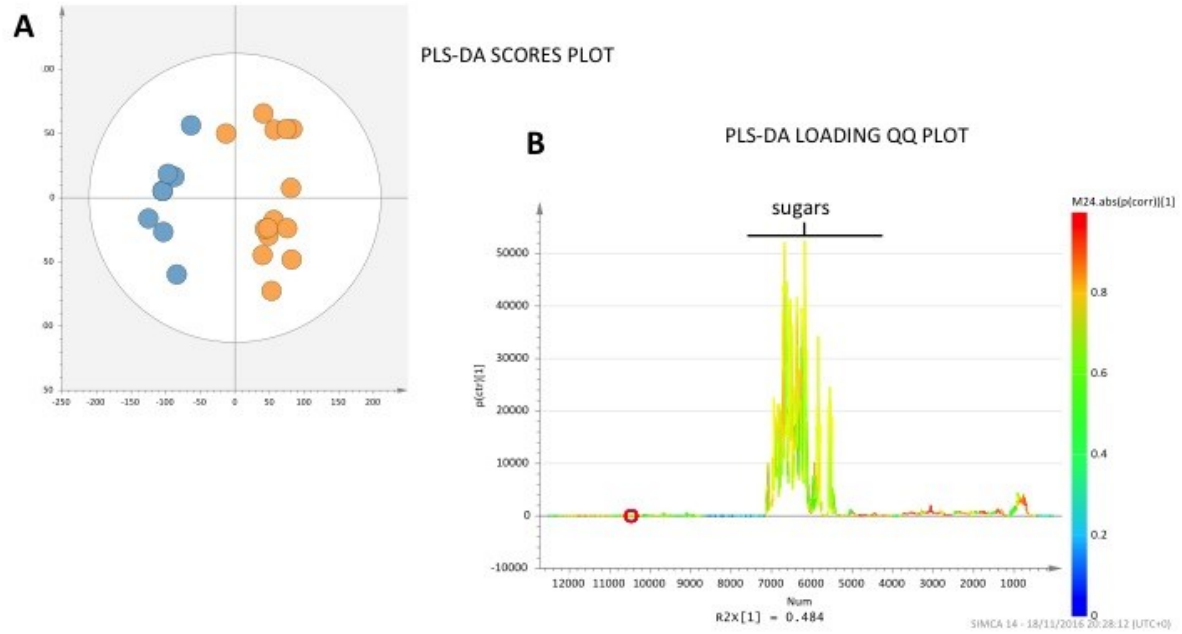


Figure 3.20. (A) Cross-validated (PLS-DA) scores plot of ^{1}H-NMR gastric metabolic profiles following a high carbohydrate meal.
Each dot represents the metabolic profile of one gastric sample and they are highlighted in blue pre-meal (t=-15 minutes) and orange post-meal (t=90-120 minutes).
(**B) Loading plots of the ^{1}H-NMR gastric metabolic profiles following a high carbohydrate meal.**
Metabolites highlighted in red are those that are significantly different when comparing time point t=-15 minutes vs t=90-120 minutes.

The PLS-DA scores plot of the duodenal samples shows clustering when comparing the pre- versus post-carbohydrate meal (t=-15 minutes vs t=90-150 minutes) (Figure 3.20A). Following the high carbohydrate meal, there are clear increases in the sugars region post-meal as was seen in the gastric analysis (Figure 3.20B). In addition, interestingly there are increases in the short-chain fatty acid butyrate in the duodenum following the carbohydrate meal, which was an unexpected find (figure 3.20B). The amino acids glutamine and glutamate were also present post meal in the duodenum following the high carbohydrate meal (figure 3.20B).

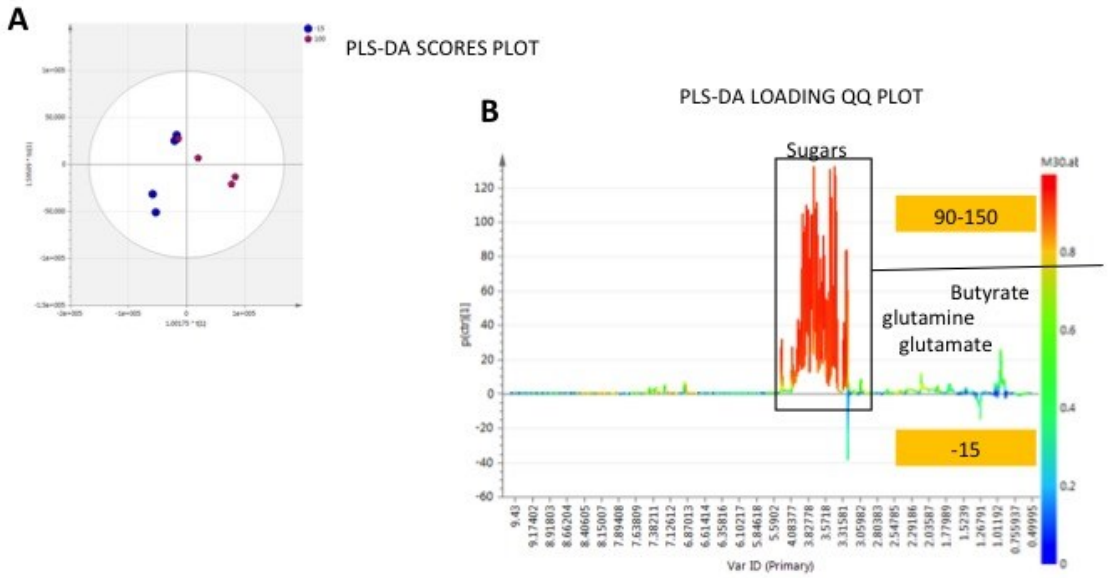


Figure 3.21. (A) PLS-DA scores plot of ^{1}H-NMR duodenal metabolic profiles following a high carbohydrate meal.
Each dot represents the metabolic profile of one duodenal sample and they are highlighted in blue pre-meal (t=-15 minutes) and red post-meal (t=90-150 minutes) following a high carbohydrate meal. (**B) Loading plots of the ^{1}H-NMR duodenal metabolic profiles following a high carbohydrate meal.** Metabolites highlighted in red are those that are significantly different when comparing time point t=-15 minutes vs t=90-150 minutes.

Following on from this, the changes seen were compared between meals again using OPLS-DA analysis.

Orthogonal projections to latent structures (OPLS) is a modification of the PLS regression analysis method. The OPLS method can separate the systematic variation in the *X* variable into two parts: (1) that which is linearly related to *Y*, and (2) that which is orthogonal to *Y*. OPLS-DA evaluates variations in frame areas between groups and was thus used to compare changes pre- and post-meals within the same meal group.

Results comparing duodenal samples following administration of the carbohydrate and protein meals are shown in figure 3.21. Glucose significantly increased after carbohydrate intake while

acetate and butyrate significantly increased after protein intake.

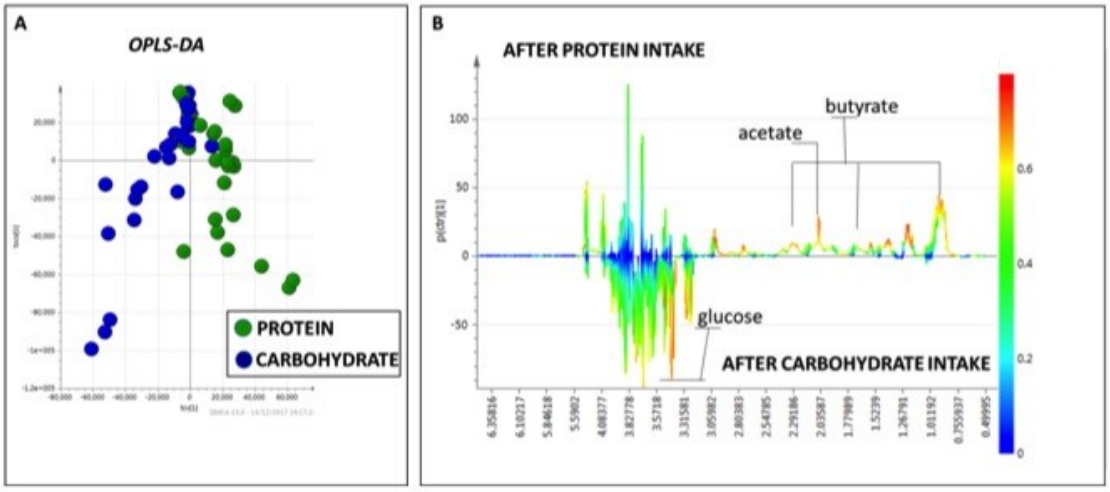


Figure 3.22. (A) Cross-validated OPLS-DA scores plot of ^{1}H-NMR duodenal metabolic profiles.
Each dot represents the metabolic profile of one duodenal sample and they are highlighted in green after protein intake and in blue after carbohydrate intake.
(**B) Loading plots of the ^{1}H-NMR duodenal metabolic profiles.**
Metabolites highlighted in red are the ones that are significantly different when comparing protein intake to carbohydrate intake.

Linear regression of the gastric samples to measures of GLP-1 showed significant increases in tyrosine, acetone, glucose and taurine correlated with GLP-1 release following the high protein meal.

3.5. **Discussion**

This study set out to investigate factors associated with the early changes seen in gut hormone release following meals of altered macronutrient intake.

There were clear differences in gut hormone release between the different test meals. The high protein meal resulted in a higher GLP-1 response compared to the high carbohydrate or fat meal. This is in line with other human studies, which find that protein has a greater and more persistent effect on circulating GLP-1 levels than other macronutrients (van der Klaauw et al., 2013, Dougkas and Ostman, 2016, Shah et al., 2017). Macronutrient content is thought to play an important role in the regulation of food intake. In rodent models, isocaloric oral protein loads were most effective in

reducing subsequent food intake, compared to carbohydrate (Bensaid et al., 2002). In humans, even a high-protein snack results in a delay in subsequent food intake compared to isocaloric high carbohydrate or high fat snacks (Marmonier et al., 2000). It is thus possible that the satiating effect of protein may at least partly be due to its increased effect on GLP-1 release. The actions of GLP-1 on satiation may be due to its ability to signal to central brain areas such as the arcuate nucleus and its ability to inhibit gastrointestinal motility (Holst, 2007a).

Interestingly, in this study gastric emptying seemed to initially be faster, but over the whole three hour period was slowest following the high protein meal. This slower gastric emptying may be a result of the increased GLP-1 release, as GLP-1 is known to strongly inhibit gastric emptying (Imeryuz et al., 1997). It is also possible that the delay in gastric emptying may be driving the later increased GLP-1 release observed with the high protein meal, when nutrients may be directly being released into the duodenum from the stomach over an extended time period, resulting in the more persistent elevation of GLP-1 levels. One of the limitations of this study was that the gastric emptying data was collected independently of the main study (due to the paracetamol being used as a marker of gastric emptying interfering with the metabolomics analysis) without measuring gut hormones (although the group studied all took part in the main study). Although we would expect a similar effect on GLP-1 release to be occurring, it is not possible to determine how closely GLP-1 and gastric emptying rates correlate.

In contrast to the GLP-1 secretory profile, the levels of the other major incretin, GIP, were highest following the high carbohydrate meal. GIP is predominantly an incretin and is thought to act synergistically with GLP-1 to stimulate insulin release. GIP appears to act directly on pancreatic islets to stimulate insulin secretion, with its activity being glucose-dependent (Siegel et al., 1992). Thus the higher GIP secretion seen with the high carbohydrate meal is not unexpected. Although the amount of GIP produced depends largely on the amount of carbohydrate ingested, fat intake also stimulates GIP release (Elliott et al., 1993).

There appear to be no significant differences in the levels of the anorectic hormones PYY and PP between the test meals. The literature is conflicting in this area, particularly when it comes to PYY. High fat meals have been reported to result in an increased PYY response compared to high carbohydrate meals (Murray et al., 2006, Essah et al., 2007), but this is in contrast to work done by Batterham et al on normal weight and obese participants, which found the highest levels of PYY were observed following ingestion of high protein meals compared to meals rich in other macronutrients (Batterham et al., 2006b). The in-house assay used to measure PYY in this study measures total PYY and thus levels reflect both PYY_{1-36} and PYY_{3-36} concentrations. Although the

main circulating postprandial form of PYY is thought to be PYY_{3-36} in humans (due to rapid cleaving of the Pro2-Ile3 bond by the enzyme DPP-IV), both forms will be present. Work by Batterham suggests circulating $PYY_{3–36}$ is an endogenous satiety factor, mediating this effect through the Y2R subtype (Batterham and Bloom, 2003). This work however has not been replicated by others (Tschop et al., 2004). Many groups have since found PYY_{3-36} reduces food intake, although this effect may not be observed when animals are stressed (Halatchev et al., 2004). In addition, high levels of PYY_{3-36} in humans can lead to nausea, which may contribute to the reduction in food intake (Degen et al., 2005, le Roux et al., 2008). The lack of difference seen in this study may be due to both PYY and PP having other physiological roles apart from satiety. Both hormones have effects on bile acid and exocrine pancreatic secretion (Adrian et al., 1985b, Langlois et al., 1990) and both high fat and high protein meals are potent stimulators of pancreatic secretion.

CCK also plays an important role in the regulation of pancreatic secretion, with CCK release typically reported to be particularly stimulated by fat and protein (Brennan et al., 2012, Wells et al., 1997). It was therefore unexpected that circulating CCK levels were higher in response to the high carbohydrate meal compared to the high fat and high protein meal. These findings may be related to the types of carbohydrate, fat and protein being used. In addition, CCK is a notoriously difficult hormone to measure for a number of reasons. There are multiple different isoforms of CCK. CCK and gastrin are similar in structure with a common amidated C-terminal tetrapeptide sequence (Johnsen, 1998), and thus extraction steps are required to measure CCK alone. Plasma concentrations of the particular forms of CCK thought to be bioactive and physiologically important are low, meaning assays need to be highly sensitive.

Post-prandial ghrelin levels were suppressed to a similar magnitude by all three test meals. However, there was a trend for the high carbohydrate meal to cause a greater suppression, and a trend for ghrelin levels to rise again more quickly in fat compared to carbohydrate and protein-rich meals. There is some work to suggest high carbohydrate meals suppress ghrelin to a greater extent than other macronutrients (Foster-Schubert et al., 2008). However other work suggests protein can suppress post-prandial circulating ghrelin levels more potently and/or for longer than carbohydrates (Al Awar et al., 2005, Blom et al., 2006, Tannous dit El Khoury et al., 2006, Bowen et al., 2006a, Bowen et al., 2006b, Leidy et al., 2007, Foster-Schubert et al., 2008). Quicker gut transit times and more rapid gastric emptying following carbohydrate ingestion (compared to fat and protein) may have resulted in the trend for a greater suppression of ghrelin in this study. Glucose is also known to inhibit ghrelin secretion (Broglio et al., 2004, Gao et al., 2011), which may be another contributing factor to the increased ghrelin suppression with the high carbohydrate meal. The carbohydrate

portion of the high carbohydrate meal was a glucose polymer, so this may have played a role in the increased ghrelin suppression, which possibly may not have occurred with other carbohydrate sources.

The endocrine pancreas plays a vital role in the regulation of glucose homeostasis. In response to glucose levels, pancreatic beta cells secrete insulin to suppress gluconeogenesis, increase glycogenesis and to transport glucose into tissues. The high carbohydrate meal was therefore expected to result in a greater insulin response than both the high fat and high protein meals. The high protein meal also resulted in a large insulin response, which was not as pronounced as for the high carbohydrate meal, but perhaps larger than that expected to maintain glucose homeostasis for relatively low glucose concentrations of the high protein meal. This may have been due to the increased GLP-1 release following the high protein meal. In addition to its anorectic effects, GLP-1 also acts as an incretin, potentiating glucose-stimulated insulin release following oral nutrient intake. The protein meal resulted in sufficient insulin release to significantly reduce blood glucose levels. This drop in blood glucose is likely to have stimulated the observed release of glucagon from the pancreas in order to prevent hypoglycaemia, though there is also evidence that, for example, amino acids can directly stimulate glucagon release from the pancreas (Eisenstein and Strack, 1978, Greenfield et al., 2009, Guttler et al., 1978). It is possible that some of the weight loss effects of protein are driven by glucagon; recently, glucagon has been linked to increased energy expenditure (Salem et al., 2016).

This study found no statistically significant differences in secretin, somatostatin and neurotensin secretion in response to the test meals. These hormones are primarily thought to influence digestive processes, rather than appetite or glucose homeostasis. Secretin stimulates the production of bicarbonate from the pancreas and bile production, and inhibits gastric acid secretion from the gastric parietal cells of the stomach. Its role in satiety remains unclear. Somatostatin inhibits gastrin release and regulates gastric acid secretion. Somatostatin secretion has been reported to be increased following ingestion of high protein and high fat meals compared to high carbohydrate meals, which may reflect its inhibitory effects on CCK and secretin (Ensinck et al., 1990, Herzig et al., 1994, Vatn et al., 1980). However, we did not observe this effect, which may require larger meals and/or higher numbers of subjects to be detected. Neurotensin secretion increased following ingestion of high protein and fat meals. Neurotensin is predominantly located in specialized enteroendocrine cells of the small intestine (Polak et al., 1977) and is secreted in response to fat ingestion (Ferris et al., 1981). Neurotensin mediates lipid uptake in intestinal cells, and humans with obesity have increased levels of pro-neurotensin whilst those without obesity who have high levels

of pro-neurotensin have double the risk of developing obesity later in life (Li et al., 2016). Though its role in food intake has yet to be fully characterized, there is some evidence that neurotensin reduces food intake (Cooke et al., 2009, Boules et al., 2000).

One of the limitations with the analysis of the gut hormones was the issue of missing data. One participant missed one of the three meals, and hence had to be excluded from the analysis. In order to overcome this issue and be able to use the remaining data from this participant, a statistician is currently in the process of analyzing some of the data using multilevel linear regression methods, and some preliminary data from this is included in appendices 7 and 8.

Although work from the analysis of the metabolites is still ongoing, it can be seen that this is likely to be a useful mechanistic tool in understanding the role of various metabolites in response to dietary changes. One of the limitations in the identification of metabolites was the volume of duodenal sample obtained, which meant that every time point could not be identified individually and some samples had to be pooled over a couple of adjacent time-points. The technique has however been shown to be valid with good clustering of the QCs with both gastric and duodenal samples. Clearly differences between the meals have been visualized, and these changes have been shown to vary over the time course of the study according to meal, particularly with the gastric samples.

Following a high carbohydrate meal, there were significant increases in the SCFA butyrate as well as the amino acids glutamine and glutamate within the duodenum. In addition, following a high protein meal there was a significantly higher concentration of butyrate and acetate when compared to the high carbohydrate meal. The presence of SCFAs within the duodenum was somewhat unexpected and further analysis is being carried out to identify whether there are other SCFAs present within the duodenum. Classically acetate and butyrate are thought to be microbial metabolites, naturally present in high concentrations in the lumen of the large intestine following fermentation of non-digestible carbohydrates (Pryde et al., 2002, Kleessen et al., 1997). Interestingly, both acetate and butyrate can also be formed from glutamate (Smith and Macfarlane, 1997), which was one of the other significant metabolites identified in the duodenum following the high carbohydrate meal. This further supports a role for microbial fermentation in the duodenum. Glutamine can be converted to glutamate by gut microbiota and is also taken up by specific microbial populations in order to increase their growth (Shoaie et al., 2013). Butyrate is utilized by the microbiota themselves for the synthesis of bacterial cell components and also serves as the primary energy source of colonic epithelial cells (Morrison and Preston, 2016, Dumas, 2011). Its exact role within the duodenum however remains to be elucidated.

The gut microbiome is thought to play a role in the gastrointestinal and metabolic response to diet, though it is often considered that such effects are chronic and/or mediated mainly through the colonic microbiota. However, metabolites such as butyrate identified in the stomach and duodenum are likely to be products of microbial action. The microbial populations in both the stomach and the duodenum from this cohort of people are currently being further investigated to see if there are acute changes pre- and post-meal following each of the meals and also to investigate exactly which populations are present and whether they could be responsible for the microbial metabolites identified. Although the microbiome has been well characterised in the large intestine with good evidence to suggest it is involved in the regulation of energy homeostasis (Backhed et al., 2004, Turnbaugh et al., 2006), far less is understood about the role of the proximal small intestinal gut microbiome in such processes. There are known roles of the small intestinal microbiota such as their involvement in the production of amino acids if they are cannot be provided by the diet itself (Raj et al., 2008, Torrallardona et al., 2003). The microbial mass increases the more distal one travels down the gastrointestinal tract; the stomach is home to 10^{2-3} cells/mL effluent, the duodenum to 10^{3-4} cells/mL effluent, the jejunum to 10^{4-5} cells/mL effluent, the ileum to 10^{8} cells/mL effluent and the colon to 10^{11} cells/mL effluent (Walter and Ley, 2011). Low levels of microbiota limit competition for nutrients: the microbiota are at an advantage to the host due to their small surface-to-volume ratios and proximity to the nutrients. Indeed, when bacterial biomass is too high in the small intestine, malabsorption of nutrients can occur (Almeida et al., 2008). The body naturally has lower levels of microbiota in the stomach and small intestine partly due to the low pH of gastric contents and rapid transit times, which tend to limit microbial growth (Kanno et al., 2009). In addition, bile salts, which are released into the duodenum via the bile, are strongly bactericidal (Watanabe et al., 2017). The body also limits the bacterial populations by the release of immunoglobulins (mainly IgA) which recognise and bind to the dominant microbes present, limiting their activity (Hapfelmeier et al., 2010). Thus, although there are fewer microbial populations within the small intestine, they are exposed to more varied and higher concentrations of nutrients, and if they survive the body's natural defence mechanisms, they are likely to be highly metabolically active organisms.

Within modern human populations, the change in diet over time is thought to influence the relative abundances of the types of microbes present in the gut (Walker et al., 2011). In addition, microbial activity within the gut differs in both herbivorous and carnivorous mammals, potentially reflecting trade-offs between carbohydrate and protein fermentation (David et al., 2014). Although there strong evidence for the role of the colonic microbiota on the fermentation of non-digestible carbohydrates yielding SCFAs (Wong et al., 2006, Morrison and Preston, 2016) and the effect of

these products on the release of gut hormones such as GLP-1 and PYY (Tolhurst et al., 2012, Psichas et al., 2015, Frost et al., 2014), little is known about the effects of the gut microbiota on nutrients in the proximal small intestine. It is possible that despite the significant findings in this study, the small sample size may mean this is not necessarily representative of the general population. However as this was a pilot study, no power calculation was possible.

Although further analyses are being done, one of the correlations from the study was that of glucose, tyrosine, acetone and taurine with GLP-1 following a high protein meal. The correlation of glucose and GLP-1 is well described in the literature; glucose is known to stimulate GLP-1 release (Kreymann et al., 1987). It is unsurprising that certain amino acids are higher following the high protein meal compared to the high carbohydrate meal. Little is known about the effect of tyrosine on anorectic gut hormone release. There is however evidence that other aromatic amino acids such as L-phenylalanine stimulate the release of GLP-1 (Alamshah et al., 2017). Studies looking at tyrosine directly are hindered by the poor solubility of tyrosine in water. Acetone is another product of bacterial fermentation, which interestingly occurs when the pH is below 5, suggesting it may be occurring in the stomach. There is good evidence for products of bacterial fermentation resulting in the release of anorectic gut hormones such as GLP-1, however these have been mainly focused on fermentation within the caecum and colon (Everard and Cani, 2014). The exact role for taurine in the gut remains unclear. There is suggestion that it can regulate the gut micro-ecology by inhibiting the growth of harmful bacteria, accelerating the production of SCFAs (Yu et al., 2016). Certainly further work looking at these metabolites in relation to GLP-1 release is warranted.

The nature of the study was relatively invasive, with two enteral tubes being inserted on day 1 of the study. Given the discomfort of the tubes, it is possible that some of the participants may have shown some anxiety during the study, however our visual analogue scales did not pick this up. In rodent models, stress has an anorexogenic effect (Ortolani et al., 2014). In humans, stress is also thought to influence the gut microbiome, which in turn may impact gut hormone release (Vrieze et al., 2010). If this is the case, the variation in the make-up of the gut microbiome between participants may result in differences in gut hormone secretion profiles. In order to limit the effects of stress on the results of this study, the enteral tubes were placed on day 1 with no further studies taking part on that day, allowing participants to acclimatize to the tubes prior to commencing the studies on day 2.

The macronutrient sources of the test meal components may be a possible confounder in this study. There is evidence that some gut hormones are released in preference to certain forms of specific macronutrients. We used whey protein as the protein portion, double cream as the fat portion and

a glucose polymer as the carbohydrate portion. It may be that other forms of protein, fat and carbohydrate may yield different results.

This study provides a comprehensive profile of gut hormones released in response to the ingestion of meals high in specific macronutrients. Further work is currently underway to determine the significance of the findings from the metabolite analysis and to correlate the metabolites that have been identified following specific meals with hormonal release using linear regression methods.

In the future it may be possible to test certain metabolites and the effects on gut hormone release and appetite based on the results of this study. It will be interesting to also look further at the effects on the gut microbiome, the composition of which has been shown to influence gut hormone release, and the time course of any changes picked up. Based on these findings, it may be possible to design specific foods that drive satiety, which may address the dietary prevention of obesity.

Chapter 4:

Discussion

Obesity, its associated complications and mortality are major worldwide public health issues. There is an urgent need for effective treatments, as current management options are either largely ineffective in the long-term or limited by risks and side effects. The only known treatment that is highly effective is bariatric surgery, but its utility is limited in the face of high economic cost and major anaesthetic and post-operative risks and complications (Maggard et al., 2005). Diet and lifestyle measures are always first-line treatment options. There has been a great deal of work looking at the effect of macronutrient diets and satiety. High protein diets reduce food intake and result in weight loss superior to that achieved with other macronutrients (Porrini et al., 1997, Reid and Hetherington, 1997, Trigazis et al., 1997, Bensaid et al., 2002, Anderson and Moore, 2004, Halton and Hu, 2004), but are difficult to adhere to in the longer-term. The exact mechanisms by which a high protein diet controls appetite remain unclear. Determining the mechanisms by which protein drives satiation and satiety would facilitate the hijacking of these systems as an alternative treatment option in the management of obesity. Evidence suggests that the amino acid products of protein digestion may play a role in regulating appetite and satiety. Different proteins result in different levels of satiety which may be a result of the amino acid composition of that particular type of protein (Veldhorst et al., 2008).

Preliminary work carried out in our laboratory and by others show that individual amino acids including L-cysteine, L-arginine and L-phenylalanine have anorectic effects in rodents (McGavigan et al., 2015, Alamshah et al., 2016a, Alamshah et al., 2017, Jordi et al., 2013b, Blouet and Schwartz, 2012). The work outlined in this thesis investigated the tolerability of L-arginine in humans and its effect on appetite and gut hormone release in humans. In addition, this thesis investigated the metabolites associated with gut hormone release following high protein, high fat and high carbohydrate meals.

L-arginine has been shown to stimulate the release of the anorectic gut hormones GLP-1 and PYY both *in vitro* and *in vivo,* and to chronically reduce food intake in rodents. There are known amino acid sensing receptors in the gut that respond to amino acids (Wellendorph et al., 2005, Conigrave and Brown, 2006, Nelson et al., 2002). It is possible that this direct sensing of L-arginine in the distal small intestine may trigger effects on gut hormone release (coinciding with the postprandial rise seen in the study outlined in this thesis), which may then result in a reduction in subsequent food intake. In the studies outlined in this thesis, oral administration of L-arginine to healthy human volunteers was well tolerated and significantly increased the release of plasma GLP-1 and PYY following an *ad libitum* meal. Food diaries showed a trend towards lower energy intake and fat intake following an acute dose of L-arginine. The post-prandial rise in anorectic gut hormones may

be responsible for the potential effects on subsequent self-reported energy intake and fat intake immediately following the study period. However, oral L-arginine did not reduce acute food intake at the dose given in the small number of participants that it was administered to. It is possible that if the *ad* libitum meal was given later in the study it may have resulted in a reduction in food intake. At such a later time point, L-arginine might be directly sensed by the large numbers of GLP-1- and PYY-expressing L-cells in the ileum, resulting in a greater release of anorectic gut hormones and thus a reduction in food intake.

However, the mechanisms underlying the anorectic effect of L-arginine are unclear, as evidence in rodents suggests it is not mediated by GLP-1 and PYY (Alamshah et al., 2016a). Amino acid transporter systems play a vital role in amino acid transport across cellular membranes. There is evidence to suggest that in addition to their transport activity, transporter systems have receptor-like activities. For example, the SNAT2 transporter has been implicated in GLP-1 release induced by L-glutamine from murine primary intestinal cultures (Tolhurst et al., 2011). Furthermore, L-arginine-induced insulin release from the pancreatic beta-cells has been demonstrated to be mediated by the electrogenic transport of L-arginine into the beta-cells resulting in membrane depolarization and consequent insulin release (Smith et al., 1997). Whether L-arginine also reduces food intake in animal models by an action on transporter systems is unknown.

The effects of L-arginine on glucose homeostasis are well characterised in the literature and were not further investigated as part of this thesis. L-arginine is known to acutely stimulate both insulin and glucagon release in humans (Palmer et al., 1975). The exact mechanism remains unclear. There is evidence to suggest L-arginine stimulates GLP-1 release to improve insulin sensitivity and glucose tolerance in rodents (Clemmensen et al., 2013b). As discussed above, other studies suggest that L-arginine stimulates insulin release directly from the beta cells of the pancreas via an electrogenic mechanism (Smith et al., 1997, Thams and Capito, 1999). The doses of L-arginine given to stimulate insulin release in the literature were much higher than the doses used as part of this study. A major limitation to further work looking at L-arginine in humans was the maximum dose able to be administered with the amino acid being considered a “foodstuff” rather than a “drug” according to the MHRA. From a practical point of view, regulatory requirements for studies that involve foods (non Clinical Trials of an Investigational Medicinal Products (non-CTIMPs)) rather than drugs (CTIMPs) are fewer, the costs are lower and the review process is more straightforward. In the longer-term, studies involving foods can result in products on the market sooner than studies involving drugs.

In order to further investigate the gut hormone release pattern seen with both high protein meals and specific amino acids, further work was undertaken to determine the metabolites potentially responsible for this, as outlined in chapter 3. There is a rapid rise in anorectic gut hormones following ingestion of a high protein meal that cannot be explained by direct amino acid sensing alone. Determining which amino acids or di- or tri-peptides or perhaps even other nutrients or combinations of nutrients may be driving this could be useful in planning further work, with the long term aim of developing products such as functional foods which manipulate intrinsic satiety mechanisms. These types of product are subject to food law rather than drug law, meaning they could be available within 5 years as opposed to manufacturing a "drug" which would take a minimum of 12 years.

Diet is one of the most influential factors on the metabolome. This study is an example of how metabolomics can be used as a mechanistic tool to explore how particular diets may exert biological effects. Identifying differences in the gastrointestinal metabolic profiles between the pre-meal and post-meal period could increase our understanding of how diet influences appetite and may highlight potential novel approaches to the management of chronic conditions such as obesity. Metabolomics data are extremely complex, and multivariate analytical techniques for data processing together with the use of high-quality analysis software is necessary to make such techniques and the insights they provide more accessible to non-experts. The use of metabolomics has influenced pharmacological research to a great extent, paving the way for personalized medicine (van Karnebeek et al., 2018, Everett, 2017, de Toro-Martin et al., 2017). Unfortunately, cost is currently one of the major limiting factors.

This study showed a significantly greater rise in GLP-1 and glucagon following a high-protein meal compared to isocaloric meals rich in other macronutrients. The increase in certain metabolites, correlated with the increase in GLP-1 release following a high protein meal. Interestingly, some of these metabolites are likely to be the products of microbial action; in particular, the presence of SCFAs in the duodenum following the high protein meal was unexpected. SCFAs are considered to be mainly produced by the action of bacteria in the colon on fermentable carbohydrates, though there is evidence that bacteria can metabolise specific amino acids into SCFAs. Our data suggest that in fact the small intestinal microbiome is capable of generating significant quantities of SCFAs, and that they may have a role in small intestinal gut hormone release. This type of insight is an example of the new findings possible using hypothesis-free techniques such as metabolomics.

The gut microbiome has been relatively well characterised in the large intestine, with mounting evidence of its role in energy homeostasis (Backhed et al., 2004, Turnbaugh et al., 2006). However,

most studies that have been carried out on the role of the gut microbiome in energy homeostasis have focused on the colon; relatively few have focussed on the upper small intestine, which contains much fewer bacteria. The gut content presented to the microbiota influence the microbial population and the metabolites they produce (Lopez-Legarrea et al., 2014). Interestingly, microbial activity mirrors differences between herbivorous and carnivorous mammals, reflecting trade-offs between carbohydrate and protein fermentation (David et al., 2014). Previous work has shown that SCFAs can stimulate the secretion of GLP-1 and PYY (Tolhurst et al., 2012, Psichas et al., 2015, Frost et al., 2014). What is less clear is the role of the gut microbiome in the proximal small intestine, namely within the duodenum. The bacterial populations in the colon and duodenum vary in both species and number on account of a number of factors, including the effect of pH and rapid gut transit times, which limit microbial growth (Kanno et al., 2009) and the bactericidal properties of bile in the duodenum (Watanabe et al., 2017). In addition, the small intestine has natural defence mechanisms against microbial colonization, which include enzymatic digestion of the microbiota and the release of immunoglobulins (in particular IgA) into the gut lumen, which bind to and limit the activity of dominant microbiota (Hapfelmeier et al., 2010). Paneth cells within the gut secrete α-defensin, an antimicrobial peptide which regulates the composition of the intestinal microbiota (Nakamura et al., 2016). The microbiota in the large intestine have the additional protection of a mucus layer, which keeps luminal microbes away from epithelial contact; the comparable mucus layer in the small intestine is discontinuous (Jandhyala et al., 2015). However, though there are far fewer microbes within the duodenum, they are presented with nutrients in much higher concentrations, and may thus be highly metabolically active. Further work needs to be done to elucidate these bacterial populations and the mechanism by which they mediate the breakdown of macronutrients and the consequent effects on gut hormones, satiation and metabolism.

The work described in this thesis investigated the effect of L-arginine on appetite and the release of gut hormones, and the metabolites that may drive early anorectic gut hormone release. The findings reported here show that L-arginine influenced the release of anorectic gut hormones in conjunction with an *ad libitum* meal, but did not influence acute food intake. Further work is required to determine if there is a longer-term effect on appetite as food diaries suggested there might be a delayed reduction in energy and in particular fat intake. It may be useful to investigate the effects of higher doses of L-arginine, however this type of study would be classed as a clinical trial of an investigational medicinal product (CTIMP), and would be subject to further regulatory requirements than the types of study outlined in this thesis. Other nutrients may also be subject to similar issues.

A food can be regarded as functional if it has beneficial effects on target functions in the body beyond nutritional effects in a way that is relevant to health and well being and/or the reduction of disease (Danse, 1999). The concept of functional foods came about in the 1980s in Japan, when health authorities recognised that an improved quality of life must accompany increasing life expectancy for the expanding number of elderly people in the population if health care costs were to be controlled. In Japan, all functional foods must meet three requirements: foods must be present in their naturally occurring form, rather than a capsule, tablet, or powder, they must be consumed in the diet as often as daily, and they should regulate a biological process in hope of preventing or controlling disease (Hardy, 2000). Functional foods have not as yet been defined by legislation in Europe. Consumer interest in the relationship between diet and health has increased substantially in recent years. An annual $20 billion to $30 billion is spent on sales of functional foods with an estimated growth range of 8.5 percent to 20 percent annually. The global market potential for functional foods and beverages has been estimated to be worth $192 billion by 2020 (Kaur and Singh, 2017). Trends in population demographics and socio-economic changes also point to the need for foods with added health benefits. An aging population and the desire for an improved quality of life, as well as increasing healthcare costs, have stimulated governments, researchers, health professionals and the food industry to see how such changes can be managed more effectively.

The general aim of this thesis was to contribute to our knowledge on how protein mediates satiety by investigating the effect of a specific amino acid (L-arginine) on food intake and gut hormone release. The studies described in this thesis introduce the possibility of postprandial effects of L-arginine on anorectic gut hormone release and delayed effects on food intake. It is likely that a single amino acid is not responsible for mediating protein induced satiety, and it is more plausible that a combination of amino acids, likely in concert with other nutrients, is responsible for the effects of a high protein meal on food intake and gut hormone release. Hence further work was done to try and establish metabolites responsible for early gut hormone release following a high protein meal. By determining these metabolites, future work could focus on investigating effects of specific amino acids or other metabolites, either alone or in combination, on appetite. If successful, this could lead to a safe, low-risk, practical and effective therapeutic treatment for weight management.

References

The ... world congress on Controversies to Consensus in Diabetes, Obesity and Hypertension.

ABBOTT, C. R., MONTEIRO, M., SMALL, C. J., SAJEDI, A., SMITH, K. L., PARKINSON, J. R., GHATEI, M. A. & BLOOM, S. R. 2005a. The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain Res,* 1044**,** 127-31.

ABBOTT, C. R., SMALL, C. J., KENNEDY, A. R., NEARY, N. M., SAJEDI, A., GHATEI, M. A. & BLOOM, S. R. 2005b. Blockade of the neuropeptide Y Y2 receptor with the specific antagonist BIIE0246 attenuates the effect of endogenous and exogenous peptide YY(3-36) on food intake. *Brain Res,* 1043**,** 139-44.

ABELLO, J., YE, F., BOSSHARD, A., BERNARD, C., CUBER, J. C. & CHAYVIALLE, J. A. 1994. Stimulation of glucagon-like peptide-1 secretion by muscarinic agonist in a murine intestinal endocrine cell line. *Endocrinology,* 134**,** 2011-7.

ABETE, I., ASTRUP, A., MARTINEZ, J. A., THORSDOTTIR, I. & ZULET, M. A. 2010. Obesity and the metabolic syndrome: role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. *Nutr Rev,* 68**,** 214-31.

ABOU-SAMRA, R., KEERSMAEKERS, L., BRIENZA, D., MUKHERJEE, R. & MACE, K. 2011. Effect of different protein sources on satiation and short-term satiety when consumed as a starter. *Nutr J,* 10**,** 139.

ADRIAN, T. E., FERRI, G. L., BACARESE-HAMILTON, A. J., FUESSL, H. S., POLAK, J. M. & BLOOM, S. R. 1985a. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology,* 89**,** 1070-7.

ADRIAN, T. E., SAVAGE, A. P., SAGOR, G. R., ALLEN, J. M., BACARESE-HAMILTON, A. J., TATEMOTO, K., POLAK, J. M. & BLOOM, S. R. 1985b. Effect of peptide YY on gastric, pancreatic, and biliary function in humans. *Gastroenterology,* 89**,** 494-9.

AFSHIN, A., FOROUZANFAR, M. H., REITSMA, M. B., SUR, P., ESTEP, K., LEE, A., MARCZAK, L., MOKDAD, A. H., MORADI-LAKEH, M., NAGHAVI, M., SALAMA, J. S., VOS, T., ABATE, K. H., ABBAFATI, C., AHMED, M. B., AL-ALY, Z., ALKERWI, A., AL-RADDADI, R., AMARE, A. T., AMBERBIR, A., AMEGAH, A. K., AMINI, E., AMROCK, S. M., ANJANA, R. M., ARNLOV, J., ASAYESH, H., BANERJEE, A., BARAC, A., BAYE, E., BENNETT, D. A., BEYENE, A. S., BIADGILIGN, S., BIRYUKOV, S., BJERTNESS, E., BONEYA, D. J., CAMPOS-NONATO, I., CARRERO, J. J., CECILIO, P., CERCY, K., CIOBANU, L. G., CORNABY, L., DAMTEW, S. A., DANDONA, L., DANDONA, R., DHARMARATNE, S. D., DUNCAN, B. B., ESHRATI, B., ESTEGHAMATI, A., FEIGIN, V. L., FERNANDES, J. C., FURST, T., GEBREHIWOT, T. T., GOLD, A., GONA, P. N., GOTO, A.,

HABTEWOLD, T. D., HADUSH, K. T., HAFEZI-NEJAD, N., HAY, S. I., HORINO, M., ISLAMI, F., KAMAL, R., KASAEIAN, A., KATIKIREDDI, S. V., KENGNE, A. P., KESAVACHANDRAN, C. N., KHADER, Y. S., KHANG, Y. H., KHUBCHANDANI, J., KIM, D., KIM, Y. J., KINFU, Y., KOSEN, S., KU, T., DEFO, B. K., KUMAR, G. A., LARSON, H. J., LEINSALU, M., LIANG, X., LIM, S. S., LIU, P., LOPEZ, A. D., LOZANO, R., MAJEED, A., MALEKZADEH, R., MALTA, D. C., MAZIDI, M., MCALINDEN, C., MCGARVEY, S. T., MENGISTU, D. T., MENSAH, G. A., MENSINK, G. B. M., MEZGEBE, H. B., MIRRAKHIMOV, E. M., MUELLER, U. O., NOUBIAP, J. J., OBERMEYER, C. M., OGBO, F. A., OWOLABI, M. O., PATTON, G. C., et al. 2017. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med,* 377**,** 13-27.

AHLMAN, H. & NILSSON 2001. The gut as the largest endocrine organ in the body. *Ann Oncol,* 12 Suppl 2**,** S63-8.

AL AWAR, R., OBEID, O., HWALLA, N. & AZAR, S. 2005. Postprandial acylated ghrelin status following fat and protein manipulation of meals in healthy young women. *Clin Sci (Lond),* 109**,** 405-11.

ALAMSHAH, A., MCGAVIGAN, A. K., SPRECKLEY, E., KINSEY-JONES, J. S., AMIN, A., TOUGH, I. R., O'HARA, H. C., MOOLLA, A., BANKS, K., FRANCE, R., HYBERG, G., NORTON, M., CHEONG, W., LEHMANN, A., BLOOM, S. R., COX, H. M. & MURPHY, K. G. 2016a. L-Arginine promotes gut hormone release and reduces food intake in rodents. *Diabetes Obes Metab*.

ALAMSHAH, A., MCGAVIGAN, A. K., SPRECKLEY, E., KINSEY-JONES, J. S., AMIN, A., TOUGH, I. R., O'HARA, H. C., MOOLLA, A., BANKS, K., FRANCE, R., HYBERG, G., NORTON, M., CHEONG, W., LEHMANN, A., BLOOM, S. R., COX, H. M. & MURPHY, K. G. 2016b. L-arginine promotes gut hormone release and reduces food intake in rodents. *Diabetes Obes Metab,* 18**,** 508-18.

ALAMSHAH, A., SPRECKLEY, E., NORTON, M., KINSEY-JONES, J. S., AMIN, A., RAMGULAM, A., CAO, Y., JOHNSON, R., SALEH, K., AKALESTOU, E., MALIK, Z., GONZALEZ-ABUIN, N., JOMARD, A., AMARSI, R., MOOLLA, A., SARGENT, P. R., GRAY, G. W., BLOOM, S. R. & MURPHY, K. G. 2017. l-phenylalanine modulates gut hormone release and glucose tolerance, and suppresses food intake through the calcium-sensing receptor in rodents. *Int J Obes (Lond),* 41**,** 1693-1701.

ALBINA, J. E., MILLS, C. D., HENRY, W. L., JR. & CALDWELL, M. D. 1989. Regulation of macrophage physiology by L-arginine: role of the oxidative L-arginine deiminase pathway. *J Immunol,* 143**,** 3641-6.

ALMEIDA, J. A., KIM, R., STOITA, A., MCIVER, C. J., KURTOVIC, J. & RIORDAN, S. M. 2008. Lactose malabsorption in the elderly: role of small intestinal bacterial overgrowth. *Scand J Gastroenterol,* 43**,** 146-54.

AMIN, A. & MURPHY, K. G. 2012. Nutritional sensing and its utility in treating obesity. *Expert Review of Endocrinology & Metabolism,* 7**,** 209-221.

ANDERSON, G. H. & MOORE, S. E. 2004. Dietary proteins in the regulation of food intake and body weight in humans. *J Nutr,* 134**,** 974S-9S.

ANDERSON, G. H., TECIMER, S. N., SHAH, D. & ZAFAR, T. A. 2004. Protein source, quantity, and time of consumption determine the effect of proteins on short-term food intake in young men. *J Nutr,* 134**,** 3011-5.

ANINI, Y. & BRUBAKER, P. L. 2003. Muscarinic receptors control glucagon-like peptide 1 secretion by human endocrine L cells. *Endocrinology,* 144**,** 3244-50.

ANINI, Y., FU-CHENG, X., CUBER, J. C., KERVRAN, A., CHARIOT, J. & ROZ, C. 1999. Comparison of the postprandial release of peptide YY and proglucagon-derived peptides in the rat. *Pflugers Arch,* 438**,** 299-306.

ASAKAWA, A., INUI, A., KAGA, T., KATSUURA, G., FUJIMIYA, M., FUJINO, M. A. & KASUGA, M. 2003. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut,* 52**,** 947-52.

AULAK, K. S., LIU, J., WU, J., HYATT, S. L., PUPPI, M., HENNING, S. J. & HATZOGLOU, M. 1996. Molecular sites of regulation of expression of the rat cationic amino acid transporter gene. *J Biol Chem,* 271**,** 29799-806.

AZPIROZ, F. & MALAGELADA, J. R. 1987. Importance of vagal input in maintaining gastric tone in the dog. *J Physiol,* 384**,** 511-24.

BACKHED, F., DING, H., WANG, T., HOOPER, L. V., KOH, G. Y., NAGY, A., SEMENKOVICH, C. F. & GORDON, J. I. 2004. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A,* 101**,** 15718-23.

BADMAN, M. K. & FLIER, J. S. 2005. The gut and energy balance: visceral allies in the obesity wars. *Science,* 307**,** 1909-14.

BAGGIO, L. L., HUANG, Q., BROWN, T. J. & DRUCKER, D. J. 2004a. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology,* 127**,** 546-58.

BAGGIO, L. L., HUANG, Q., BROWN, T. J. & DRUCKER, D. J. 2004b. A recombinant human glucagon-like peptide (GLP)-1-albumin protein (albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. *Diabetes,* 53**,** 2492-500.

BAI, M., TRIVEDI, S. & BROWN, E. M. 1998. Dimerization of the extracellular calcium-sensing receptor (CaR) on the cell surface of CaR-transfected HEK293 cells. *J Biol Chem,* 273**,** 23605-10.

BAILEY, E. F. 2008. A tasty morsel: the role of the dorsal vagal complex in the regulation of food intake and swallowing. Focus on "BDNF/TrkB signaling interacts with GABAergic system to inhibit rhythmic swallowing in the rat," by Bariohay et al. *Am J Physiol Regul Integr Comp Physiol,* 295**,** R1048-9.

BALK, E. M. & LICHTENSTEIN, A. H. 2017. Omega-3 Fatty Acids and Cardiovascular Disease: Summary of the 2016 Agency of Healthcare Research and Quality Evidence Review. *Nutrients,* 9.

BANDYOPADHYAY, S., TFELT-HANSEN, J. & CHATTOPADHYAY, N. 2010. Diverse roles of extracellular calcium-sensing receptor in the central nervous system. *J Neurosci Res,* 88**,** 2073-82.

BANKS, W. A., OWEN, J. B. & ERICKSON, M. A. 2012. Insulin in the brain: there and back again. *Pharmacol Ther,* 136**,** 82-93.

BARNETT, B. P., HWANG, Y., TAYLOR, M. S., KIRCHNER, H., PFLUGER, P. T., BERNARD, V., LIN, Y. Y., BOWERS, E. M., MUKHERJEE, C., SONG, W. J., LONGO, P. A., LEAHY, D. J., HUSSAIN, M. A., TSCHOP, M. H., BOEKE, J. D. & COLE, P. A. 2010. Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor. *Science,* 330**,** 1689-92.

BARRACHINA, M. D., MARTINEZ, V., WANG, L., WEI, J. Y. & TACHE, Y. 1997. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. *Proc Natl Acad Sci U S A,* 94**,** 10455-60.

BASKIN, D. G., FIGLEWICZ LATTEMANN, D., SEELEY, R. J., WOODS, S. C., PORTE, D. & SCHWARTZ, M. W. 1999. Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. *Brain Research,* 848**,** 114-123.

BASSIL, M. S., HWALLA, N. & OBEID, O. A. 2007. Meal pattern of male rats maintained on histidine-, leucine-, or tyrosine-supplemented diet. *Obesity (Silver Spring),* 15**,** 616-23.

BATTERHAM, R. L. & BLOOM, S. R. 2003. The gut hormone peptide YY regulates appetite. *Ann N Y Acad Sci,* 994**,** 162-8.

BATTERHAM, R. L., COHEN, M. A., ELLIS, S. M., LE ROUX, C. W., WITHERS, D. J., FROST, G. S., GHATEI, M. A. & BLOOM, S. R. 2003. Inhibition of Food Intake in Obese Subjects by Peptide YY3–36. *New England Journal of Medicine,* 349**,** 941-948.

BATTERHAM, R. L., COWLEY, M. A., SMALL, C. J., HERZOG, H., COHEN, M. A., DAKIN, C. L., WREN, A. M., BRYNES, A. E., LOW, M. J., GHATEI, M. A., CONE, R. D. & BLOOM, S. R. 2002. Gut hormone PYY3-36 physiologically inhibits food intake. *Nature,* 418**,** 650-654.

BATTERHAM, R. L., FFYTCHE, D. H., ROSENTHAL, J. M., ZELAYA, F. O., BARKER, G. J., WITHERS, D. J. & WILLIAMS, S. C. 2007. PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. *Nature,* 450**,** 106-9.

BATTERHAM, R. L., HEFFRON, H., KAPOOR, S., CHIVERS, J. E., CHANDARANA, K., HERZOG, H., LE ROUX, C. W., THOMAS, E. L., BELL, J. D. & WITHERS, D. J. 2006a. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metab,* 4**,** 223-233.

BATTERHAM, R. L., HEFFRON, H., KAPOOR, S., CHIVERS, J. E., CHANDARANA, K., HERZOG, H., LE ROUX, C. W., THOMAS, E. L., BELL, J. D. & WITHERS, D. J. 2006b. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metab,* 4**,** 223-33.

BEDNAREK, M. A., FEIGHNER, S. D., PONG, S. S., MCKEE, K. K., HRENIUK, D. L., SILVA, M. V., WARREN, V. A., HOWARD, A. D., VAN DER PLOEG, L. H. & HECK, J. V. 2000. Structure-function studies on the new growth hormone-releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor 1a. *J Med Chem,* 43**,** 4370-6.

BEGG, D. P. & WOODS, S. C. 2012. The Central Insulin System and Energy Balance. *In:* JOOST, H.-G. (ed.) *Appetite Control.* Berlin, Heidelberg: Springer Berlin Heidelberg.

BEGLINGER, S., DREWE, J., SCHIRRA, J., GOKE, B., D'AMATO, M. & BEGLINGER, C. 2010. Role of fat hydrolysis in regulating glucagon-like Peptide-1 secretion. *J Clin Endocrinol Metab,* 95**,** 879-86.

BEINFELD, M. C., MEYER, D. K., ESKAY, R. L., JENSEN, R. T. & BROWNSTEIN, M. J. 1981. The distribution of cholecystokinin immunoreactivity in the central nervous system of the rat as determined by radioimmunoassay. *Brain Research,* 212**,** 51-57.

BENOIT, S. C., KEMP, C. J., ELIAS, C. F., ABPLANALP, W., HERMAN, J. P., MIGRENNE, S., LEFEVRE, A. L., CRUCIANI-GUGLIELMACCI, C., MAGNAN, C., YU, F., NISWENDER, K., IRANI, B. G., HOLLAND, W. L. & CLEGG, D. J. 2009. Palmitic acid mediates hypothalamic insulin resistance by altering PKC-theta subcellular localization in rodents. *J Clin Invest,* 119**,** 2577-89.

BENSAID, A., TOME, D., GIETZEN, D., EVEN, P., MORENS, C., GAUSSERES, N. & FROMENTIN, G. 2002. Protein is more potent than carbohydrate for reducing appetite in rats. *Physiol Behav,* 75**,** 577-82.

BENSAID, A., TOME, D., L'HEUREUX-BOURDON, D., EVEN, P., GIETZEN, D., MORENS, C., GAUDICHON, C., LARUE-ACHAGIOTIS, C. & FROMENTIN, G. 2003. A high-protein diet enhances satiety without conditioned taste aversion in the rat. *Physiol Behav,* 78**,** 311-20.

BERTHOUD, H. R. 2002. Multiple neural systems controlling food intake and body weight. *Neuroscience and biobehavioral reviews,* 26**,** 393-428.

BERTHOUD, H. R., BLACKSHAW, L. A., BROOKES, S. J. & GRUNDY, D. 2004. Neuroanatomy of extrinsic afferents supplying the gastrointestinal tract. *Neurogastroenterol Motil,* 16 Suppl 1**,** 28-33.

BEZENCON, C., LE COUTRE, J. & DAMAK, S. 2007. Taste-signaling proteins are coexpressed in solitary intestinal epithelial cells. *Chem Senses,* 32**,** 41-9.

BHAVSAR, S., WATKINS, J. & YOUNG, A. 1998. Synergy between amylin and cholecystokinin for inhibition of food intake in mice. *Physiol Behav,* 64**,** 557-61.

BLAIS, A., HUNEAU, J. F., MAGRUM, L. J., KOEHNLE, T. J., SHARP, J. W., TOME, D. & GIETZEN, D. W. 2003. Threonine deprivation rapidly activates the system A amino acid transporter in primary cultures of rat neurons from the essential amino acid sensor in the anterior piriform cortex. *J Nutr,* 133**,** 2156-64.

BLOM, W. A., LLUCH, A., STAFLEU, A., VINOY, S., HOLST, J. J., SCHAAFSMA, G. & HENDRIKS, H. F. 2006. Effect of a high-protein breakfast on the postprandial ghrelin response. *Am J Clin Nutr,* 83**,** 211-20.

BLOUET, C., JO, Y. H., LI, X. & SCHWARTZ, G. J. 2009. Mediobasal hypothalamic leucine sensing regulates food intake through activation of a hypothalamus-brainstem circuit. *J Neurosci,* 29**,** 8302-11.

BLOUET, C. & SCHWARTZ, G. J. 2010. Hypothalamic nutrient sensing in the control of energy homeostasis. *Behav Brain Res,* 209**,** 1-12.

BLOUET, C. & SCHWARTZ, G. J. 2012. Brainstem nutrient sensing in the nucleus of the solitary tract inhibits feeding. *Cell Metab,* 16**,** 579-87.

BOEY, D., LIN, S., KARL, T., BALDOCK, P., LEE, N., ENRIQUEZ, R., COUZENS, M., SLACK, K., DALLMANN, R., SAINSBURY, A. & HERZOG, H. 2006. Peptide YY ablation in mice leads to the development of hyperinsulinaemia and obesity. *Diabetologia,* 49**,** 1360-70.

BOGLE, R. G., MONCADA, S., PEARSON, J. D. & MANN, G. E. 1992. Identification of inhibitors of nitric oxide synthase that do not interact with the endothelial cell L-arginine transporter. *Br J Pharmacol,* 105**,** 768-70.

BOULES, M., CUSACK, B., ZHAO, L., FAUQ, A., MCCORMICK, D. J. & RICHELSON, E. 2000. A novel neurotensin peptide analog given extracranially decreases food intake and weight in rodents. *Brain Res,* 865**,** 35-44.

BOURET, S. G., DRAPER, S. J. & SIMERLY, R. B. 2004. Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. *J Neurosci,* 24**,** 2797-805.

BOWEN, J., NOAKES, M. & CLIFTON, P. M. 2006a. Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake. *J Clin Endocrinol Metab,* 91**,** 2913-9.

BOWEN, J., NOAKES, M., TRENERRY, C. & CLIFTON, P. M. 2006b. Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *J Clin Endocrinol Metab,* 91**,** 1477-83.

BRAY, G. A., NIELSEN, S. J. & POPKIN, B. M. 2004. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *The American journal of clinical nutrition,* 79**,** 537-43.

BRENNAN, I. M., LUSCOMBE-MARSH, N. D., SEIMON, R. V., OTTO, B., HOROWITZ, M., WISHART, J. M. & FEINLE-BISSET, C. 2012. Effects of fat, protein, and carbohydrate and protein load on appetite, plasma cholecystokinin, peptide YY, and ghrelin, and energy intake in lean and obese men. *Am J Physiol Gastrointest Liver Physiol,* 303**,** G129-40.

BRISCOE, C. P., TADAYYON, M., ANDREWS, J. L., BENSON, W. G., CHAMBERS, J. K., EILERT, M. M., ELLIS, C., ELSHOURBAGY, N. A., GOETZ, A. S., MINNICK, D. T., MURDOCK, P. R., SAULS, H. R., JR., SHABON, U., SPINAGE, L. D., STRUM, J. C., SZEKERES, P. G., TAN, K. B., WAY, J. M., IGNAR, D. M., WILSON, S. & MUIR, A. I. 2003. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J Biol Chem,* 278**,** 11303-11.

BROBERGER, C., LANDRY, M., WONG, H., WALSH, J. N. & HOKFELT, T. 1997. Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. *Neuroendocrinology,* 66**,** 393-408.

BROER, S. 2008. Amino acid transport across mammalian intestinal and renal epithelia. *Physiol Rev,* 88**,** 249-86.

BROGLIO, F., GOTTERO, C., PRODAM, F., DESTEFANIS, S., GAUNA, C., ME, E., RIGANTI, F., VIVENZA, D., RAPA, A., MARTINA, V., ARVAT, E., BONA, G., VAN DER LELY, A. J. & GHIGO, E. 2004. Ghrelin secretion is inhibited by glucose load and insulin-induced hypoglycaemia but unaffected by glucagon and arginine in humans. *Clin Endocrinol (Oxf),* 61**,** 503-9.

BROOKS, L., VIARDOT, A., TSAKMAKI, A., STOLARCZYK, E., HOWARD, J. K., CANI, P. D., EVERARD, A., SLEETH, M. L., PSICHAS, A., ANASTASOVSKAJ, J., BELL, J. D., BELL-ANDERSON, K., MACKAY, C. R., GHATEI, M. A., BLOOM, S. R., FROST, G. & BEWICK, G. A. 2017. Fermentable carbohydrate stimulates FFAR2-dependent colonic PYY cell expansion to increase satiety. *Mol Metab,* 6**,** 48-60.

BROSNAN, J. T. & BROSNAN, M. E. 2006. Branched-chain amino acids: enzyme and substrate regulation. *J Nutr,* 136**,** 207S-11S.

BROWN, A. J., GOLDSWORTHY, S. M., BARNES, A. A., EILERT, M. M., TCHEANG, L., DANIELS, D., MUIR, A. I., WIGGLESWORTH, M. J., KINGHORN, I., FRASER, N. J., PIKE, N. B., STRUM, J. C.,

STEPLEWSKI, K. M., MURDOCK, P. R., HOLDER, J. C., MARSHALL, F. H., SZEKERES, P. G., WILSON, S., IGNAR, D. M., FOORD, S. M., WISE, A. & DOWELL, S. J. 2003. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem,* 278**,** 11312-9.

BROWN, E. M. & MACLEOD, R. J. 2001. Extracellular Calcium Sensing and Extracellular Calcium Signaling. *Physiological Reviews,* 81**,** 239-297.

BRUBAKER, P. L. & ANINI, Y. 2003. Direct and indirect mechanisms regulating secretion of glucagon-like peptide-1 and glucagon-like peptide-2. *Can J Physiol Pharmacol,* 81**,** 1005-12.

BRUCE, J. I., YANG, X., FERGUSON, C. J., ELLIOTT, A. C., STEWARD, M. C., CASE, R. M. & RICCARDI, D. 1999. Molecular and functional identification of a Ca2+ (polyvalent cation)-sensing receptor in rat pancreas. *J Biol Chem,* 274**,** 20561-8.

BRUNING, J. C., GAUTAM, D., BURKS, D. J., GILLETTE, J., SCHUBERT, M., ORBAN, P. C., KLEIN, R., KRONE, W., MULLER-WIELAND, D. & KAHN, C. R. 2000. Role of brain insulin receptor in control of body weight and reproduction. *Science,* 289**,** 2122-5.

BUCHAN, A. M., POLAK, J. M., SOLCIA, E., CAPELLA, C., HUDSON, D. & PEARSE, A. G. 1978. Electron immunohistochemical evidence for the human intestinal I cell as the source of CCK. *Gut,* 19**,** 403-7.

BURANT, C. F., TAKEDA, J., BROT-LAROCHE, E., BELL, G. I. & DAVIDSON, N. O. 1992. Fructose transporter in human spermatozoa and small intestine is GLUT5. *J Biol Chem,* 267**,** 14523-6.

BUSQUE, S. M., KERSTETTER, J. E., GEIBEL, J. P. & INSOGNA, K. 2005. L-type amino acids stimulate gastric acid secretion by activation of the calcium-sensing receptor in parietal cells. *Am J Physiol Gastrointest Liver Physiol,* 289**,** G664-9.

BUTLAND, B. J., S; KOPELMAN, P; MCPHERSON, K; THOMAS, S; MARDELL, J; PARRY, V. 2008. *Foresight Tackling Obesities: Future Choices - Project report* [Online]. [Accessed].

CALBET, J. A. & HOLST, J. J. 2004. Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. *Eur J Nutr,* 43**,** 127-39.

CANAFF, L., PETIT, J. L., KISIEL, M., WATSON, P. H., GASCON-BARRE, M. & HENDY, G. N. 2001. Extracellular calcium-sensing receptor is expressed in rat hepatocytes. coupling to intracellular calcium mobilization and stimulation of bile flow. *J Biol Chem,* 276**,** 4070-9.

CARRASQUER, G., ZHANG, J. Y., GENG, Z., REHM, W. S. & SCHWARTZ, M. 1993. Electrogenicity of the frog gastric mucosa proton pump based on polarization responses in the presence of H(+)-secretion inhibitors. *Biochim Biophys Acta,* 1149**,** 195-201.

CASPI, L., WANG, P. Y. & LAM, T. K. 2007. A balance of lipid-sensing mechanisms in the brain and liver. *Cell Metab,* 6**,** 99-104.

CECIL, J. E., TAVENDALE, R., WATT, P., HETHERINGTON, M. M. & PALMER, C. N. 2008. An obesity-associated FTO gene variant and increased energy intake in children. *N Engl J Med,* 359**,** 2558-66.

CHALLIS, B. G., PINNOCK, S. B., COLL, A. P., CARTER, R. N., DICKSON, S. L. & O'RAHILLY, S. 2003. Acute effects of PYY3-36 on food intake and hypothalamic neuropeptide expression in the mouse. *Biochem Biophys Res Commun,* 311**,** 915-9.

CHANG, W., PRATT, S., CHEN, T. H., NEMETH, E., HUANG, Z. & SHOBACK, D. 1998. Coupling of calcium receptors to inositol phosphate and cyclic AMP generation in mammalian cells and Xenopus laevis oocytes and immunodetection of receptor protein by region-specific antipeptide antisera. *J Bone Miner Res,* 13**,** 570-80.

CHAUDHRI, O., SMALL, C. & BLOOM, S. 2006. Gastrointestinal hormones regulating appetite. *Philos Trans R Soc Lond B Biol Sci,* 361**,** 1187-209.

CHAUDHRI, O. B., SALEM, V., MURPHY, K. G. & BLOOM, S. R. 2008a. Gastrointestinal satiety signals. *Annu Rev Physiol,* 70**,** 239-55.

CHAUDHRI, O. B., WYNNE, K. & BLOOM, S. R. 2008b. Can gut hormones control appetite and prevent obesity? *Diabetes Care,* 31 Suppl 2**,** S284-9.

CHEN, C. J., BARNETT, J. V., CONGO, D. A. & BROWN, E. M. 1989. Divalent cations suppress 3',5'-adenosine monophosphate accumulation by stimulating a pertussis toxin-sensitive guanine nucleotide-binding protein in cultured bovine parathyroid cells. *Endocrinology,* 124**,** 233-9.

CHEN, H., CHARLAT, O., TARTAGLIA, L. A., WOOLF, E. A., WENG, X., ELLIS, S. J., LAKEY, N. D., CULPEPPER, J., MOORE, K. J., BREITBART, R. E., DUYK, G. M., TEPPER, R. I. & MORGENSTERN, J. P. 1996. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell,* 84**,** 491-5.

CHEN, H. Y., TRUMBAUER, M. E., CHEN, A. S., WEINGARTH, D. T., ADAMS, J. R., FRAZIER, E. G., SHEN, Z., MARSH, D. J., FEIGHNER, S. D., GUAN, X. M., YE, Z., NARGUND, R. P., SMITH, R. G., VAN DER PLOEG, L. H., HOWARD, A. D., MACNEIL, D. J. & QIAN, S. 2004. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology,* 145**,** 2607-12.

CHEN, Q. & REIMER, R. A. 2009. Dairy protein and leucine alter GLP-1 release and mRNA of genes involved in intestinal lipid metabolism in vitro. *Nutrition,* 25**,** 340-9.

CHEUNG, M. K., GULATI, P., O'RAHILLY, S. & YEO, G. S. 2013. FTO expression is regulated by availability of essential amino acids. *Int J Obes (Lond),* 37**,** 744-7.

CHOI, S., LEE, M., SHIU, A. L., YO, S. J. & APONTE, G. W. 2007a. Identification of a protein hydrolysate responsive G protein-coupled receptor in enterocytes. *Am J Physiol Gastrointest Liver Physiol,* 292**,** G98-G112.

CHOI, S., LEE, M., SHIU, A. L., YO, S. J., HALLDEN, G. & APONTE, G. W. 2007b. GPR93 activation by protein hydrolysate induces CCK transcription and secretion in STC-1 cells. *Am J Physiol Gastrointest Liver Physiol,* 292**,** G1366-75.

CHRISTIANSEN, B., HANSEN, K. B., WELLENDORPH, P. & BRAUNER-OSBORNE, H. 2007. Pharmacological characterization of mouse GPRC6A, an L-alpha-amino-acid receptor modulated by divalent cations. *Br J Pharmacol,* 150**,** 798-807.

CLARK, J. T., SAHU, A., KALRA, P. S., BALASUBRAMANIAM, A. & KALRA, S. P. 1987. Neuropeptide Y (NPY)-induced feeding behavior in female rats: comparison with human NPY ([Met17]NPY), NPY analog ([norLeu4]NPY) and peptide YY. *Regul Pept,* 17**,** 31-9.

CLEMMENSEN, C., SMAJILOVIC, S., MADSEN, A. N., KLEIN, A. B., HOLST, B. & BRAUNER-OSBORNE, H. 2013a. Increased susceptibility to diet-induced obesity in GPRC6A receptor knockout mice. *J Endocrinol,* 217**,** 151-60.

CLEMMENSEN, C., SMAJILOVIC, S., SMITH, E. P., WOODS, S. C., BRAUNER-OSBORNE, H., SEELEY, R. J., D'ALESSIO, D. A. & RYAN, K. K. 2013b. Oral L-arginine stimulates GLP-1 secretion to improve glucose tolerance in male mice. *Endocrinology,* 154**,** 3978-83.

CLOSS, E. I., BOISSEL, J. P., HABERMEIER, A. & ROTMANN, A. 2006. Structure and function of cationic amino acid transporters (CATs). *J Membr Biol,* 213**,** 67-77.

COHEN, M. A., ELLIS, S. M., LE ROUX, C. W., BATTERHAM, R. L., PARK, A., PATTERSON, M., FROST, G. S., GHATEI, M. A. & BLOOM, S. R. 2003. Oxyntomodulin suppresses appetite and reduces food intake in humans. *J Clin Endocrinol Metab,* 88**,** 4696-701.

CONE, R. D., COWLEY, M. A., BUTLER, A. A., FAN, W., MARKS, D. L. & LOW, M. J. 2001. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity,* 25 Suppl 5**,** S63-7.

CONIGRAVE, A. D., FRANKS, A. H., BROWN, E. M. & QUINN, S. J. 2002. L-amino acid sensing by the calcium-sensing receptor: a general mechanism for coupling protein and calcium metabolism? *Eur J Clin Nutr,* 56**,** 1072-80.

CONIGRAVE, A. D., QUINN, S. J. & BROWN, E. M. 2000. L-amino acid sensing by the extracellular Ca2+-sensing receptor. *Proc Natl Acad Sci U S A,* 97**,** 4814-9.

CONSIDINE, R. V., SINHA, M. K., HEIMAN, M. L., KRIAUCIUNAS, A., STEPHENS, T. W., NYCE, M. R., OHANNESIAN, J. P., MARCO, C. C., MCKEE, L. J., BAUER, T. L. & ET AL. 1996. Serum

immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med,* 334**,** 292-5.

COOKE, J. H., PATTERSON, M., PATEL, S. R., SMITH, K. L., GHATEI, M. A., BLOOM, S. R. & MURPHY, K. G. 2009. Peripheral and central administration of xenin and neurotensin suppress food intake in rodents. *Obesity (Silver Spring),* 17**,** 1135-43.

CORP, E. S., MCQUADE, J., KRASNICKI, S. & CONZE, D. B. 2001. Feeding after fourth ventricular administration of neuropeptide Y receptor agonists in rats. *Peptides,* 22**,** 493-9.

CORP, E. S., MCQUADE, J., MORAN, T. H. & SMITH, G. P. 1993. Characterization of type A and type B CCK receptor binding sites in rat vagus nerve. *Brain Res,* 623**,** 161-6.

CORP, E. S., MELVILLE, L. D., GREENBERG, D., GIBBS, J. & SMITH, G. P. 1990. Effect of fourth ventricular neuropeptide Y and peptide YY on ingestive and other behaviors. *Am J Physiol,* 259**,** R317-23.

COTA, D., PROULX, K., SMITH, K. A., KOZMA, S. C., THOMAS, G., WOODS, S. C. & SEELEY, R. J. 2006. Hypothalamic mTOR signaling regulates food intake. *Science,* 312**,** 927-30.

COVASA, M., GRAHN, J. & RITTER, R. C. 2000. Reduced hindbrain and enteric neuronal response to intestinal oleate in rats maintained on high-fat diet. *Auton Neurosci,* 84**,** 8-18.

CRAWLEY, J. N. & BEINFELD, M. C. 1983. Rapid development of tolerance to the behavioural actions of cholecystokinin. *Nature,* 302**,** 703-6.

DAKIN, C. L., GUNN, I., SMALL, C. J., EDWARDS, C. M., HAY, D. L., SMITH, D. M., GHATEI, M. A. & BLOOM, S. R. 2001. Oxyntomodulin inhibits food intake in the rat. *Endocrinology,* 142**,** 4244-50.

DAKIN, C. L., SMALL, C. J., BATTERHAM, R. L., NEARY, N. M., COHEN, M. A., PATTERSON, M., GHATEI, M. A. & BLOOM, S. R. 2004. Peripheral oxyntomodulin reduces food intake and body weight gain in rats. *Endocrinology,* 145**,** 2687-95.

DALY, K., AL-RAMMAHI, M., MORAN, A., MARCELLO, M., NINOMIYA, Y. & SHIRAZI-BEECHEY, S. P. 2013. Sensing of amino acids by the gut-expressed taste receptor T1R1-T1R3 stimulates CCK secretion. *Am J Physiol Gastrointest Liver Physiol,* 304**,** G271-82.

DANSE, B. 1999. Scientific Concepts of Functional Foods in Europe Consensus Document. . *British Journal of Nutrition,* 81**,** S1-S27.

DATE, Y., KOJIMA, M., HOSODA, H., SAWAGUCHI, A., MONDAL, M. S., SUGANUMA, T., MATSUKURA, S., KANGAWA, K. & NAKAZATO, M. 2000. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology,* 141**,** 4255-61.

DATE, Y., MURAKAMI, N., TOSHINAI, K., MATSUKURA, S., NIIJIMA, A., MATSUO, H., KANGAWA, K. & NAKAZATO, M. 2002. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology,* 123**,** 1120-8.

DAVID, L. A., MAURICE, C. F., CARMODY, R. N., GOOTENBERG, D. B., BUTTON, J. E., WOLFE, B. E., LING, A. V., DEVLIN, A. S., VARMA, Y., FISCHBACH, M. A., BIDDINGER, S. B., DUTTON, R. J. & TURNBAUGH, P. J. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature,* 505**,** 559-63.

DAVIS, J. D., WIRTSHAFTER, D., ASIN, K. E. & BRIEF, D. 1981. Sustained intracerebroventricular infusion of brain fuels reduces body weight and food intake in rats. *Science,* 212**,** 81-3.

DE BACKER, I., HUSSAIN, S. S., BLOOM, S. R. & GARDINER, J. V. 2016. Insights into the role of neuronal glucokinase. *Am J Physiol Endocrinol Metab,* 311**,** E42-55.

DE SILVA, A. & BLOOM, S. R. 2012. Gut Hormones and Appetite Control: A Focus on PYY and GLP-1 as Therapeutic Targets in Obesity. *Gut Liver,* 6**,** 10-20.

DE TORO-MARTIN, J., ARSENAULT, B. J., DESPRES, J. P. & VOHL, M. C. 2017. Precision Nutrition: A Review of Personalized Nutritional Approaches for the Prevention and Management of Metabolic Syndrome. *Nutrients,* 9.

DEACON, C. F., KNUDSEN, L. B., MADSEN, K., WIBERG, F. C., JACOBSEN, O. & HOLST, J. J. 1998. Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity. *Diabetologia,* 41**,** 271-8.

DEGEN, L., OESCH, S., CASANOVA, M., GRAF, S., KETTERER, S., DREWE, J. & BEGLINGER, C. 2005. Effect of peptide YY3-36 on food intake in humans. *Gastroenterology,* 129**,** 1430-6.

DIAKOGIANNAKI, E., PAIS, R., TOLHURST, G., PARKER, H. E., HORSCROFT, J., RAUSCHER, B., ZIETEK, T., DANIEL, H., GRIBBLE, F. M. & REIMANN, F. 2013. Oligopeptides stimulate glucagon-like peptide-1 secretion in mice through proton-coupled uptake and the calcium-sensing receptor. *Diabetologia,* 56**,** 2688-96.

DIEPVENS, K., HABERER, D. & WESTERTERP-PLANTENGA, M. 2008. Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans. *Int J Obes (Lond),* 32**,** 510-8.

DIMITRIADIS, G., MITROU, P., LAMBADIARI, V., MARATOU, E. & RAPTIS, S. A. 2011. Insulin effects in muscle and adipose tissue. *Diabetes Res Clin Pract,* 93 Suppl 1**,** S52-9.

DONA, A. C., JIMENEZ, B., SCHAFER, H., HUMPFER, E., SPRAUL, M., LEWIS, M. R., PEARCE, J. T., HOLMES, E., LINDON, J. C. & NICHOLSON, J. K. 2014. Precision high-throughput proton NMR spectroscopy of human urine, serum, and plasma for large-scale metabolic phenotyping. *Anal Chem,* 86**,** 9887-94.

DOUGKAS, A. & OSTMAN, E. 2016. Protein-Enriched Liquid Preloads Varying in Macronutrient Content Modulate Appetite and Appetite-Regulating Hormones in Healthy Adults. *J Nutr,* 146**,** 637-45.

DRUCKER, D. J. 2006. The biology of incretin hormones. *Cell Metab,* 3**,** 153-65.

DUE, A., LARSEN, T. M., HERMANSEN, K., STENDER, S., HOLST, J. J., TOUBRO, S., MARTINUSSEN, T. & ASTRUP, A. 2008. Comparison of the effects on insulin resistance and glucose tolerance of 6-mo high-monounsaturated-fat, low-fat, and control diets. *Am J Clin Nutr,* 87**,** 855-62.

DUFNER, M. M., KIRCHHOFF, P., REMY, C., HAFNER, P., MULLER, M. K., CHENG, S. X., TANG, L. Q., HEBERT, S. C., GEIBEL, J. P. & WAGNER, C. A. 2005. The calcium-sensing receptor acts as a modulator of gastric acid secretion in freshly isolated human gastric glands. *Am J Physiol Gastrointest Liver Physiol,* 289**,** G1084-90.

DUMAS, M. E. 2011. The microbial-mammalian metabolic axis: beyond simple metabolism. *Cell Metab,* 13**,** 489-90.

DUMONT, Y., FOURNIER, A., ST-PIERRE, S. & QUIRION, R. 1995. Characterization of neuropeptide Y binding sites in rat brain membrane preparations using [125I][Leu31,Pro34]peptide YY and [125I]peptide YY3-36 as selective Y1 and Y2 radioligands. *J Pharmacol Exp Ther,* 272**,** 673-80.

DYER, J., SALMON, K. S., ZIBRIK, L. & SHIRAZI-BEECHEY, S. P. 2005. Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells. *Biochem Soc Trans,* 33**,** 302-5.

DYER, J., VAYRO, S., KING, T. P. & SHIRAZI-BEECHEY, S. P. 2003. Glucose sensing in the intestinal epithelium. *European journal of biochemistry / FEBS,* 270**,** 3377-88.

EBERLEIN, G. A., EYSSELEIN, V. E., SCHAEFFER, M., LAYER, P., GRANDT, D., GOEBELL, H., NIEBEL, W., DAVIS, M., LEE, T. D., SHIVELY, J. E. & ET AL. 1989. A new molecular form of PYY: structural characterization of human PYY(3-36) and PYY(1-36). *Peptides,* 10**,** 797-803.

EFEYAN, A., COMB, W. C. & SABATINI, D. M. 2015. Nutrient-sensing mechanisms and pathways. *Nature,* 517**,** 302-10.

EGEROD, K. L., ENGELSTOFT, M. S., GRUNDDAL, K. V., NOHR, M. K., SECHER, A., SAKATA, I., PEDERSEN, J., WINDELOV, J. A., FUCHTBAUER, E. M., OLSEN, J., SUNDLER, F., CHRISTENSEN, J. P., WIERUP, N., OLSEN, J. V., HOLST, J. J., ZIGMAN, J. M., POULSEN, S. S. & SCHWARTZ, T. W. 2012. A major lineage of enteroendocrine cells coexpress CCK, secretin, GIP, GLP-1, PYY, and neurotensin but not somatostatin. *Endocrinology,* 153**,** 5782-95.

EISENSTEIN, A. B. & STRACK, I. 1978. Amino acid stimulation of glucagon secretion by perifused islets of high-protein-fed rats. *Diabetes,* 27**,** 370-6.

EISENSTEIN, J., ROBERTS, S. B., DALLAL, G. & SALTZMAN, E. 2002. High-protein weight-loss diets: are they safe and do they work? A review of the experimental and epidemiologic data. *Nutr Rev,* 60**,** 189-200.

EISSELE, R., GOKE, R., WILLEMER, S., HARTHUS, H. P., VERMEER, H., ARNOLD, R. & GOKE, B. 1992. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur J Clin Invest,* 22**,** 283-91.

EKBLAD, E. & SUNDLER, F. 2002. Distribution of pancreatic polypeptide and peptide YY. *Peptides,* 23**,** 251-61.

ELIAS, C. F., SAPER, C. B., MARATOS-FLIER, E., TRITOS, N. A., LEE, C., KELLY, J., TATRO, J. B., HOFFMAN, G. E., OLLMANN, M. M., BARSH, G. S., SAKURAI, T., YANAGISAWA, M. & ELMQUIST, J. K. 1998. Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *J Comp Neurol,* 402**,** 442-59.

ELLIOTT, R. M., MORGAN, L. M., TREDGER, J. A., DEACON, S., WRIGHT, J. & MARKS, V. 1993. Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol,* 138**,** 159-66.

ENSINCK, J. W., VOGEL, R. E., LASCHANSKY, E. C. & FRANCIS, B. H. 1990. Effect of ingested carbohydrate, fat, and protein on the release of somatostatin-28 in humans. *Gastroenterology,* 98**,** 633-8.

ESKO, T., HIRSCHHORN, J. N., FELDMAN, H. A., HSU, Y. H., DEIK, A. A., CLISH, C. B., EBBELING, C. B. & LUDWIG, D. S. 2017. Metabolomic profiles as reliable biomarkers of dietary composition. *Am J Clin Nutr,* 105**,** 547-554.

ESSAH, P. A., LEVY, J. R., SISTRUN, S. N., KELLY, S. M. & NESTLER, J. E. 2007. Effect of macronutrient composition on postprandial peptide YY levels. *J Clin Endocrinol Metab,* 92**,** 4052-5.

EVANS, C. E., GREENWOOD, D. C., THREAPLETON, D. E., GALE, C. P., CLEGHORN, C. L. & BURLEY, V. J. 2017. Glycemic index, glycemic load, and blood pressure: a systematic review and meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition,* 105**,** 1176-1190.

EVERARD, A. & CANI, P. D. 2014. Gut microbiota and GLP-1. *Rev Endocr Metab Disord,* 15**,** 189-96.

EVERETT, J. R. 2017. NMR-based pharmacometabonomics: A new paradigm for personalised or precision medicine. *Prog Nucl Magn Reson Spectrosc,* 102-103**,** 1-14.

EYSSELEIN, V. E., REEVE, J. R., JR. & EBERLEIN, G. 1986. Cholecystokinin--gene structure, and molecular forms in tissue and blood. *Z Gastroenterol,* 24**,** 645-59.

FAIPOUX, R., TOME, D., GOUGIS, S., DARCEL, N. & FROMENTIN, G. 2008. Proteins activate satiety-related neuronal pathways in the brainstem and hypothalamus of rats. *J Nutr,* 138**,** 1172-8.

FEHMANN, H. C., JIANG, J., SCHWEINFURTH, J., WHEELER, M. B., BOYD, A. E., 3RD & GOKE, B. 1994. Stable expression of the rat GLP-I receptor in CHO cells: activation and binding characteristics utilizing GLP-I(7-36)-amide, oxyntomodulin, exendin-4, and exendin(9-39). *Peptides,* 15**,** 453-6.

FERRIS, C. F., HAMMER, R. A. & LEEMAN, S. E. 1981. Elevation of plasma neurotensin during lipid perfusion of rat small intestine. *Peptides,* 2 Suppl 2**,** 263-6.

FIORAMONTI, X., CONTIE, S., SONG, Z., ROUTH, V. H., LORSIGNOL, A. & PENICAUD, L. 2007. Characterization of glucosensing neuron subpopulations in the arcuate nucleus: integration in neuropeptide Y and pro-opio melanocortin networks? *Diabetes,* 56**,** 1219-27.

FLIER, J. S. 1998. Clinical review 94: What's in a name? In search of leptin's physiologic role. *J Clin Endocrinol Metab,* 83**,** 1407-13.

FLINT, A., RABEN, A., ASTRUP, A. & HOLST, J. J. 1998. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest,* 101**,** 515-20.

FLOYD, J. C., JR., FAJANS, S. S., CONN, J. W., KNOPF, R. F. & RULL, J. 1966. Stimulation of insulin secretion by amino acids. *J Clin Invest,* 45**,** 1487-502.

FOSTER-SCHUBERT, K. E., OVERDUIN, J., PRUDOM, C. E., LIU, J., CALLAHAN, H. S., GAYLINN, B. D., THORNER, M. O. & CUMMINGS, D. E. 2008. Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. *J Clin Endocrinol Metab,* 93**,** 1971-9.

FRANCHI-GAZZOLA, R., VISIGALLI, R., BUSSOLATI, O., DALL'ASTA, V. & GAZZOLA, G. C. 1999. Adaptive increase of amino acid transport system A requires ERK1/2 activation. *J Biol Chem,* 274**,** 28922-8.

FRAYLING, T. M., TIMPSON, N. J., WEEDON, M. N., ZEGGINI, E., FREATHY, R. M., LINDGREN, C. M., PERRY, J. R., ELLIOTT, K. S., LANGO, H., RAYNER, N. W., SHIELDS, B., HARRIES, L. W., BARRETT, J. C., ELLARD, S., GROVES, C. J., KNIGHT, B., PATCH, A. M., NESS, A. R., EBRAHIM, S., LAWLOR, D. A., RING, S. M., BEN-SHLOMO, Y., JARVELIN, M. R., SOVIO, U., BENNETT, A. J., MELZER, D., FERRUCCI, L., LOOS, R. J., BARROSO, I., WAREHAM, N. J., KARPE, F., OWEN, K. R., CARDON, L. R., WALKER, M., HITMAN, G. A., PALMER, C. N., DONEY, A. S., MORRIS, A. D., SMITH, G. D., HATTERSLEY, A. T. & MCCARTHY, M. I. 2007. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science,* 316**,** 889-94.

FREEMAN, H. J. & KIM, Y. S. 1978. Digestion and absorption of protein. *Annu Rev Med,* 29**,** 99-116.

FROMENTIN, G., DARCEL, N., CHAUMONTET, C., MARSSET-BAGLIERI, A., NADKARNI, N. & TOME, D. 2012. Peripheral and central mechanisms involved in the control of food intake by dietary amino acids and proteins. *Nutr Res Rev,* 25**,** 29-39.

FROST, G., SLEETH, M. L., SAHURI-ARISOYLU, M., LIZARBE, B., CERDAN, S., BRODY, L., ANASTASOVSKA, J., GHOURAB, S., HANKIR, M., ZHANG, S., CARLING, D., SWANN, J. R., GIBSON, G., VIARDOT, A., MORRISON, D., LOUISE THOMAS, E. & BELL, J. D. 2014. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun,* 5**,** 3611.

FURNESS, J. B., CALLAGHAN, B. P., RIVERA, L. R. & CHO, H. J. 2014. The enteric nervous system and gastrointestinal innervation: integrated local and central control. *Adv Exp Med Biol,* 817**,** 39-71.

FURNESS, J. B., JONES, C., NURGALI, K. & CLERC, N. 2004. Intrinsic primary afferent neurons and nerve circuits within the intestine. *Prog Neurobiol,* 72**,** 143-64.

GALLWITZ, B. & SCHMIDT, W. E. 1997. [GLP-1 receptor gen "knock out" causes glucose intolerance, but no alterations of eating behavior]. *Z Gastroenterol,* 35**,** 655-8.

GAO, J., GUO, Y., WANG, J., WANG, Z., JIN, X., CHENG, C., LI, Y. & LI, K. 2011. Spectroscopic analyses on interaction of o-Vanillin-D-Phenylalanine, o-Vanillin-L-Tyrosine and o-Vanillin-L-Levodopa Schiff Bases with bovine serum albumin (BSA). *Spectrochim Acta A Mol Biomol Spectrosc,* 78**,** 1278-86.

GARCIA-PEREZ, I., POSMA, J. M., GIBSON, R., CHAMBERS, E. S., HANSEN, T. H., VESTERGAARD, H., HANSEN, T., BECKMANN, M., PEDERSEN, O., ELLIOTT, P., STAMLER, J., NICHOLSON, J. K., DRAPER, J., MATHERS, J. C., HOLMES, E. & FROST, G. 2017. Objective assessment of dietary patterns by use of metabolic phenotyping: a randomised, controlled, crossover trial. *Lancet Diabetes Endocrinol,* 5**,** 184-195.

GERAEDTS, M. C., TAKAHASHI, T., VIGUES, S., MARKWARDT, M. L., NKOBENA, A., COCKERHAM, R. E., HAJNAL, A., DOTSON, C. D., RIZZO, M. A. & MUNGER, S. D. 2012. Transformation of postingestive glucose responses after deletion of sweet taste receptor subunits or gastric bypass surgery. *Am J Physiol Endocrinol Metab,* 303**,** E464-74.

GERKEN, T., GIRARD, C. A., TUNG, Y. C., WEBBY, C. J., SAUDEK, V., HEWITSON, K. S., YEO, G. S., MCDONOUGH, M. A., CUNLIFFE, S., MCNEILL, L. A., GALVANOVSKIS, J., RORSMAN, P., ROBINS, P., PRIEUR, X., COLL, A. P., MA, M., JOVANOVIC, Z., FAROOQI, I. S., SEDGWICK, B., BARROSO, I., LINDAHL, T., PONTING, C. P., ASHCROFT, F. M., O'RAHILLY, S. & SCHOFIELD, C. J. 2007. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science,* 318**,** 1469-72.

GHANBARI, R., ANWAR, F., ALKHARFY, K. M., GILANI, A. H. & SAARI, N. 2012. Valuable nutrients and functional bioactives in different parts of olive (Olea europaea L.)-a review. *Int J Mol Sci,* 13**,** 3291-340.

GIBBS, J., YOUNG, R. C. & SMITH, G. P. 1973. Cholecystokinin decreases food intake in rats. *J Comp Physiol Psychol,* 84**,** 488-95.

GOFF, L. M., COWLAND, D. E., HOOPER, L. & FROST, G. S. 2013. Low glycaemic index diets and blood lipids: a systematic review and meta-analysis of randomised controlled trials. *Nutr Metab Cardiovasc Dis,* 23**,** 1-10.

GOKE, R., FEHMANN, H. C., LINN, T., SCHMIDT, H., KRAUSE, M., ENG, J. & GOKE, B. 1993. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. *J Biol Chem,* 268**,** 19650-5.

GOTO, K., KASAOKA, S., TAKIZAWA, M., OGAWA, M., TSUCHIYA, T. & NAKAJIMA, S. 2007. Bitter taste and blood glucose are not involved in the suppressive effect of dietary histidine on food intake. *Neurosci Lett,* 420**,** 106-9.

GREENBERG, D., SMITH, G. P. & GIBBS, J. 1990. Intraduodenal infusions of fats elicit satiety in sham-feeding rats. *Am J Physiol,* 259**,** R110-8.

GREENFIELD, J. R., FAROOQI, I. S., KEOGH, J. M., HENNING, E., HABIB, A. M., BLACKWOOD, A., REIMANN, F., HOLST, J. J. & GRIBBLE, F. M. 2009. Oral glutamine increases circulating glucagon-like peptide 1, glucagon, and insulin concentrations in lean, obese, and type 2 diabetic subjects. *Am J Clin Nutr,* 89**,** 106-113.

GROSS, L. S., LI, L., FORD, E. S. & LIU, S. 2004. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. *The American journal of clinical nutrition,* 79**,** 774-9.

GULATI, P., CHEUNG, M. K., ANTROBUS, R., CHURCH, C. D., HARDING, H. P., TUNG, Y. C., RIMMINGTON, D., MA, M., RON, D., LEHNER, P. J., ASHCROFT, F. M., COX, R. D., COLL, A. P., O'RAHILLY, S. & YEO, G. S. 2013. Role for the obesity-related FTO gene in the cellular sensing of amino acids. *Proc Natl Acad Sci U S A,* 110**,** 2557-62.

GUTTLER, F., KUHL, C., PEDERSEN, L. & PABY, P. 1978. Effects of oral phenylalanine load on plasma glucagon, insulin, amino acid and glucose concentrations in man. *Scand J Clin Lab Invest,* 38**,** 255-60.

GUTZWILLER, J. P., GOKE, B., DREWE, J., HILDEBRAND, P., KETTERER, S., HANDSCHIN, D., WINTERHALDER, R., CONEN, D. & BEGLINGER, C. 1999. Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut,* 44**,** 81-6.

HABIB, A. M., RICHARDS, P., CAIRNS, L. S., ROGERS, G. J., BANNON, C. A., PARKER, H. E., MORLEY, T. C., YEO, G. S., REIMANN, F. & GRIBBLE, F. M. 2012. Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. *Endocrinology,* 153**,** 3054-65.

HAID, D. C., JORDAN-BIEGGER, C., WIDMAYER, P. & BREER, H. 2012. Receptors responsive to protein breakdown products in g-cells and d-cells of mouse, swine and human. *Front Physiol,* 3**,** 65.

HALATCHEV, I. G., ELLACOTT, K. L., FAN, W. & CONE, R. D. 2004. Peptide YY3-36 inhibits food intake in mice through a melanocortin-4 receptor-independent mechanism. *Endocrinology,* 145**,** 2585-90.

HALFORD, J. C. & HARROLD, J. A. 2012. Satiety-enhancing products for appetite control: science and regulation of functional foods for weight management. *Proc Nutr Soc,* 71**,** 350-62.

HALL, W. L., MILLWARD, D. J., LONG, S. J. & MORGAN, L. M. 2003. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *Br J Nutr,* 89**,** 239-48.

HALTON, T. L. & HU, F. B. 2004. The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *J Am Coll Nutr,* 23**,** 373-85.

HAMER, H. M., JONKERS, D., VENEMA, K., VANHOUTVIN, S., TROOST, F. J. & BRUMMER, R. J. 2008. Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther,* 27**,** 104-19.

HANDLOGTEN, M. E., HUANG, C., SHIRAISHI, N., AWATA, H. & MILLER, R. T. 2001. The Ca2+-sensing receptor activates cytosolic phospholipase A2 via a Gqalpha -dependent ERK-independent pathway. *J Biol Chem,* 276**,** 13941-8.

HANNAH, J. S., DUBEY, A. K. & HANSEN, B. C. 1990. Postingestional effects of a high-protein diet on the regulation of food intake in monkeys. *Am J Clin Nutr,* 52**,** 320-5.

HAO, S., SHARP, J. W., ROSS-INTA, C. M., MCDANIEL, B. J., ANTHONY, T. G., WEK, R. C., CAVENER, D. R., MCGRATH, B. C., RUDELL, J. B., KOEHNLE, T. J. & GIETZEN, D. W. 2005. Uncharged tRNA and sensing of amino acid deficiency in mammalian piriform cortex. *Science,* 307**,** 1776-8.

HAPFELMEIER, S., LAWSON, M. A., SLACK, E., KIRUNDI, J. K., STOEL, M., HEIKENWALDER, M., CAHENZLI, J., VELYKOREDKO, Y., BALMER, M. L., ENDT, K., GEUKING, M. B., CURTISS, R., 3RD, MCCOY, K. D. & MACPHERSON, A. J. 2010. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. *Science,* 328**,** 1705-9.

HARA, T., KIMURA, I., INOUE, D., ICHIMURA, A. & HIRASAWA, A. 2013. Free fatty acid receptors and their role in regulation of energy metabolism. *Rev Physiol Biochem Pharmacol,* 164**,** 77-116.

HARDY, G. 2000. Nutraceuticals and functional foods: introduction and meaning. *Nutrition,* 16**,** 688-9.

HARPER, A. E. & PETERS, J. C. 1989. Protein intake, brain amino acid and serotonin concentrations and protein self-selection. *J Nutr,* 119**,** 677-89.

HASLER, C. M. 2000. The changing face of functional foods. *J Am Coll Nutr,* 19**,** 499S-506S.

HAUPT, A., THAMER, C., STAIGER, H., TSCHRITTER, O., KIRCHHOFF, K., MACHICAO, F., HARING, H. U., STEFAN, N. & FRITSCHE, A. 2009. Variation in the FTO gene influences food intake but not energy expenditure. *Exp Clin Endocrinol Diabetes,* 117**,** 194-7.

HE, W., LAM, T. K., OBICI, S. & ROSSETTI, L. 2006. Molecular disruption of hypothalamic nutrient sensing induces obesity. *Nat Neurosci,* 9**,** 227-33.

HENQUIN, J. C., DUFRANE, D. & NENQUIN, M. 2006. Nutrient control of insulin secretion in isolated normal human islets. *Diabetes,* 55**,** 3470-7.

HERRMANN, C., GOKE, R., RICHTER, G., FEHMANN, H. C., ARNOLD, R. & GOKE, B. 1995. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion,* 56**,** 117-26.

HERZIG, K. H., LOUIE, D. S. & OWYANG, C. 1994. Somatostatin inhibits CCK release by inhibiting secretion and action of CCK-releasing peptide. *Am J Physiol,* 266**,** G1156-61.

HEYMSFIELD, S. B., GREENBERG, A. S., FUJIOKA, K., DIXON, R. M., KUSHNER, R., HUNT, T., LUBINA, J. A., PATANE, J., SELF, B., HUNT, P. & MCCAMISH, M. 1999. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA,* 282**,** 1568-75.

HILL, J. O. & PETERS, J. C. 2002. Biomarkers and functional foods for obesity and diabetes. *Br J Nutr,* 88 Suppl 2**,** S213-8.

HIRA, T., NAKAJIMA, S., ETO, Y. & HARA, H. 2008. Calcium-sensing receptor mediates phenylalanine-induced cholecystokinin secretion in enteroendocrine STC-1 cells. *FEBS Journal,* 275**,** 4620-4626.

HIRASAWA, A., TSUMAYA, K., AWAJI, T., KATSUMA, S., ADACHI, T., YAMADA, M., SUGIMOTO, Y., MIYAZAKI, S. & TSUJIMOTO, G. 2005. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med,* 11**,** 90-4.

HO, C., CONNER, D. A., POLLAK, M. R., LADD, D. J., KIFOR, O., WARREN, H. B., BROWN, E. M., SEIDMAN, J. G. & SEIDMAN, C. E. 1995. A mouse model of human familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Nat Genet,* 11**,** 389-94.

HOFER, D., ASAN, E. & DRENCKHAHN, D. 1999. Chemosensory Perception in the Gut. *News Physiol Sci,* 14**,** 18-23.

HOLST, J. J. 1999. Glucagon-like Peptide 1 (GLP-1): An Intestinal Hormone, Signalling Nutritional Abundance, with an Unusual Therapeutic Potential. *Trends Endocrinol Metab,* 10**,** 229-235.

HOLST, J. J. 2004. Treatment of type 2 diabetes mellitus with agonists of the GLP-1 receptor or DPP-IV inhibitors. *Expert Opin Emerg Drugs,* 9**,** 155-66.

HOLST, J. J. 2007a. The physiology of glucagon-like peptide 1. *Physiol Rev,* 87**,** 1409-39.

HOLST, J. J. 2007b. The physiology of glucagon-like peptide 1. *Physiol Rev.,* 87**,** 1409-1439.

HOROWITZ, M. & DENT, J. 1991. Disordered gastric emptying: mechanical basis, assessment and treatment. *Baillieres Clin Gastroenterol,* 5**,** 371-407.

HOSODA, H., DOI, K., NAGAYA, N., OKUMURA, H., NAKAGAWA, E., ENOMOTO, M., ONO, F. & KANGAWA, K. 2004. Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. *Clin Chem,* 50**,** 1077-80.

HOSOKAWA, H., NINOMIYA, H., SAWAMURA, T., SUGIMOTO, Y., ICHIKAWA, A., FUJIWARA, K. & MASAKI, T. 1999. Neuron-specific expression of cationic amino acid transporter 3 in the adult rat brain. *Brain Res,* 838**,** 158-65.

HUSSAIN, S., RICHARDSON, E., MA, Y., HOLTON, C., DE BACKER, I., BUCKLEY, N., DHILLO, W., BEWICK, G., ZHANG, S., CARLING, D., BLOOM, S. & GARDINER, J. 2015. Glucokinase activity in the arcuate nucleus regulates glucose intake. *J Clin Invest,* 125**,** 337-49.

HUTCHISON, J. B., DIMALINE, R. & DOCKRAY, G. J. 1981. Neuropeptides in the gut: Quantification and characterization of cholecystokinin octapeptide-, bombesin- and vasoactive intestinal polypeptide-like immunoreactivities in the myenteric plexus of the guinea-pig small intestine. *Peptides,* 2**,** 23-30.

IBRAHIM, N., BOSCH, M. A., SMART, J. L., QIU, J., RUBINSTEIN, M., RONNEKLEIV, O. K., LOW, M. J. & KELLY, M. J. 2003. Hypothalamic proopiomelanocortin neurons are glucose responsive and express K(ATP) channels. *Endocrinology,* 144**,** 1331-40.

IMERYUZ, N., YEGEN, B. C., BOZKURT, A., COSKUN, T., VILLANUEVA-PENACARRILLO, M. L. & ULUSOY, N. B. 1997. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am J Physiol,* 273**,** G920-7.

INOKI, K., LI, Y., XU, T. & GUAN, K. L. 2003. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev,* 17**,** 1829-34.

JANDHYALA, S. M., TALUKDAR, R., SUBRAMANYAM, C., VUYYURU, H., SASIKALA, M. & NAGESHWAR REDDY, D. 2015. Role of the normal gut microbiota. *World J Gastroenterol,* 21**,** 8787-803.

JANG, H.-J., KOKRASHVILI, Z., THEODORAKIS, M. J., CARLSON, O. D., KIM, B.-J., ZHOU, J., KIM, H. H., XU, X., CHAN, S. L., JUHASZOVA, M., BERNIER, M., MOSINGER, B., MARGOLSKEE, R. F. & EGAN, J. M. 2007a. Gut-expressed gustducin and taste receptors regulate secretion of

glucagon-like peptide-1. *Proceedings of the National Academy of Sciences,* 104**,** 15069-15074.

JANG, H. J., KOKRASHVILI, Z., THEODORAKIS, M. J., CARLSON, O. D., KIM, B. J., ZHOU, J., KIM, H. H., XU, X., CHAN, S. L., JUHASZOVA, M., BERNIER, M., MOSINGER, B., MARGOLSKEE, R. F. & EGAN, J. M. 2007b. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci U S A,* 104**,** 15069-74.

JANSSEN, S. & DEPOORTERE, I. 2013. Nutrient sensing in the gut: new roads to therapeutics? *Trends in Endocrinology & Metabolism,* 24**,** 92-100.

JEAN, C., ROME, S., MATHE, V., HUNEAU, J. F., AATTOURI, N., FROMENTIN, G., ACHAGIOTIS, C. L. & TOME, D. 2001. Metabolic evidence for adaptation to a high protein diet in rats. *J Nutr,* 131**,** 91-8.

JOHNSEN, A. H. 1998. Phylogeny of the cholecystokinin/gastrin family. *Front Neuroendocrinol,* 19**,** 73-99.

JOHNSEN, L. J. A. H. 2001. Post-translational modifications of heterologously expressed cholecystokinin in Saccharomyces cerevisiae. *Scandinavian Journal of Clinical and Laboratory Investigation,* 61**,** 87-92.

JOHNSON, R. J., SEGAL, M. S., SAUTIN, Y., NAKAGAWA, T., FEIG, D. I., KANG, D. H., GERSCH, M. S., BENNER, S. & SANCHEZ-LOZADA, L. G. 2007. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *The American journal of clinical nutrition,* 86**,** 899-906.

JORDI, J., HERZOG, B., CAMARGO, S. M., BOYLE, C. N., LUTZ, T. A. & VERREY, F. 2013a. Specific amino acids inhibit food intake via the area postrema or vagal afferents. *J Physiol,* 591**,** 5611-21.

JORDI, J., HERZOG, B., CAMARGO, S. M., BOYLE, C. N., LUTZ, T. A. & VERREY, F. 2013b. Specific amino acids inhibit food intake via the area postrema or vagal afferents. *J Physiol,* 591**,** 5611-21.

JOURNEL, M., CHAUMONTET, C., DARCEL, N., FROMENTIN, G. & TOME, D. 2012. Brain responses to high-protein diets. *Adv Nutr,* 3**,** 322-9.

JOYNER, K., SMITH, G. P. & GIBBS, J. 1993. Abdominal vagotomy decreases the satiating potency of CCK-8 in sham and real feeding. *Am J Physiol,* 264**,** R912-6.

JUANOLA-FALGARONA, M., SALAS-SALVADO, J., IBARROLA-JURADO, N., RABASSA-SOLER, A., DIAZ-LOPEZ, A., GUASCH-FERRE, M., HERNANDEZ-ALONSO, P., BALANZA, R. & BULLO, M. 2014. Effect of the glycemic index of the diet on weight loss, modulation of satiety, inflammation, and other metabolic risk factors: a randomized controlled trial. *Am J Clin Nutr,* 100**,** 27-35.

KAHN, B. B., ALQUIER, T., CARLING, D. & HARDIE, D. G. 2005. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab,* 1**,** 15-25.

KAHN, S. E., PRIGEON, R. L., MCCULLOCH, D. K., BOYKO, E. J., BERGMAN, R. N., SCHWARTZ, M. W., NEIFING, J. L., WARD, W. K., BEARD, J. C., PALMER, J. P. & ET AL. 1993. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes,* 42**,** 1663-72.

KALRA, S. P., DUBE, M. G., PU, S., XU, B., HORVATH, T. L. & KALRA, P. S. 1999. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocrine reviews,* 20**,** 68-100.

KAMP, F., GUO, W., SOUTO, R., PILCH, P. F., CORKEY, B. E. & HAMILTON, J. A. 2003. Rapid flip-flop of oleic acid across the plasma membrane of adipocytes. *J Biol Chem,* 278**,** 7988-95.

KANAMORI, K., ROSS, B. D. & KONDRAT, R. W. 1998. Rate of glutamate synthesis from leucine in rat brain measured in vivo by 15N NMR. *J Neurochem,* 70**,** 1304-15.

KANNO, T., MATSUKI, T., OKA, M., UTSUNOMIYA, H., INADA, K., MAGARI, H., INOUE, I., MAEKITA, T., UEDA, K., ENOMOTO, S., IGUCHI, M., YANAOKA, K., TAMAI, H., AKIMOTO, S., NOMOTO, K., TANAKA, R. & ICHINOSE, M. 2009. Gastric acid reduction leads to an alteration in lower intestinal microflora. *Biochem Biophys Res Commun,* 381**,** 666-70.

KARAKI, S., MITSUI, R., HAYASHI, H., KATO, I., SUGIYA, H., IWANAGA, T., FURNESS, J. B. & KUWAHARA, A. 2006. Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. *Cell Tissue Res,* 324**,** 353-60.

KAUR, N. & SINGH, D. P. 2017. Deciphering the consumer behaviour facets of functional foods: A literature review. *Appetite,* 112**,** 167-187.

KELESIDIS, T., KELESIDIS, I., CHOU, S. & MANTZOROS, C. S. 2010. Narrative Review: The Role of Leptin in Human Physiology: Emerging Clinical Applications. *Annals of internal medicine,* 152**,** 93-100.

KELLEY, A. E., BAKSHI, V. P., HABER, S. N., STEININGER, T. L., WILL, M. J. & ZHANG, M. 2002. Opioid modulation of taste hedonics within the ventral striatum. *Physiology & behavior,* 76**,** 365-77.

KELLY, T., YANG, W., CHEN, C. S., REYNOLDS, K. & HE, J. 2008. Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond),* 32**,** 1431-7.

KIMBALL, S. R. & JEFFERSON, L. S. 2006. New functions for amino acids: effects on gene transcription and translation. *Am J Clin Nutr,* 83**,** 500S-507S.

KINSEY-JONES, J. S., ALAMSHAH, A., MCGAVIGAN, A. K., SPRECKLEY, E., BANKS, K., CERECEDA MONTEOLIVA, N., NORTON, M., BEWICK, G. A. & MURPHY, K. G. 2015. GPRC6a is not required for the effects of a high-protein diet on body weight in mice. *Obesity (Silver Spring),* 23**,** 1194-200.

KINZIG, K. P., HARGRAVE, S. L., HYUN, J. & MORAN, T. H. 2007. Energy balance and hypothalamic effects of a high-protein/low-carbohydrate diet. *Physiol Behav,* 92**,** 454-60.

KISSILEFF, H. R., PI-SUNYER, F. X., THORNTON, J. & SMITH, G. P. 1981. C-terminal octapeptide of cholecystokinin decreases food intake in man. *Am J Clin Nutr,* 34**,** 154-60.

KLEESSEN, B., STOOF, G., PROLL, J., SCHMIEDL, D., NOACK, J. & BLAUT, M. 1997. Feeding resistant starch affects fecal and cecal microflora and short-chain fatty acids in rats. *J Anim Sci,* 75**,** 2453-62.

KODA, S., DATE, Y., MURAKAMI, N., SHIMBARA, T., HANADA, T., TOSHINAI, K., NIIJIMA, A., FURUYA, M., INOMATA, N., OSUYE, K. & NAKAZATO, M. 2005. The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. *Endocrinology,* 146**,** 2369-75.

KOEHNLE, T. J., RUSSELL, M. C. & GIETZEN, D. W. 2003. Rats rapidly reject diets deficient in essential amino acids. *J Nutr,* 133**,** 2331-5.

KOJIMA, M., HOSODA, H., DATE, Y., NAKAZATO, M., MATSUO, H. & KANGAWA, K. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature,* 402**,** 656-60.

KOJIMA, M. & KANGAWA, K. 2005. Ghrelin: Structure and Function. *Physiological Reviews,* 85**,** 495-522.

KOKRASHVILI, Z., MOSINGER, B. & MARGOLSKEE, R. F. 2009. Taste signaling elements expressed in gut enteroendocrine cells regulate nutrient-responsive secretion of gut hormones. *Am J Clin Nutr,* 90**,** 822S-825S.

KREYMANN, B., WILLIAMS, G., GHATEI, M. A. & BLOOM, S. R. 1987. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet,* 2**,** 1300-4.

KUANG, D., YAO, Y., LAM, J., TSUSHIMA, R. G. & HAMPSON, D. R. 2005. Cloning and characterization of a family C orphan G-protein coupled receptor. *J Neurochem,* 93**,** 383-91.

LAM, C. K., CHARI, M. & LAM, T. K. 2009. CNS regulation of glucose homeostasis. *Physiology (Bethesda),* 24**,** 159-70.

LAM, T. K., GUTIERREZ-JUAREZ, R., POCAI, A. & ROSSETTI, L. 2005a. Regulation of blood glucose by hypothalamic pyruvate metabolism. *Science,* 309**,** 943-7.

LAM, T. K., POCAI, A., GUTIERREZ-JUAREZ, R., OBICI, S., BRYAN, J., AGUILAR-BRYAN, L., SCHWARTZ, G. J. & ROSSETTI, L. 2005b. Hypothalamic sensing of circulating fatty acids is required for glucose homeostasis. *Nat Med,* 11**,** 320-7.

LAM, T. K., SCHWARTZ, G. J. & ROSSETTI, L. 2005c. Hypothalamic sensing of fatty acids. *Nat Neurosci,* 8**,** 579-84.

LAM, T. K., YOSHII, H., HABER, C. A., BOGDANOVIC, E., LAM, L., FANTUS, I. G. & GIACCA, A. 2002. Free fatty acid-induced hepatic insulin resistance: a potential role for protein kinase C-delta. *Am J Physiol Endocrinol Metab,* 283**,** E682-91.

LANGLOIS, A., CORRING, T., LEVENEZ, F., CUBER, J. C. & CHAYVIALLE, J. A. 1990. Effects of pancreatic polypeptide on biliary flow and bile acid secretion stimulated by secretin and cholecystokinin in the conscious pig. *Regul Pept,* 27**,** 139-47.

LARSEN, P. J., TANG-CHRISTENSEN, M., HOLST, J. J. & ORSKOV, C. 1997. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience,* 77**,** 257-70.

LAVIN, J. H., WITTERT, G. A., ANDREWS, J., YEAP, B., WISHART, J. M., MORRIS, H. A., MORLEY, J. E., HOROWITZ, M. & READ, N. W. 1998. Interaction of insulin, glucagon-like peptide 1, gastric inhibitory polypeptide, and appetite in response to intraduodenal carbohydrate. *The American journal of clinical nutrition,* 68**,** 591-8.

LE GALL, M., TOBIN, V., STOLARCZYK, E., DALET, V., LETURQUE, A. & BROT-LAROCHE, E. 2007. Sugar sensing by enterocytes combines polarity, membrane bound detectors and sugar metabolism. *J Cell Physiol,* 213**,** 834-43.

LE POUL, E., LOISON, C., STRUYF, S., SPRINGAEL, J. Y., LANNOY, V., DECOBECQ, M. E., BREZILLON, S., DUPRIEZ, V., VASSART, G., VAN DAMME, J., PARMENTIER, M. & DETHEUX, M. 2003. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem,* 278**,** 25481-9.

LE ROUX, C. W., BORG, C. M., MURPHY, K. G., VINCENT, R. P., GHATEI, M. A. & BLOOM, S. R. 2008. Supraphysiological doses of intravenous PYY3-36 cause nausea, but no additional reduction in food intake. *Ann Clin Biochem,* 45**,** 93-5.

LE ROUX, C. W., PATTERSON, M., VINCENT, R. P., HUNT, C., GHATEI, M. A. & BLOOM, S. R. 2005. Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. *J Clin Endocrinol Metab,* 90**,** 1068-71.

LEE, G. H., PROENCA, R., MONTEZ, J. M., CARROLL, K. M., DARVISHZADEH, J. G., LEE, J. I. & FRIEDMAN, J. M. 1996. Abnormal splicing of the leptin receptor in diabetic mice. *Nature,* 379**,** 632-5.

LEIDY, H. J., MATTES, R. D. & CAMPBELL, W. W. 2007. Effects of acute and chronic protein intake on metabolism, appetite, and ghrelin during weight loss. *Obesity (Silver Spring),* 15**,** 1215-25.

LEJEUNE, M. P., WESTERTERP, K. R., ADAM, T. C., LUSCOMBE-MARSH, N. D. & WESTERTERP-PLANTENGA, M. S. 2006. Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety,

and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. *Am J Clin Nutr,* 83**,** 89-94.

LEUNG, P. M. & ROGERS, Q. R. 1971. Importance of prepyriform cortex in food-intake response of rats to amino acids. *Am J Physiol,* 221**,** 929-35.

LEVINE, A. S., KOTZ, C. M. & GOSNELL, B. A. 2003. Sugars: hedonic aspects, neuroregulation, and energy balance. *The American journal of clinical nutrition,* 78**,** 834S-842S.

LEY, R. E., TURNBAUGH, P. J., KLEIN, S. & GORDON, J. I. 2006. Microbial ecology: human gut microbes associated with obesity. *Nature,* 444**,** 1022-3.

LI, J., SONG, J., ZAYTSEVA, Y. Y., LIU, Y., RYCHAHOU, P., JIANG, K., STARR, M. E., KIM, J. T., HARRIS, J. W., YIANNIKOURIS, F. B., KATZ, W. S., NILSSON, P. M., ORHO-MELANDER, M., CHEN, J., ZHU, H., FAHRENHOLZ, T., HIGASHI, R. M., GAO, T., MORRIS, A. J., CASSIS, L. A., FAN, T. W., WEISS, H. L., DOBNER, P. R., MELANDER, O., JIA, J. & EVERS, B. M. 2016. An obligatory role for neurotensin in high-fat-diet-induced obesity. *Nature,* 533**,** 411-5.

LI, X., STASZEWSKI, L., XU, H., DURICK, K., ZOLLER, M. & ADLER, E. 2002. Human receptors for sweet and umami taste. *Proc Natl Acad Sci U S A,* 99**,** 4692-6.

LIEVERSE, R. J., JANSEN, J. B., MASCLEE, A. A., ROVATI, L. C. & LAMERS, C. B. 1994. Effect of a low dose of intraduodenal fat on satiety in humans: studies using the type A cholecystokinin receptor antagonist loxiglumide. *Gut,* 35**,** 501-5.

LIOU, A. P., SEI, Y., ZHAO, X., FENG, J., LU, X., THOMAS, C., PECHHOLD, S., RAYBOULD, H. E. & WANK, S. A. 2011. The extracellular calcium-sensing receptor is required for cholecystokinin secretion in response to L-phenylalanine in acutely isolated intestinal I cells. *Am J Physiol Gastrointest Liver Physiol,* 300**,** G538-46.

LIU, L. & BARAJAS, L. 1993. Nitric oxide synthase immunoreactive neurons in the rat kidney. *Neurosci Lett,* 161**,** 145-8.

LIU, R. H. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr,* 78**,** 517S-520S.

LLUIS, F., FUJIMURA, M., GOMEZ, G., SALVA, J. A., GREELEY, G. H., JR. & THOMPSON, J. C. 1989. [Cellular localization, half-life, and secretion of peptide YY]. *Rev Esp Fisiol,* 45**,** 377-84.

LOBO, V., PATIL, A., PHATAK, A. & CHANDRA, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev,* 4**,** 118-26.

LOMENICK, J. P., MELGUIZO, M. S., MITCHELL, S. L., SUMMAR, M. L. & ANDERSON, J. W. 2009. Effects of meals high in carbohydrate, protein, and fat on ghrelin and peptide YY secretion in prepubertal children. *J Clin Endocrinol Metab,* 94**,** 4463-71.

LOPEZ-LEGARREA, P., FULLER, N. R., ZULET, M. A., MARTINEZ, J. A. & CATERSON, I. D. 2014. The influence of Mediterranean, carbohydrate and high protein diets on gut microbiota composition in the treatment of obesity and associated inflammatory state. *Asia Pac J Clin Nutr,* 23**,** 360-8.

LOPEZ, M., LAGE, R., SAHA, A. K., PEREZ-TILVE, D., VAZQUEZ, M. J., VARELA, L., SANGIAO-ALVARELLOS, S., TOVAR, S., RAGHAY, K., RODRIGUEZ-CUENCA, S., DEOLIVEIRA, R. M., CASTANEDA, T., DATTA, R., DONG, J. Z., CULLER, M., SLEEMAN, M. W., ALVAREZ, C. V., GALLEGO, R., LELLIOTT, C. J., CARLING, D., TSCHOP, M. H., DIEGUEZ, C. & VIDAL-PUIG, A. 2008. Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell Metab,* 7**,** 389-99.

LOVEJOY, J. C., CHAMPAGNE, C. M., SMITH, S. R., DELANY, J. P., BRAY, G. A., LEFEVRE, M., DENKINS, Y. M. & ROOD, J. C. 2001. Relationship of dietary fat and serum cholesterol ester and phospholipid fatty acids to markers of insulin resistance in men and women with a range of glucose tolerance. *Metabolism,* 50**,** 86-92.

LUHOVYY, B. L., AKHAVAN, T. & ANDERSON, G. H. 2007. Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr,* 26**,** 704S-12S.

LUSCOMBE, N. D., CLIFTON, P. M., NOAKES, M., FARNSWORTH, E. & WITTERT, G. 2003. Effect of a high-protein, energy-restricted diet on weight loss and energy expenditure after weight stabilization in hyperinsulinemic subjects. *Int J Obes Relat Metab Disord,* 27**,** 582-90.

LUSCOMBE, N. D., CLIFTON, P. M., NOAKES, M., PARKER, B. & WITTERT, G. 2002. Effects of energy-restricted diets containing increased protein on weight loss, resting energy expenditure, and the thermic effect of feeding in type 2 diabetes. *Diabetes Care,* 25**,** 652-7.

MA, D., TAO, B., WARASHINA, S., KOTANI, S., LU, L., KAPLAMADZHIEV, D. B., MORI, Y., TONCHEV, A. B. & YAMASHIMA, T. 2007. Expression of free fatty acid receptor GPR40 in the central nervous system of adult monkeys. *Neurosci Res,* 58**,** 394-401.

MACE, O. J., AFFLECK, J., PATEL, N. & KELLETT, G. L. 2007. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. *J Physiol,* 582**,** 379-92.

MACE, O. J., LISTER, N., MORGAN, E., SHEPHERD, E., AFFLECK, J., HELLIWELL, P., BRONK, J. R., KELLETT, G. L., MEREDITH, D., BOYD, R., PIERI, M., BAILEY, P. D., PETTCREW, R. & FOLEY, D. 2009. An energy supply network of nutrient absorption coordinated by calcium and T1R taste receptors in rat small intestine. *J Physiol,* 587**,** 195-210.

MACE, O. J., SCHINDLER, M. & PATEL, S. 2012. The regulation of K- and L-cell activity by GLUT2 and the calcium-sensing receptor CasR in rat small intestine. *J Physiol,* 590**,** 2917-36.

MAFFEI, M., HALAAS, J., RAVUSSIN, E., PRATLEY, R. E., LEE, G. H., ZHANG, Y., FEI, H., KIM, S., LALLONE, R., RANGANATHAN, S. & ET AL. 1995. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med,* 1**,** 1155-61.

MAGGARD, M. A., SHUGARMAN, L. R., SUTTORP, M., MAGLIONE, M., SUGERMAN, H. J., LIVINGSTON, E. H., NGUYEN, N. T., LI, Z., MOJICA, W. A., HILTON, L., RHODES, S., MORTON, S. C. & SHEKELLE, P. G. 2005. Meta-analysis: surgical treatment of obesity. *Ann Intern Med,* 142**,** 547-59.

MARGOLSKEE, R. F., DYER, J., KOKRASHVILI, Z., SALMON, K. S., ILEGEMS, E., DALY, K., MAILLET, E. L., NINOMIYA, Y., MOSINGER, B. & SHIRAZI-BEECHEY, S. P. 2007. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. *Proc Natl Acad Sci U S A,* 104**,** 15075-80.

MARKS, J. L., PORTE, D., JR., STAHL, W. L. & BASKIN, D. G. 1990. Localization of insulin receptor mRNA in rat brain by in situ hybridization. *Endocrinology,* 127**,** 3234-6.

MARMONIER, C., CHAPELOT, D. & LOUIS-SYLVESTRE, J. 2000. Effects of macronutrient content and energy density of snacks consumed in a satiety state on the onset of the next meal. *Appetite,* 34**,** 161-8.

MARTY, N., DALLAPORTA, M. & THORENS, B. 2007. Brain glucose sensing, counterregulation, and energy homeostasis. *Physiology (Bethesda),* 22**,** 241-51.

MATARESE, G. 2000. Leptin and the immune system: how nutritional status influences the immune response. *Eur Cytokine Netw,* 11**,** 7-14.

MATSCHINSKY, F. M. 2002. Regulation of pancreatic beta-cell glucokinase: from basics to therapeutics. *Diabetes,* 51 Suppl 3**,** S394-404.

MATZINGER, D., DEGEN, L., DREWE, J., MEULI, J., DUEBENDORFER, R., RUCKSTUHL, N., D'AMATO, M., ROVATI, L. & BEGLINGER, C. 2000. The role of long chain fatty acids in regulating food intake and cholecystokinin release in humans. *Gut,* 46**,** 688-693.

MCGARRY, J. D. 2002. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes,* 51**,** 7-18.

MCGAVIGAN, A. K., O'HARA, H. C., AMIN, A., KINSEY-JONES, J., SPRECKLEY, E., ALAMSHAH, A., AGAHI, A., BANKS, K., FRANCE, R., HYBERG, G., WONG, C., BEWICK, G. A., GARDINER, J. V., LEHMANN, A., MARTIN, N. M., GHATEI, M. A., BLOOM, S. R. & MURPHY, K. G. 2015. L-cysteine suppresses ghrelin and reduces appetite in rodents and humans. *Int J Obes (Lond),* 39**,** 447-55.

MCKNIGHT, J. R., SATTERFIELD, M. C., JOBGEN, W. S., SMITH, S. B., SPENCER, T. E., MEININGER, C. J., MCNEAL, C. J. & WU, G. 2010. Beneficial effects of L-arginine on reducing obesity: potential mechanisms and important implications for human health. *Amino Acids,* 39**,** 349-57.

MEEK, C. L., REIMANN, F., PARK, A. J. & GRIBBLE, F. M. 2015. Can encapsulated glutamine increase GLP-1 secretion, improve glucose tolerance, and reduce meal size in healthy volunteers? A randomised, placebo-controlled, cross-over trial. *Lancet,* 385 Suppl 1**,** S68.

MEERAN, K., O'SHEA, D., EDWARDS, C. M., TURTON, M. D., HEATH, M. M., GUNN, I., ABUSNANA, S., ROSSI, M., SMALL, C. J., GOLDSTONE, A. P., TAYLOR, G. M., SUNTER, D., STEERE, J., CHOI, S. J., GHATEI, M. A. & BLOOM, S. R. 1999. Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. *Endocrinology,* 140**,** 244-50.

MELLINKOFF, S. M., FRANKLAND, M., BOYLE, D. & GREIPEL, M. 1956. Relationship between serum amino acid concentration and fluctuations in appetite. *J Appl Physiol,* 8**,** 535-8.

MELNICK, I. V., PRICE, C. J. & COLMERS, W. F. 2011. Glucosensing in parvocellular neurons of the rat hypothalamic paraventricular nucleus. *Eur J Neurosci,* 34**,** 272-82.

MENTLEIN, R., DAHMS, P., GRANDT, D. & KRUGER, R. 1993. Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. *Regul Pept,* 49**,** 133-44.

MEYER, J. H. & KELLY, G. A. 1976. Canine pancreatic responses to intestinally perfused proteins and protein digests. *Am J Physiol,* 231**,** 682-91.

MIKKELSEN, P. B., TOUBRO, S. & ASTRUP, A. 2000. Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. *Am J Clin Nutr,* 72**,** 1135-41.

MINOKOSHI, Y., ALQUIER, T., FURUKAWA, N., KIM, Y. B., LEE, A., XUE, B., MU, J., FOUFELLE, F., FERRE, P., BIRNBAUM, M. J., STUCK, B. J. & KAHN, B. B. 2004. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature,* 428**,** 569-74.

MITHIEUX, G., MISERY, P., MAGNAN, C., PILLOT, B., GAUTIER-STEIN, A., BERNARD, C., RAJAS, F. & ZITOUN, C. 2005. Portal sensing of intestinal gluconeogenesis is a mechanistic link in the diminution of food intake induced by diet protein. *Cell Metab,* 2**,** 321-9.

MITRAKOU, A., RYAN, C., VENEMAN, T., MOKAN, M., JENSSEN, T., KISS, I., DURRANT, J., CRYER, P. & GERICH, J. 1991. Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol,* 260**,** E67-74.

MIYAUCHI, S., HIRASAWA, A., IGA, T., LIU, N., ITSUBO, C., SADAKANE, K., HARA, T. & TSUJIMOTO, G. 2009. Distribution and regulation of protein expression of the free fatty acid receptor GPR120. *Naunyn Schmiedebergs Arch Pharmacol,* 379**,** 427-34.

MORAN, T. H., BALDESSARINI, A. R., SALORIO, C. F., LOWERY, T. & SCHWARTZ, G. J. 1997. Vagal afferent and efferent contributions to the inhibition of food intake by cholecystokinin. *Am J Physiol,* 272**,** R1245-51.

MORAN, T. H. & KINZIG, K. P. 2004. Gastrointestinal satiety signals II. Cholecystokinin. *Am J Physiol Gastrointest Liver Physiol,* 286**,** G183-8.

MORAN, T. H., NORGREN, R., CROSBY, R. J. & MCHUGH, P. R. 1990. Central and peripheral vagal transport of cholecystokinin binding sites occurs in afferent fibers. *Brain Res,* 526**,** 95-102.

MORENS, C., GAUDICHON, C., FROMENTIN, G., MARSSET-BAGLIERI, A., BENSAID, A., LARUE-ACHAGIOTIS, C., LUENGO, C. & TOME, D. 2001. Daily delivery of dietary nitrogen to the periphery is stable in rats adapted to increased protein intake. *Am J Physiol Endocrinol Metab,* 281**,** E826-36.

MORENS, C., GAUDICHON, C., METGES, C. C., FROMENTIN, G., BAGLIERI, A., EVEN, P. C., HUNEAU, J. F. & TOME, D. 2000. A high-protein meal exceeds anabolic and catabolic capacities in rats adapted to a normal protein diet. *J Nutr,* 130**,** 2312-21.

MORGAN, K., OBICI, S. & ROSSETTI, L. 2004. Hypothalamic responses to long-chain fatty acids are nutritionally regulated. *J Biol Chem,* 279**,** 31139-48.

MORRISON, D. J. & PRESTON, T. 2016. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes,* 7**,** 189-200.

MOUNTJOY, P. D., BAILEY, S. J. & RUTTER, G. A. 2007. Inhibition by glucose or leptin of hypothalamic neurons expressing neuropeptide Y requires changes in AMP-activated protein kinase activity. *Diabetologia,* 50**,** 168-77.

MUROYA, S., YADA, T., SHIODA, S. & TAKIGAWA, M. 1999. Glucose-sensitive neurons in the rat arcuate nucleus contain neuropeptide Y. *Neurosci Lett,* 264**,** 113-6.

MURPHY, K. G. & BLOOM, S. R. 2006. Gut hormones and the regulation of energy homeostasis. *Nature,* 444**,** 854-9.

MURRAY, C. D., LE ROUX, C. W., GOUVEIA, C., BASSETT, P., GHATEI, M. A., BLOOM, S. R., EMMANUEL, A. V. & GABE, S. M. 2006. The effect of different macronutrient infusions on appetite, ghrelin and peptide YY in parenterally fed patients. *Clin Nutr,* 25**,** 626-33.

MUURAHAINEN, N., KISSILEFF, H. R., DEROGATIS, A. J. & PI-SUNYER, F. X. 1988. Effects of cholecystokinin-octapeptide (CCK-8) on food intake and gastric emptying in man. *Physiol Behav,* 44**,** 645-9.

NAIR, K. S., SCHWARTZ, R. G. & WELLE, S. 1992. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am J Physiol,* 263**,** E928-34.

NAKAMURA, K., SAKURAGI, N., TAKAKUWA, A. & AYABE, T. 2016. Paneth cell alpha-defensins and enteric microbiota in health and disease. *Biosci Microbiota Food Health,* 35**,** 57-67.

NCD-RISC 2016. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet,* 387**,** 1377-1396.

NELSON, G., CHANDRASHEKAR, J., HOON, M. A., FENG, L., ZHAO, G., RYBA, N. J. & ZUKER, C. S. 2002. An amino-acid taste receptor. *Nature,* 416**,** 199-202.

NELSON, G., HOON, M. A., CHANDRASHEKAR, J., ZHANG, Y., RYBA, N. J. & ZUKER, C. S. 2001. Mammalian sweet taste receptors. *Cell,* 106**,** 381-90.

NEMOZ-GAILLARD, E., BERNARD, C., ABELLO, J., CORDIER-BUSSAT, M., CHAYVIALLE, J. A. & CUBER, J. C. 1998. Regulation of cholecystokinin secretion by peptones and peptidomimetic antibiotics in STC-1 cells. *Endocrinology,* 139**,** 932-8.

NIIJIMA, A. 2000. Reflex effects of oral, gastrointestinal and hepatoportal glutamate sensors on vagal nerve activity. *J Nutr,* 130**,** 971S-3S.

NILSSON, M., STENBERG, M., FRID, A. H., HOLST, J. J. & BJORCK, I. M. 2004. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins. *Am J Clin Nutr,* 80**,** 1246-53.

NILSSON, N. E., KOTARSKY, K., OWMAN, C. & OLDE, B. 2003. Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. *Biochem Biophys Res Commun,* 303**,** 1047-52.

NISHI, T., HARA, H., HIRA, T. & TOMITA, F. 2001. Dietary protein peptic hydrolysates stimulate cholecystokinin release via direct sensing by rat intestinal mucosal cells. *Exp Biol Med (Maywood),* 226**,** 1031-6.

OBICI, S., FENG, Z., MORGAN, K., STEIN, D., KARKANIAS, G. & ROSSETTI, L. 2002. Central administration of oleic acid inhibits glucose production and food intake. *Diabetes,* 51**,** 271-5.

OOMURA, Y. & YOSHIMATSU, H. 1984. Neural network of glucose monitoring system. *J Auton Nerv Syst,* 10**,** 359-72.

ORSKOV, C., RABENHOJ, L., WETTERGREN, A., KOFOD, H. & HOLST, J. J. 1994. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes,* 43**,** 535-9.

ORTOLANI, D., GARCIA, M. C., MELO-THOMAS, L. & SPADARI-BRATFISCH, R. C. 2014. Stress-induced endocrine response and anxiety: the effects of comfort food in rats. *Stress,* 17**,** 211-8.

OYA, M., KITAGUCHI, T., PAIS, R., REIMANN, F., GRIBBLE, F. & TSUBOI, T. 2013. The G protein-coupled receptor family C group 6 subtype A (GPRC6A) receptor is involved in amino acid-induced glucagon-like peptide-1 secretion from GLUTag cells. *J Biol Chem,* 288**,** 4513-21.

OYA, M., SUZUKI, H., WATANABE, Y., SATO, M. & TSUBOI, T. 2011. Amino acid taste receptor regulates insulin secretion in pancreatic beta-cell line MIN6 cells. *Genes Cells,* 16**,** 608-16.

PALMER, J. P., WALTER, R. M. & ENSINCK, J. W. 1975. Arginine-stimulated acute phase of insulin and glucagon secretion. I. in normal man. *Diabetes,* 24**,** 735-40.

PALMER, R. M., ASHTON, D. S. & MONCADA, S. 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature,* 333**,** 664-6.

PANKSEPP, J. & ROSSI, J., 3RD 1981. D-glucose infusions into the basal ventromedial hypothalamus and feeding. *Behav Brain Res,* 3**,** 381-92.

PARKINSON, J. R., CHAUDHRI, O. B., KUO, Y. T., FIELD, B. C., HERLIHY, A. H., DHILLO, W. S., GHATEI, M. A., BLOOM, S. R. & BELL, J. D. 2009. Differential patterns of neuronal activation in the brainstem and hypothalamus following peripheral injection of GLP-1, oxyntomodulin and lithium chloride in mice detected by manganese-enhanced magnetic resonance imaging (MEMRI). *Neuroimage,* 44**,** 1022-31.

PELCHAT, M. L., JOHNSON, A., CHAN, R., VALDEZ, J. & RAGLAND, J. D. 2004. Images of desire: food-craving activation during fMRI. *NeuroImage,* 23**,** 1486-93.

PELLEYMOUNTER, M. A., CULLEN, M. J., BAKER, M. B., HECHT, R., WINTERS, D., BOONE, T. & COLLINS, F. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science,* 269**,** 540-3.

PENHOAT, A., MUTEL, E., AMIGO-CORREIG, M., PILLOT, B., STEFANUTTI, A., RAJAS, F. & MITHIEUX, G. 2011. Protein-induced satiety is abolished in the absence of intestinal gluconeogenesis. *Physiol Behav,* 105**,** 89-93.

PERBONI, S. & INUI, A. 2010. Appetite and gastrointestinal motility: Role of ghrelin-family peptides. *Clinical Nutrition,* 29**,** 227-234.

PERSEGHIN, G., REGALIA, E., BATTEZZATI, A., VERGANI, S., PULVIRENTI, A., TERRUZZI, I., BARATTI, D., BOZZETTI, F., MAZZAFERRO, V. & LUZI, L. 1997. Regulation of glucose homeostasis in humans with denervated livers. *J Clin Invest,* 100**,** 931-41.

PEUHKURI, K., SIHVOLA, N. & KORPELA, R. 2011. Dietary proteins and food-related reward signals. *Food & nutrition research,* 55.

PFANNKUCHE, H. & GABEL, G. 2009. Glucose, epithelium, and enteric nervous system: dialogue in the dark. *J Anim Physiol Anim Nutr (Berl),* 93**,** 277-86.

PI-SUNYER, X., ASTRUP, A., FUJIOKA, K., GREENWAY, F., HALPERN, A., KREMPF, M., LAU, D. C., LE ROUX, C. W., VIOLANTE ORTIZ, R., JENSEN, C. B. & WILDING, J. P. 2015. A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. *N Engl J Med,* 373**,** 11-22.

PI-SUNYER, X., KISSILEFF, H. R., THORNTON, J. & SMITH, G. P. 1982. C-terminal octapeptide of cholecystokinin decreases food intake in obese men. *Physiol Behav,* 29**,** 627-30.

PI, M., CHEN, L., HUANG, M. Z., ZHU, W., RINGHOFER, B., LUO, J., CHRISTENSON, L., LI, B., ZHANG, J., JACKSON, P. D., FABER, P., BRUNDEN, K. R., HARRINGTON, J. J. & QUARLES, L. D. 2008. GPRC6A null mice exhibit osteopenia, feminization and metabolic syndrome. *PLoS One,* 3**,** e3858.

PI, M., FABER, P., EKEMA, G., JACKSON, P. D., TING, A., WANG, N., FONTILLA-POOLE, M., MAYS, R. W., BRUNDEN, K. R., HARRINGTON, J. J. & QUARLES, L. D. 2005. Identification of a novel extracellular cation-sensing G-protein-coupled receptor. *J Biol Chem,* 280**,** 40201-9.

PI, M., WU, Y., LENCHIK, N. I., GERLING, I. & QUARLES, L. D. 2012. GPRC6A mediates the effects of L-arginine on insulin secretion in mouse pancreatic islets. *Endocrinology,* 153**,** 4608-15.

POCAI, A., CARRINGTON, P. E., ADAMS, J. R., WRIGHT, M., EIERMANN, G., ZHU, L., DU, X., PETROV, A., LASSMAN, M. E., JIANG, G., LIU, F., MILLER, C., TOTA, L. M., ZHOU, G., ZHANG, X., SOUNTIS, M. M., SANTOPRETE, A., CAPITO, E., CHICCHI, G. G., THORNBERRY, N., BIANCHI, E., PESSI, A., MARSH, D. J. & SINHAROY, R. 2009. Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. *Diabetes,* 58**,** 2258-66.

POCAI, A., LAM, T. K., OBICI, S., GUTIERREZ-JUAREZ, R., MUSE, E. D., ARDUINI, A. & ROSSETTI, L. 2006. Restoration of hypothalamic lipid sensing normalizes energy and glucose homeostasis in overfed rats. *J Clin Invest,* 116**,** 1081-91.

POCAI, A., OBICI, S., SCHWARTZ, G. J. & ROSSETTI, L. 2005. A brain-liver circuit regulates glucose homeostasis. *Cell Metab,* 1**,** 53-61.

POLAK, J. M., SULLIVAN, S. N., BLOOM, S. R., BUCHAN, A. M., FACER, P., BROWN, M. R. & PEARSE, A. G. 1977. Specific localisation of neurotensin to the N cell in human intestine by radioimmunoassay and immunocytochemistry. *Nature,* 270**,** 183-4.

POPPITT, S. D., MCCORMACK, D. & BUFFENSTEIN, R. 1998. Short-term effects of macronutrient preloads on appetite and energy intake in lean women. *Physiol Behav,* 64**,** 279-85.

PORRINI, M., CROVETTI, R., TESTOLIN, G. & SILVA, S. 1995. Evaluation of satiety sensations and food intake after different preloads. *Appetite,* 25**,** 17-30.

PORRINI, M., SANTANGELO, A., CROVETTI, R., RISO, P., TESTOLIN, G. & BLUNDELL, J. E. 1997. Weight, protein, fat, and timing of preloads affect food intake. *Physiol Behav,* 62**,** 563-70.

POSMA, J. M., GARCIA-PEREZ, I., HEATON, J. C., BURDISSO, P., MATHERS, J. C., DRAPER, J., LEWIS, M., LINDON, J. C., FROST, G., HOLMES, E. & NICHOLSON, J. K. 2017. Integrated Analytical and Statistical Two-Dimensional Spectroscopy Strategy for Metabolite Identification: Application to Dietary Biomarkers. *Anal Chem,* 89**,** 3300-3309.

POTIER, M., DARCEL, N. & TOME, D. 2009. Protein, amino acids and the control of food intake. *Curr Opin Clin Nutr Metab Care,* 12**,** 54-8.

PROUD, C. G. 2004. mTOR-mediated regulation of translation factors by amino acids. *Biochem Biophys Res Commun,* 313**,** 429-36.

PRYDE, S. E., DUNCAN, S. H., HOLD, G. L., STEWART, C. S. & FLINT, H. J. 2002. The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett,* 217**,** 133-9.

PSICHAS, A., SLEETH, M. L., MURPHY, K. G., BROOKS, L., BEWICK, G. A., HANYALOGLU, A. C., GHATEI, M. A., BLOOM, S. R. & FROST, G. 2015. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int J Obes (Lond),* 39**,** 424-9.

RABIER, D. & KAMOUN, P. 1995. Metabolism of citrulline in man. *Amino Acids,* 9**,** 299-316.

RAIJMAN, L. 1974. Citrulline synthesis in rat tissues and liver content of carbamoyl phosphate and ornithine. *Biochem J,* 138**,** 225-32.

RAJ, T., DILEEP, U., VAZ, M., FULLER, M. F. & KURPAD, A. V. 2008. Intestinal microbial contribution to metabolic leucine input in adult men. *J Nutr,* 138**,** 2217-21.

RASSCHAERT, J. & MALAISSE, W. J. 1999. Expression of the calcium-sensing receptor in pancreatic islet B-cells. *Biochem Biophys Res Commun,* 264**,** 615-8.

RAY, J. M., SQUIRES, P. E., CURTIS, S. B., MELOCHE, M. R. & BUCHAN, A. M. 1997. Expression of the calcium-sensing receptor on human antral gastrin cells in culture. *J Clin Invest,* 99**,** 2328-33.

RAYBOULD, H. E. 1998. Does Your Gut Taste? Sensory Transduction in the Gastrointestinal Tract. *News Physiol Sci,* 13**,** 275-280.

RAYBOULD, H. E. 2007. Sensing of glucose in the gastrointestinal tract. *Autonomic neuroscience : basic & clinical,* 133**,** 86-90.

RAYBOULD, H. E. & HOLZER, H. 1992. Dual capsaicin-sensitive afferent pathways mediate inhibition of gastric emptying in rat induced by intestinal carbohydrate. *Neurosci Lett,* 141**,** 236-8.

RECABARREN, S. E., JOFRE, A., LOBOS, A., ORELLANA, P. & PARILO, J. 1996. Effect of arginine and ornithine infusions on luteinizing hormone secretion in prepubertal ewes. *J Anim Sci,* 74**,** 162-6.

REEVE, J. R., JR., GREEN, G. M., CHEW, P., EYSSELEIN, V. E. & KEIRE, D. A. 2003. CCK-58 is the only detectable endocrine form of cholecystokinin in rat. *Am J Physiol Gastrointest Liver Physiol,* 285**,** G255-65.

REID, M. & HETHERINGTON, M. 1997. Relative effects of carbohydrates and protein on satiety -- a review of methodology. *Neurosci Biobehav Rev,* 21**,** 295-308.

REIMANN, F., WILLIAMS, L., DA SILVA XAVIER, G., RUTTER, G. A. & GRIBBLE, F. M. 2004. Glutamine potently stimulates glucagon-like peptide-1 secretion from GLUTag cells. *Diabetologia,* 47**,** 1592-601.

REIMER, R. A. 2006. Meat hydrolysate and essential amino acid-induced glucagon-like peptide-1 secretion, in the human NCI-H716 enteroendocrine cell line, is regulated by extracellular signal-regulated kinase1/2 and p38 mitogen-activated protein kinases. *J Endocrinol,* 191**,** 159-70.

REN, X., ZHOU, L., TERWILLIGER, R., NEWTON, S. S. & DE ARAUJO, I. E. 2009. Sweet taste signaling functions as a hypothalamic glucose sensor. *Front Integr Neurosci,* 3**,** 12.

REYES, A. A., KARL, I. E. & KLAHR, S. 1994. Role of arginine in health and in renal disease. *Am J Physiol,* 267**,** F331-46.

ROBERGE, J. N. & BRUBAKER, P. L. 1993. Regulation of intestinal proglucagon-derived peptide secretion by glucose-dependent insulinotropic peptide in a novel enteroendocrine loop. *Endocrinology,* 133**,** 233-40.

ROSS, R., WANG, P. Y., CHARI, M., LAM, C. K., CASPI, L., ONO, H., MUSE, E. D., LI, X., GUTIERREZ-JUAREZ, R., LIGHT, P. E., SCHWARTZ, G. J., ROSSETTI, L. & LAM, T. K. 2008. Hypothalamic protein kinase C regulates glucose production. *Diabetes,* 57**,** 2061-5.

ROZE, C., COUTURIER, D., CHARIOT, J. & DEBRAY, C. 1977. Inhibition of gastric electrical and mechanical activity by intraduodenal agents in pigs and the effects of vagotomy. *Digestion,* 15**,** 526-39.

RUIZ, C. J., WRAY, K., DELAY, E., MARGOLSKEE, R. F. & KINNAMON, S. C. 2003. Behavioral evidence for a role of alpha-gustducin in glutamate taste. *Chem Senses,* 28**,** 573-9.

SALEM, V., IZZI-ENGBEAYA, C., COELLO, C., THOMAS, D. B., CHAMBERS, E. S., COMNINOS, A. N., BUCKLEY, A., WIN, Z., AL-NAHHAS, A., RABINER, E. A., GUNN, R. N., BUDGE, H., SYMONDS, M. E., BLOOM, S. R., TAN, T. M. & DHILLO, W. S. 2016. Glucagon increases energy expenditure independently of brown adipose tissue activation in humans. *Diabetes Obes Metab,* 18**,** 72-81.

SAN GABRIEL, A., UNEYAMA, H., MAEKAWA, T. & TORII, K. 2009. The calcium-sensing receptor in taste tissue. *Biochem Biophys Res Commun,* 378**,** 414-8.

SARGRAD, K. R., HOMKO, C., MOZZOLI, M. & BODEN, G. 2005. Effect of high protein vs high carbohydrate intake on insulin sensitivity, body weight, hemoglobin A1c, and blood pressure

in patients with type 2 diabetes mellitus. *Journal of the American Dietetic Association,* 105**,** 573-80.

SAVAGE, A. P., ADRIAN, T. E., CAROLAN, G., CHATTERJEE, V. K. & BLOOM, S. R. 1987. Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. *Gut,* 28**,** 166-70.

SCARPIGNATO, C., VARGA, G. & CORRADI, C. 1993. Effect of CCK and its antagonists on gastric emptying. *Journal of Physiology-Paris,* 87**,** 291-300.

SCHIRRA, J., KATSCHINSKI, M., WEIDMANN, C., SCHAFER, T., WANK, U., ARNOLD, R. & GOKE, B. 1996. Gastric emptying and release of incretin hormones after glucose ingestion in humans. *J Clin Invest,* 97**,** 92-103.

SCHWARTZ, G. J. 2000. The role of gastrointestinal vagal afferents in the control of food intake: current prospects. *Nutrition,* 16**,** 866-73.

SCHWARTZ, G. J. 2006. Integrative capacity of the caudal brainstem in the control of food intake. *Philos Trans R Soc Lond B Biol Sci,* 361**,** 1275-80.

SCHWARTZ, G. J., BERKOW, G., MCHUGH, P. R. & MORAN, T. H. 1993. Gastric branch vagotomy blocks nutrient and cholecystokinin-induced suppression of gastric emptying. *Am J Physiol,* 264**,** R630-7.

SCHWARTZ, M. W., WOODS, S. C., PORTE, D., JR., SEELEY, R. J. & BASKIN, D. G. 2000. Central nervous system control of food intake. *Nature,* 404**,** 661-71.

SCOTT, M. M., PERELLO, M., CHUANG, J. C., SAKATA, I., GAUTRON, L., LEE, C. E., LAUZON, D., ELMQUIST, J. K. & ZIGMAN, J. M. 2012. Hindbrain ghrelin receptor signaling is sufficient to maintain fasting glucose. *PLoS One,* 7**,** e44089.

SCROCCHI, L. A., HILL, M. E., SALEH, J., PERKINS, B. & DRUCKER, D. J. 2000. Elimination of glucagon-like peptide 1R signaling does not modify weight gain and islet adaptation in mice with combined disruption of leptin and GLP-1 action. *Diabetes,* 49**,** 1552-60.

SHAH, M., JAFFERY, M., ADAMS-HUET, B., FRANKLIN, B., OLIVER, J. & MITCHELL, J. 2017. Effect of meal composition on postprandial lipid concentrations and lipoprotein particle numbers: A randomized cross-over study. *PLoS One,* 12**,** e0172732.

SHARP, J. W., ROSS, C. M., KOEHNLE, T. J. & GIETZEN, D. W. 2004. Phosphorylation of Ca2+/calmodulin-dependent protein kinase type ii and the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (ampa) receptor in response to a threonine-devoid diet. *Neuroscience,* 126**,** 1053-62.

SHEARMAN, L. P., WANG, S. P., HELMLING, S., STRIBLING, D. S., MAZUR, P., GE, L., WANG, L., KLUSSMANN, S., MACINTYRE, D. E., HOWARD, A. D. & STRACK, A. M. 2006. Ghrelin

neutralization by a ribonucleic acid-SPM ameliorates obesity in diet-induced obese mice. *Endocrinology,* 147**,** 1517-26.

SHEININ, Y., KALLAY, E., WRBA, F., KRIWANEK, S., PETERLIK, M. & CROSS, H. S. 2000. Immunocytochemical localization of the extracellular calcium-sensing receptor in normal and malignant human large intestinal mucosa. *J Histochem Cytochem,* 48**,** 595-602.

SHOAIE, S., KARLSSON, F., MARDINOGLU, A., NOOKAEW, I., BORDEL, S. & NIELSEN, J. 2013. Understanding the interactions between bacteria in the human gut through metabolic modeling. *Sci Rep,* 3**,** 2532.

SHUGHRUE, P. J., LANE, M. V. & MERCHENTHALER, I. 1996. Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. *Endocrinology,* 137**,** 5159-62.

SIDHU, S. S., THOMPSON, D. G., WARHURST, G., CASE, R. M. & BENSON, R. S. 2000. Fatty acid-induced cholecystokinin secretion and changes in intracellular Ca2+ in two enteroendocrine cell lines, STC-1 and GLUTag. *J Physiol,* 528 Pt 1**,** 165-76.

SIEGEL, E. G., SCHULZE, A., SCHMIDT, W. E. & CREUTZFELDT, W. 1992. Comparison of the effect of GIP and GLP-1 (7-36amide) on insulin release from rat pancreatic islets. *Eur J Clin Invest,* 22**,** 154-7.

SILVER, I. A. & ERECINSKA, M. 1998. Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons. *J Neurophysiol,* 79**,** 1733-45.

SJOSTROM, L., NARBRO, K., SJOSTROM, C. D., KARASON, K., LARSSON, B., WEDEL, H., LYSTIG, T., SULLIVAN, M., BOUCHARD, C., CARLSSON, B., BENGTSSON, C., DAHLGREN, S., GUMMESSON, A., JACOBSON, P., KARLSSON, J., LINDROOS, A. K., LONROTH, H., NASLUND, I., OLBERS, T., STENLOF, K., TORGERSON, J., AGREN, G. & CARLSSON, L. M. 2007. Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med,* 357**,** 741-52.

SLOTH, B., DAVIDSEN, L., HOLST, J. J., FLINT, A. & ASTRUP, A. 2007. Effect of subcutaneous injections of PYY1-36 and PYY3-36 on appetite, ad libitum energy intake, and plasma free fatty acid concentration in obese males. *Am J Physiol Endocrinol Metab,* 293**,** E604-9.

SMAJILOVIC, S., CLEMMENSEN, C., JOHANSEN, L. D., WELLENDORPH, P., HOLST, J. J., THAMS, P. G., OGO, E. & BRAUNER-OSBORNE, H. 2013. The L-alpha-amino acid receptor GPRC6A is expressed in the islets of Langerhans but is not involved in L-arginine-induced insulin release. *Amino Acids,* 44**,** 383-90.

SMAJILOVIC, S., WELLENDORPH, P. & BRAUNER-OSBORNE, H. 2014. Promiscuous seven transmembrane receptors sensing L-alpha-amino acids. *Curr Pharm Des,* 20**,** 2693-702.

SMEETS, A. J., SOENEN, S., LUSCOMBE-MARSH, N. D., UELAND, O. & WESTERTERP-PLANTENGA, M. S. 2008. Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch. *J Nutr,* 138**,** 698-702.

SMITH, E. A. & MACFARLANE, G. T. 1997. Dissimilatory amino Acid metabolism in human colonic bacteria. *Anaerobe,* 3**,** 327-37.

SMITH, G. P., JEROME, C., CUSHIN, B. J., ETERNO, R. & SIMANSKY, K. J. 1981. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science,* 213**,** 1036-7.

SMITH, P. A., SAKURA, H., COLES, B., GUMMERSON, N., PROKS, P. & ASHCROFT, F. M. 1997. Electrogenic arginine transport mediates stimulus-secretion coupling in mouse pancreatic beta-cells. *J Physiol,* 499 (Pt 3)**,** 625-35.

SPRECKLEY, E. & MURPHY, K. G. 2015. The L-Cell in Nutritional Sensing and the Regulation of Appetite. *Front Nutr,* 2**,** 23.

SQUIRES, P. E., HARRIS, T. E., PERSAUD, S. J., CURTIS, S. B., BUCHAN, A. M. & JONES, P. M. 2000. The extracellular calcium-sensing receptor on human beta-cells negatively modulates insulin secretion. *Diabetes,* 49**,** 409-17.

STANLEY, S., WYNNE, K., MCGOWAN, B. & BLOOM, S. 2005. Hormonal regulation of food intake. *Physiol Rev,* 85**,** 1131-58.

STEINERT, R. E. & BEGLINGER, C. 2011. Nutrient sensing in the gut: interactions between chemosensory cells, visceral afferents and the secretion of satiation peptides. *Physiology & Behavior,* In Press, Corrected Proof.

STOCKER, H., RADIMERSKI, T., SCHINDELHOLZ, B., WITTWER, F., BELAWAT, P., DARAM, P., BREUER, S., THOMAS, G. & HAFEN, E. 2003. Rheb is an essential regulator of S6K in controlling cell growth in Drosophila. *Nat Cell Biol,* 5**,** 559-65.

STOECKEL, L. E., WELLER, R. E., GIDDINGS, M. & COX, J. E. 2008. Peptide YY levels are associated with appetite suppression in response to long-chain fatty acids. *Physiol Behav,* 93**,** 289-95.

STOLL, B., HENRY, J., REEDS, P. J., YU, H., JAHOOR, F. & BURRIN, D. G. 1998. Catabolism Dominates the First-Pass Intestinal Metabolism of Dietary Essential Amino Acids in Milk Protein-Fed Piglets. *The Journal of Nutrition,* 128**,** 606-614.

STUBBS, R. J., O'REILLY, L. M., JOHNSTONE, A. M., HARRISON, C. L., CLARK, H., FRANKLIN, M. F., REID, C. A. & MAZLAN, N. 1999. Description and evaluation of an experimental model to examine changes in selection between high-protein, high-carbohydrate and high-fat foods in humans. *Eur J Clin Nutr,* 53**,** 13-21.

SUN, Y., BUTTE, N. F., GARCIA, J. M. & SMITH, R. G. 2008. Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. *Endocrinology,* 149**,** 843-50.

SYMONDS, E. L., PEIRIS, M., PAGE, A. J., CHIA, B., DOGRA, H., MASDING, A., GALANAKIS, V., ATIBA, M., BULMER, D., YOUNG, R. L. & BLACKSHAW, L. A. 2015. Mechanisms of activation of mouse and human enteroendocrine cells by nutrients. *Gut,* 64**,** 618-26.

TABORSKY, G. J., JR., AHREN, B. & HAVEL, P. J. 1998. Autonomic mediation of glucagon secretion during hypoglycemia: implications for impaired alpha-cell responses in type 1 diabetes. *Diabetes,* 47**,** 995-1005.

TANG, C., BIEMOND, I. & LAMERS, C. B. 1996. Cholecystokinin receptors in human pancreas and gallbladder muscle: a comparative study. *Gastroenterology,* 111**,** 1621-6.

TANGPHAO, O., CHALON, S., COULSTON, A. M., MORENO, H., JR., CHAN, J. R., COOKE, J. P., HOFFMAN, B. B. & BLASCHKE, T. F. 1999. L-arginine and nitric oxide-related compounds in plasma: comparison of normal and arginine-free diets in a 24-h crossover study. *Vasc Med,* 4**,** 27-32.

TANNOUS DIT EL KHOURY, D., OBEID, O., AZAR, S. T. & HWALLA, N. 2006. Variations in postprandial ghrelin status following ingestion of high-carbohydrate, high-fat, and high-protein meals in males. *Ann Nutr Metab,* 50**,** 260-9.

TAPPY, L. 1996. Thermic effect of food and sympathetic nervous system activity in humans. *Reprod Nutr Dev,* 36**,** 391-7.

TARTAGLIA, L. A., DEMBSKI, M., WENG, X., DENG, N., CULPEPPER, J., DEVOS, R., RICHARDS, G. J., CAMPFIELD, L. A., CLARK, F. T., DEEDS, J., MUIR, C., SANKER, S., MORIARTY, A., MOORE, K. J., SMUTKO, J. S., MAYS, G. G., WOOL, E. A., MONROE, C. A. & TEPPER, R. I. 1995. Identification and expression cloning of a leptin receptor, OB-R. *Cell,* 83**,** 1263-71.

TEUFFEL, P., WANG, L., PRINZ, P., GOEBEL-STENGEL, M., SCHARNER, S., KOBELT, P., HOFMANN, T., ROSE, M., KLAPP, B. F., REEVE, J. R., JR. & STENGEL, A. 2015. Treatment with the ghrelin-O-acyltransferase (GOAT) inhibitor GO-CoA-Tat reduces food intake by reducing meal frequency in rats. *J Physiol Pharmacol,* 66**,** 493-503.

THAMS, P. & CAPITO, K. 1999. L-arginine stimulation of glucose-induced insulin secretion through membrane depolarization and independent of nitric oxide. *Eur J Endocrinol,* 140**,** 87-93.

THOMPSON, N. M., GILL, D. A., DAVIES, R., LOVERIDGE, N., HOUSTON, P. A., ROBINSON, I. C. & WELLS, T. 2004. Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. *Endocrinology,* 145**,** 234-42.

TOI, K., BYNUM, E., NORRIS, E. & ITANO, H. A. 1967. Studies on the chemical modification of arginine. I. The reaction of 1,2-cyclohexanedione with arginine and arginyl residues of proteins. *J Biol Chem,* 242**,** 1036-43.

TOLHURST, G., HEFFRON, H., LAM, Y. S., PARKER, H. E., HABIB, A. M., DIAKOGIANNAKI, E., CAMERON, J., GROSSE, J., REIMANN, F. & GRIBBLE, F. M. 2012. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes,* 61**,** 364-71.

TOLHURST, G., ZHENG, Y., PARKER, H. E., HABIB, A. M., REIMANN, F. & GRIBBLE, F. M. 2011. Glutamine triggers and potentiates glucagon-like peptide-1 secretion by raising cytosolic Ca2+ and cAMP. *Endocrinology,* 152**,** 405-13.

TOME, D., SCHWARZ, J., DARCEL, N. & FROMENTIN, G. 2009. Protein, amino acids, vagus nerve signaling, and the brain. *Am J Clin Nutr,* 90**,** 838S-843S.

TORDOFF, M. G., ALARCON, L. K., VALMEKI, S. & JIANG, P. 2012. T1R3: a human calcium taste receptor. *Sci Rep,* 2**,** 496.

TORGERSON, J. S., HAUPTMAN, J., BOLDRIN, M. N. & SJOSTROM, L. 2004. XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. *Diabetes Care,* 27**,** 155-61.

TORRALLARDONA, D., HARRIS, C. I. & FULLER, M. F. 2003. Pigs' gastrointestinal microflora provide them with essential amino acids. *J Nutr,* 133**,** 1127-31.

TRIGAZIS, L., ORTTMANN, A. & ANDERSON, G. H. 1997. Effect of a cholecystokinin-A receptor blocker on protein-induced food intake suppression in rats. *Am J Physiol,* 272**,** R1826-33.

TRUONG, B. G., MAGRUM, L. J. & GIETZEN, D. W. 2002. GABA(A) and GABA(B) receptors in the anterior piriform cortex modulate feeding in rats. *Brain Res,* 924**,** 1-9.

TSCHOP, M., CASTANEDA, T. R., JOOST, H. G., THONE-REINEKE, C., ORTMANN, S., KLAUS, S., HAGAN, M. M., CHANDLER, P. C., OSWALD, K. D., BENOIT, S. C., SEELEY, R. J., KINZIG, K. P., MORAN, T. H., BECK-SICKINGER, A. G., KOGLIN, N., RODGERS, R. J., BLUNDELL, J. E., ISHII, Y., BEATTIE, A. H., HOLCH, P., ALLISON, D. B., RAUN, K., MADSEN, K., WULFF, B. S., STIDSEN, C. E., BIRRINGER, M., KREUZER, O. J., SCHINDLER, M., ARNDT, K., RUDOLF, K., MARK, M., DENG, X. Y., WHITCOMB, D. C., HALEM, H., TAYLOR, J., DONG, J., DATTA, R., CULLER, M., CRANEY, S., FLORA, D., SMILEY, D. & HEIMAN, M. L. 2004. Physiology: does gut hormone PYY3-36 decrease food intake in rodents? *Nature,* 430**,** 1 p following 165; discussion 2 p following 165.

TSCHOP, M., SMILEY, D. L. & HEIMAN, M. L. 2000. Ghrelin induces adiposity in rodents. *Nature,* 407**,** 908-13.

TSCHOP, M., WEYER, C., TATARANNI, P. A., DEVANARAYAN, V., RAVUSSIN, E. & HEIMAN, M. L. 2001. Circulating ghrelin levels are decreased in human obesity. *Diabetes,* 50**,** 707-9.

TUNG, Y. C., AYUSO, E., SHAN, X., BOSCH, F., O'RAHILLY, S., COLL, A. P. & YEO, G. S. 2010. Hypothalamic-specific manipulation of Fto, the ortholog of the human obesity gene FTO, affects food intake in rats. *PLoS One,* 5**,** e8771.

TURNBAUGH, P. J., LEY, R. E., MAHOWALD, M. A., MAGRINI, V., MARDIS, E. R. & GORDON, J. I. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature,* 444**,** 1027-31.

TURTON, M. D., O'SHEA, D., GUNN, I., BEAK, S. A., EDWARDS, C. M., MEERAN, K., CHOI, S. J., TAYLOR, G. M., HEATH, M. M., LAMBERT, P. D., WILDING, J. P., SMITH, D. M., GHATEI, M. A., HERBERT, J. & BLOOM, S. R. 1996. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature,* 379**,** 69-72.

VAHL, T. P., PATY, B. W., FULLER, B. D., PRIGEON, R. L. & D'ALESSIO, D. A. 2003. Effects of GLP-1-(7–36)NH2, GLP-1-(7–37), and GLP-1- (9–36)NH2 on Intravenous Glucose Tolerance and Glucose-Induced Insulin Secretion in Healthy Humans. *The Journal of Clinical Endocrinology & Metabolism,* 88**,** 1772-1779.

VAN DER KLAAUW, A. A., KEOGH, J. M., HENNING, E., TROWSE, V. M., DHILLO, W. S., GHATEI, M. A. & FAROOQI, I. S. 2012. High protein intake stimulates postprandial GLP1 and PYY release. *Obesity*.

VAN DER KLAAUW, A. A., KEOGH, J. M., HENNING, E., TROWSE, V. M., DHILLO, W. S., GHATEI, M. A. & FAROOQI, I. S. 2013. High protein intake stimulates postprandial GLP1 and PYY release. *Obesity (Silver Spring),* 21**,** 1602-7.

VAN KARNEBEEK, C. D. M., WORTMANN, S. B., TARAILO-GRAOVAC, M., LANGEVELD, M., FERREIRA, C. R., VAN DE KAMP, J. M., HOLLAK, C. E., WASSERMAN, W. W., WATERHAM, H. R., WEVERS, R. A., HAACK, T. B., WANDERS, R. J. A. & BOYCOTT, K. M. 2018. The role of the clinician in the multi-omics era: are you ready? *J Inherit Metab Dis*.

VATN, M. H., SCHRUMPF, E., HANSSEN, K. F. & MYREN, J. 1980. A small dose of somatostatin inhibits the secretin stimulated secretion of bicarbonate, amylase, and chymotrypsin in man. *J Endocrinol Invest,* 3**,** 279-82.

VELDHORST, M., SMEETS, A., SOENEN, S., HOCHSTENBACH-WAELEN, A., HURSEL, R., DIEPVENS, K., LEJEUNE, M., LUSCOMBE-MARSH, N. & WESTERTERP-PLANTENGA, M. 2008. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol Behav,* 94**,** 300-7.

VELDHORST, M. A., NIEUWENHUIZEN, A. G., HOCHSTENBACH-WAELEN, A., VAN VUGHT, A. J., WESTERTERP, K. R., ENGELEN, M. P., BRUMMER, R. J., DEUTZ, N. E. & WESTERTERP-PLANTENGA, M. S. 2009a. Dose-dependent satiating effect of whey relative to casein or soy. *Physiol Behav,* 96**,** 675-82.

VELDHORST, M. A., NIEUWENHUIZEN, A. G., HOCHSTENBACH-WAELEN, A., WESTERTERP, K. R., ENGELEN, M. P., BRUMMER, R. J., DEUTZ, N. E. & WESTERTERP-PLANTENGA, M. S. 2009b. A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. *Clin Nutr,* 28**,** 147-55.

VELDHORST, M. A., NIEUWENHUIZEN, A. G., HOCHSTENBACH-WAELEN, A., WESTERTERP, K. R., ENGELEN, M. P., BRUMMER, R. J., DEUTZ, N. E. & WESTERTERP-PLANTENGA, M. S. 2009c. Comparison of the effects of a high- and normal-casein breakfast on satiety, 'satiety' hormones, plasma amino acids and subsequent energy intake. *Br J Nutr,* 101**,** 295-303.

VELDHORST, M. A., NIEUWENHUIZEN, A. G., HOCHSTENBACH-WAELEN, A., WESTERTERP, K. R., ENGELEN, M. P., BRUMMER, R. J., DEUTZ, N. E. & WESTERTERP-PLANTENGA, M. S. 2009d. Effects of complete whey-protein breakfasts versus whey without GMP-breakfasts on energy intake and satiety. *Appetite,* 52**,** 388-95.

VELDHORST, M. A., NIEUWENHUIZEN, A. G., HOCHSTENBACH-WAELEN, A., WESTERTERP, K. R., ENGELEN, M. P., BRUMMER, R. J., DEUTZ, N. E. & WESTERTERP-PLANTENGA, M. S. 2009e. Effects of high and normal soyprotein breakfasts on satiety and subsequent energy intake, including amino acid and 'satiety' hormone responses. *Eur J Nutr,* 48**,** 92-100.

VELDHORST, M. A., WESTERTERP-PLANTENGA, M. S. & WESTERTERP, K. R. 2009f. Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet. *Am J Clin Nutr,* 90**,** 519-26.

VELDHORST, M. A., WESTERTERP, K. R. & WESTERTERP-PLANTENGA, M. S. 2012. Gluconeogenesis and protein-induced satiety. *Br J Nutr,* 107**,** 595-600.

VIZCARRA, J. A., KIRBY, J. D., KIM, S. K. & GALYEAN, M. L. 2007. Active immunization against ghrelin decreases weight gain and alters plasma concentrations of growth hormone in growing pigs. *Domest Anim Endocrinol,* 33**,** 176-89.

VOLKOW, N. D., WANG, G. J. & BALER, R. D. 2011. Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci,* 15**,** 37-46.

VRANG, N., LARSEN, P. J., CLAUSEN, J. T. & KRISTENSEN, P. 1999. Neurochemical characterization of hypothalamic cocaine- amphetamine-regulated transcript neurons. *J Neurosci,* 19**,** RC5.

VRIEZE, A., HOLLEMAN, F., ZOETENDAL, E. G., DE VOS, W. M., HOEKSTRA, J. B. & NIEUWDORP, M. 2010. The environment within: how gut microbiota may influence metabolism and body composition. *Diabetologia,* 53**,** 606-13.

WAKABAYASHI, Y., YAMADA, E., YOSHIDA, T. & TAKAHASHI, H. 1994. Arginine becomes an essential amino acid after massive resection of rat small intestine. *J Biol Chem,* 269**,** 32667-71.

WALKER, A. W., INCE, J., DUNCAN, S. H., WEBSTER, L. M., HOLTROP, G., ZE, X., BROWN, D., STARES, M. D., SCOTT, P., BERGERAT, A., LOUIS, P., MCINTOSH, F., JOHNSTONE, A. M., LOBLEY, G. E., PARKHILL, J. & FLINT, H. J. 2011. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J,* 5**,** 220-30.

WALKER, J. B. 1979. Creatine: biosynthesis, regulation, and function. *Adv Enzymol Relat Areas Mol Biol,* 50**,** 177-242.

WALTER, J. & LEY, R. 2011. The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol,* 65**,** 411-29.

WANG, P. Y., CASPI, L., LAM, C. K., CHARI, M., LI, X., LIGHT, P. E., GUTIERREZ-JUAREZ, R., ANG, M., SCHWARTZ, G. J. & LAM, T. K. 2008. Upper intestinal lipids trigger a gut-brain-liver axis to regulate glucose production. *Nature,* 452**,** 1012-6.

WANG, Y., CHANDRA, R., SAMSA, L. A., GOOCH, B., FEE, B. E., COOK, J. M., VIGNA, S. R., GRANT, A. O. & LIDDLE, R. A. 2011. Amino acids stimulate cholecystokinin release through the Ca2+-sensing receptor. *Am J Physiol Gastrointest Liver Physiol,* 300**,** G528-37.

WATANABE, M., FUKIYA, S. & YOKOTA, A. 2017. Comprehensive evaluation of the bactericidal activities of free bile acids in the large intestine of humans and rodents. *J Lipid Res,* 58**,** 1143-1152.

WATERSON, M. J. & HORVATH, T. L. 2015. Neuronal Regulation of Energy Homeostasis: Beyond the Hypothalamus and Feeding. *Cell Metab,* 22**,** 962-70.

WAUSON, E. M., ZAGANJOR, E., LEE, A. Y., GUERRA, M. L., GHOSH, A. B., BOOKOUT, A. L., CHAMBERS, C. P., JIVAN, A., MCGLYNN, K., HUTCHISON, M. R., DEBERARDINIS, R. J. & COBB, M. H. 2012. The G protein-coupled taste receptor T1R1/T1R3 regulates mTORC1 and autophagy. *Mol Cell,* 47**,** 851-62.

WEIGLE, D. S., BREEN, P. A., MATTHYS, C. C., CALLAHAN, H. S., MEEUWS, K. E., BURDEN, V. R. & PURNELL, J. Q. 2005. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *Am J Clin Nutr,* 82**,** 41-8.

WELLENDORPH, P. & BRAUNER-OSBORNE, H. 2004. Molecular cloning, expression, and sequence analysis of GPRC6A, a novel family C G-protein-coupled receptor. *Gene,* 335**,** 37-46.

WELLENDORPH, P., BURHENNE, N., CHRISTIANSEN, B., WALTER, B., SCHMALE, H. & BRAUNER-OSBORNE, H. 2007. The rat GPRC6A: cloning and characterization. *Gene,* 396**,** 257-67.

WELLENDORPH, P., HANSEN, K. B., BALSGAARD, A., GREENWOOD, J. R., EGEBJERG, J. & BRAUNER-OSBORNE, H. 2005. Deorphanization of GPRC6A: a promiscuous L-alpha-amino acid receptor with preference for basic amino acids. *Mol Pharmacol,* 67**,** 589-97.

WELLENDORPH, P., JOHANSEN, L. D. & BRAUNER-OSBORNE, H. 2009a. Molecular pharmacology of promiscuous seven transmembrane receptors sensing organic nutrients. *Mol Pharmacol,* 76**,** 453-65.

WELLENDORPH, P., JOHANSEN, L. D. & BRAUNER-OSBORNE, H. 2010. The emerging role of promiscuous 7TM receptors as chemosensors for food intake. *Vitam Horm,* 84**,** 151-84.

WELLENDORPH, P., JOHANSEN, L. D., JENSEN, A. A., CASANOVA, E., GASSMANN, M., DEPREZ, P., CLEMENT-LACROIX, P., BETTLER, B. & BRAUNER-OSBORNE, H. 2009b. No evidence for a bone phenotype in GPRC6A knockout mice under normal physiological conditions. *J Mol Endocrinol,* 42**,** 215-23.

WELLS, A. S., READ, N. W., UVNAS-MOBERG, K. & ALSTER, P. 1997. Influences of fat and carbohydrate on postprandial sleepiness, mood, and hormones. *Physiol Behav,* 61**,** 679-86.

WEST, D. B., FEY, D. & WOODS, S. C. 1984. Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *Am J Physiol,* 246**,** R776-87.

WESTERTERP-PLANTENGA, M. S., ROLLAND, V., WILSON, S. A. & WESTERTERP, K. R. 1999. Satiety related to 24 h diet-induced thermogenesis during high protein/carbohydrate vs high fat diets measured in a respiration chamber. *Eur J Clin Nutr,* 53**,** 495-502.

WESTERTERP, K. R. 2004. Diet induced thermogenesis. *Nutrition & Metabolism,* 1**,** 5.

WHITE, M. F. 1985. The transport of cationic amino acids across the plasma membrane of mammalian cells. *Biochim Biophys Acta,* 822**,** 355-74.

WHITLOCK, G., LEWINGTON, S., SHERLIKER, P., CLARKE, R., EMBERSON, J., HALSEY, J., QIZILBASH, N., COLLINS, R. & PETO, R. 2009. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet,* 373**,** 1083-96.

WHO 2009. Global health risks: Mortality and burden of disease attributable to selected major risks.

WHO 2016. Fiscal Policies for Diet and Prevention of Noncommunicable Diseases: Technical Meeting Report.

WILKINSON, A. R. & JOHNSTON, D. 1973. Effect of truncal, selective and highly selective vagotomy on gastric emptying and intestinal transit of a food-barium meal in man. *Ann Surg,* 178**,** 190-3.

WINDMUELLER, H. G. 1982. Glutamine utilization by the small intestine. *Adv Enzymol Relat Areas Mol Biol,* 53**,** 201-37.

WINDMUELLER, H. G. & SPAETH, A. E. 1981. Source and fate of circulating citrulline. *Am J Physiol,* 241**,** E473-80.

WONG, J. M., DE SOUZA, R., KENDALL, C. W., EMAM, A. & JENKINS, D. J. 2006. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol,* 40**,** 235-43.

WOOD, W. G., WACHTER, C. & SCRIBA, P. C. 1981. Experiences using chloramine-T and 1, 3, 4, 6-tetrachloro-3 alpha, 6 alpha-diphenylglycoluril (Iodogen) for radioiodination of materials for radioimmunoassay. *J Clin Chem Clin Biochem,* 19**,** 1051-6.

WORLEY, B. & POWERS, R. 2013. Multivariate Analysis in Metabolomics. *Curr Metabolomics,* 1**,** 92-107.

WREN, A. M., SEAL, L. J., COHEN, M. A., BRYNES, A. E., FROST, G. S., MURPHY, K. G., DHILLO, W. S., GHATEI, M. A. & BLOOM, S. R. 2001a. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab,* 86**,** 5992.

WREN, A. M., SMALL, C. J., ABBOTT, C. R., DHILLO, W. S., SEAL, L. J., COHEN, M. A., BATTERHAM, R. L., TAHERI, S., STANLEY, S. A., GHATEI, M. A. & BLOOM, S. R. 2001b. Ghrelin causes hyperphagia and obesity in rats. *Diabetes,* 50**,** 2540-7.

WREN, A. M., SMALL, C. J., WARD, H. L., MURPHY, K. G., DAKIN, C. L., TAHERI, S., KENNEDY, A. R., ROBERTS, G. H., MORGAN, D. G., GHATEI, M. A. & BLOOM, S. R. 2000a. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology,* 141**,** 4325-8.

WREN, A. M., SMALL, C. J., WARD, H. L., MURPHY, K. G., DAKIN, C. L., TAHERI, S., KENNEDY, A. R., ROBERTS, G. H., MORGAN, D. G. A., GHATEI, M. A. & BLOOM, S. R. 2000b. The Novel Hypothalamic Peptide Ghrelin Stimulates Food Intake and Growth Hormone Secretion. *Endocrinology,* 141**,** 4325-4328.

WRIGHT, E. M., MARTIN, M. G. & TURK, E. 2003. Intestinal absorption in health and disease--sugars. *Best practice & research. Clinical gastroenterology,* 17**,** 943-56.

WU, G. 2009. Amino acids: metabolism, functions, and nutrition. *Amino Acids,* 37**,** 1-17.

WU, G., KNABE, D. A. & KIM, S. W. 2004. Arginine nutrition in neonatal pigs. *J Nutr,* 134**,** 2783S-2790S; discussion 2796S-2797S.

WU, G. & MORRIS, S. M., JR. 1998. Arginine metabolism: nitric oxide and beyond. *Biochem J,* 336 (Pt 1)**,** 1-17.

WYNNE, K., PARK, A. J., SMALL, C. J., MEERAN, K., GHATEI, M. A., FROST, G. S. & BLOOM, S. R. 2006. Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. *Int J Obes (Lond),* 30**,** 1729-36.

XUE, B. & KAHN, B. B. 2006. AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues. *The Journal of physiology,* 574**,** 73-83.

YANG, J., BROWN, M. S., LIANG, G., GRISHIN, N. V. & GOLDSTEIN, J. L. 2008. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell,* 132**,** 387-96.

YOUNG, S. H., REY, O., STERNINI, C. & ROZENGURT, E. 2010. Amino acid sensing by enteroendocrine STC-1 cells: role of the Na+-coupled neutral amino acid transporter 2. *Am J Physiol Cell Physiol,* 298**,** C1401-13.

YOX, D. P., BRENNER, L. & RITTER, R. C. 1992. CCK-receptor antagonists attenuate suppression of sham feeding by intestinal nutrients. *Am J Physiol,* 262**,** R554-61.

YU, H., GUO, Z., SHEN, S. & SHAN, W. 2016. Effects of taurine on gut microbiota and metabolism in mice. *Amino Acids,* 48**,** 1601-17.

ZHANG, F., CHEN, Y., HEIMAN, M. & DIMARCHI, R. 2005. Leptin: Structure, Function and Biology. *Vitamins & Hormones.* Academic Press.

ZHANG, Y., GAO, X., SAUCEDO, L. J., RU, B., EDGAR, B. A. & PAN, D. 2003a. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat Cell Biol,* 5**,** 578-81.

ZHANG, Y., HOON, M. A., CHANDRASHEKAR, J., MUELLER, K. L., COOK, B., WU, D., ZUKER, C. S. & RYBA, N. J. 2003b. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell,* 112**,** 293-301.

ZHAO, G. Q., ZHANG, Y., HOON, M. A., CHANDRASHEKAR, J., ERLENBACH, I., RYBA, N. J. & ZUKER, C. S. 2003. The receptors for mammalian sweet and umami taste. *Cell,* 115**,** 255-66.

ZHOU, B. F., STAMLER, J., DENNIS, B., MOAG-STAHLBERG, A., OKUDA, N., ROBERTSON, C., ZHAO, L., CHAN, Q., ELLIOTT, P. & GROUP, I. R. 2003. Nutrient intakes of middle-aged men and women in China, Japan, United Kingdom, and United States in the late 1990s: the INTERMAP study. *J Hum Hypertens,* 17**,** 623-30.

Appendices

Imperial College London

Version 3.0 (15/04/13), Ref Number 11/H0707/7

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INFORMATION SHEET FOR RESEARCH PARTICIPANTS

You will be given a copy of this Information Sheet and a signed copy of your consent form to keep, should you decide to participate in the study.

STUDY TITLE: THE EFFECT OF SPECIFIC AMINO ACIDS ON APPETITE

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. You are free to withdraw at any time without explanation. Thank you for reading this.

WHAT IS THE PURPOSE OF THIS STUDY?

Diets which are rich in proteins are known to suppress appetite (i.e. how hungry you feel) but the reason why this happens is unknown. Proteins are broken down by your body into smaller molecules called amino acids. This study is looking at whether specific individual amino acids also reduce appetite. This may be important in terms of controlling body weight and therefore preventing obesity. We aim to investigate this by measuring how hungry a person is after ingesting these amino acids. The study will also form part of a postgraduate research project (PhD).

WHY HAVE I BEEN INVITED?

You have been invited to take part in this study because you meet the following criteria:

- You are aged 18-60 years
- You have a body mass index (BMI) between 18.5-35 kg/m^2

If any of the following apply to you then unfortunately you are unsuitable to take part in this study:

- Treatment with any medication that is may affect your appetite and/or blood clotting
- Bleeding disorders and anticoagulant treatment
- Inborn errors of metabolism
- Abnormal liver function tests
- Current pregnancy or breast feeding (as determined by a pregnancy test)
- Having a baby within the last year
- Significant long-term disease or history of clinically significant disease of any type, in particular liver, kidney, or heart disease, any form of diabetes mellitus or psychiatric illness
- History of cancer, excluding skin cancer
- History of severe or multiple allergies, severe adverse drug reaction or low white blood cells
- Smokers
- Regular drinkers of more than three units of alcohol daily (1 unit = 300mL beer, 1 glass wine, 1 measure spirit)

- Anyone who has taken amino acid supplements either continuously or intermittently in the preceding 12 months
- History of illicit drug use or previous heavy alcohol use
- Regular intake of over-the-counter medication (other than the occasional paracetamol/aspirin)
- Blood donation within the 12 week period before the study starts

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

WHAT WILL HAPPEN TO ME IF I TAKE PART?

If you agree to take part in this study, you will first be invited to attend a health screening with one of the researchers involved in this study. This will involve a blood test after an overnight fast (no more than 20mL blood, equivalent to just over one tablespoon of blood) and a pregnancy test (if female). You will be asked to complete several short questionnaires about your eating habits, personality and mood and asked details about your past medical history. You will also be asked to taste a number of different meals which will be used later on in the study. Some of you will be asked to fill in a 3-day dietary record at home to assess your eating habits (Parts C – see below).

As long as these medical checks are satisfactory and you are still happy to participate, you will then be enrolled onto the study.

The study consists of 3 separate parts. **You do not have to take part in all 3 parts.**

Number of visits

All the study visits will take part in the Clinical Investigation Ward (NIHR/Wellcome Trust Imperial CRF) at Hammersmith Hospital as an outpatient.

For Part A, this involves one study visit.
For Part B, this involves up to 5 study visits, each separated by at least one week.
For Part C, this involves up to 10 study visits, each separated by at least one week.

Study visits

You will be asked to have nothing to eat and only water to drink from 21:00 on the evening before the study and to abstain from alcohol and strenuous exercise for 24 hours before the visit.

During the study visits you will be seated or lying on a couch and you may read or watch television.

When you arrive you will have a small plastic cannula tube inserted into a vein in one arm. A vein is the type of blood vessel commonly used for taking blood samples. You may feel some discomfort whilst the cannula is being inserted. After the cannula tube has been inserted this will be used to take blood samples. During the visit up to 45 mL or 3 tablespoons of blood will be taken over a 2.5 hour period. Blood samples will be used to measure the levels of hormones which control hunger in your body, as well as for routine tests, levels of amino acids and paracetamol levels.

The total amount of blood taken over all your visits will not be more than 470 mL over a four month period.

If you are female, you will be asked to provide us with a urine sample so that we can perform a pregnancy test.

Just before each blood sample is taken you will be asked to fill in a chart describing how hungry you feel.

Part A involves you eating a high protein meal followed by blood sampling through the cannula (as described above).

Part B involves you taking a drink containing a specific amino acid with a single dose of paracetamol followed by blood sampling through the cannula (as described above). Each study day will follow the same timetable. The only difference is that your drink will contain different amounts of amino acids.

Part C involves you taking a drink containing a specific amino acid followed by blood sampling through the cannula (as described above). A short time after taking the drink, you will be given access to a buffet meal (chosen on your screening day or a previous study day) and asked to eat until you are comfortably full.

WHAT DO I HAVE TO DO?
To take part in this study you must be willing to do the following things:

1. Attend the hospital on up to 10 separate study days
2. Have nothing to eat and only water to drink from 21:00 on the night before visits.
3. Some of you will be asked to keep a record of all food and drinks consumed over the 3 days after the screening visit (to post this back to us using a self-addressed envelope provided) (if you take part in Part C).
4. For 24 hours before each study meal you will be asked to refrain from taking strenuous exercise and drinking alcohol.

WHAT ARE WE TESTING?
We are measuring levels of hormones which influence appetite, other markers of appetite and metabolism, levels of amino acids in the bloodstream, as well as other routine blood tests.

WHAT ARE THE SIDE EFFECTS OF TAKING PART?
The amino acids consumed over the course of the study are naturally present in a normal diet. They are generally not found to be linked to any serious side effects. In healthy individuals there are very low risks of side effects occurring, which can include nausea or bloating.

WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART?
Insertion of the cannula into your arm on each of the study days may cause minor discomfort or superficial bruising.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?
Taking part in the study will provide no direct benefit for you. The information that we get from this study will help us to better understand normal appetite regulation and may help us to better treat future patients who suffer from obesity. If any of the screening questionnaires or blood tests reveal any medical problems (e.g. depression, diabetes, kidney or liver problems), your GP will be informed so that they can coordinate your further care, arrange any further tests, and refer you on to Hospital Doctors if necessary.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?
Sometimes during the course of a research project, new information becomes available about the study. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study.

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?
Once the study has finished, the results of the study can be made available to you and/or your GP should you wish. If you have any problems immediately following the study, then you should contact one of the research doctors on the numbers provided below.

WHAT IF SOMETHING GOES WRONG?
In the event of any adverse events occurring as a consequence of your participation in this study, you may be entitled to compensation through the Imperial College School of Medicine's 'No-fault' Compensation scheme.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?
All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will be kept in a secure location at Imperial College London.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?
The results are likely to be published in the year following the study. Your confidentiality will be ensured at all times and you will not be identified in any publication. At the end of the study, the results of the study can be made available to you and/or your GP should you wish.

WHO IS ORGANISING AND FUNDING THE RESEARCH?
This study is being organised by the Section of Investigative Medicine, Imperial College London.

WILL I BE REIMBURSED FOR MY TRAVEL EXPENSES/TIME?
You will receive a fixed payment to cover expenses including travel costs. This will be £10 for the screening visit and £30 per study day.

WHO HAS REVIEWED THE STUDY?
This study has been reviewed by the London – West London & GTAC Research Ethics Committee.

WHAT IF SOMETHING GOES WRONG?
Imperial College London holds insurance polices which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you wish to complain for any reason, you should approach the PALS office (PALS address: PALS manager, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, Telephone: 020 3313 3322, Fax: 020 3313 0087.

If you are harmed due to someone's negligence, then you may have grounds for legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect on the way you have been treated during the course of this study then you should immediately inform the Chief Investigator (Professor Stephen Bloom, Department of Investigative Medicine, 6th Floor Commonwealth Building, Hammersmith Hospital, Imperial College London, Du Cane Road, London, W12 0NN, U.K. Tel: 020 8383 3242). The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

CONTACT FOR FURTHER INFORMATION
If you have any questions regarding the study please contact the researchers (Hannah Greenwood or Dr. Anjali Amin) on 020 8383 4843. If you would specifically like to talk to the doctor involved in the study, Dr. Amin will be available by telephone during working hours on 020 8383 4843. The hospital switchboard (020 8383 1000) has home and mobile phone numbers for all the doctors involved in the study and can contact them at any time outside normal working hours. If you agree to take part in the study, you will also be given the contact details of the researchers.

If you experience any problems during this study, you may withdraw at any stage.
Thank you for taking the time to read this.

Imperial College London

Version 2.0 (21/11/13), Ref Number 11/H0707/7

Section of Investigative Medicine
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London W12 0NN

Participant Consent Form

STUDY TITLE: THE EFFECT OF SPECIFIC AMINO ACIDS ON APPETITE

Name of Principal Investigator: Professor Stephen Bloom

Please tick each statement:

1. I confirm that I have read and understand the participant information sheet for the above Study (Version 3.0 (15/4/13)). ☐
2. I have had the opportunity to ask questions and discuss this study. ☐
3. All my questions have been answered fully. ☐
4. I have received enough information about the study. ☐
5. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
6. I understand that sections of any of my medical notes may be looked at by responsible individuals from Imperial College London and Imperial College Healthcare NHS Trust or from regulatory authorities where it is relevant to my taking part in this research. ☐
7. I give permission for these individuals to access my records that are relevant to this research. ☐
8. I give permission for my test results to be used for this research and future ethically approved research by responsible individuals from Imperial College and Hammersmith Hospital so long as they do not contain identifying personal information. ☐
9. I give permission for any blood samples that I give during this study, to be used in future studies which have been granted suitable ethical approval. ☐
10. The compensation arrangements have been discussed with me. ☐
11. I agree to take part in the above study. ☐
12. I agree to be contacted again by the Investigators to participate in future research. ☐

Name of Subject (block capitals)	Signature	Date
Name of Person taking consent (if different from Principal Investigator)	Signature	Date

APPENDIX 3 – VISUAL ANALOGUES SCALES (BOTH STUDIES)

Initials: Date:

Code: Study visit number:

Time: 29 x 2

How hungry do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How sick do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

How pleasant would it be to eat right now?

NOT AT ALL ——————————————— EXTREMELY

How much do you think you could eat right now?

NOTHING ——————————————— A LARGE AMOUNT

How full do you feel right now?

NOT AT ALL ——————————————— EXTREMELY

11/H0707/7

Initials: Date:

Code: Study visit number:

Time: 29x2

How warm do you feel right now?

VERY COLD ——— VERY HOT

How irritable do you feel right now?

NOT AT ALL ——— EXTREMELY

How anxious do you feel right now?

NOT AT ALL ——— EXTREMELY

How sleepy do you feel right now?

NOT AT ALL ——— EXTREMELY

11/H0707/7

Imperial College London

Version 4.0 (2/2/16), Ref Number 15/LO/1060
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6th Floor Commonwealth Building
Imperial College London
Hammersmith Hospital Campus, Du Cane Road
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INFORMATION SHEET FOR RESEARCH PARTICIPANTS

You will be given a copy of this Information Sheet and a signed copy of your consent form to keep, should you decide to participate in the study.

STUDY TITLE: ASSESSMENT OF DIGESTION AND METABOLISM FOLLOWING ALTERED MACRONUTRIENT INTAKE

You are being invited to take part in a pilot research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information and it will not affect your future care. Take time to decide whether or not you wish to take part.

If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. You are free to withdraw at any time without explanation. Thank you for reading this.

WHAT IS THE PURPOSE OF THIS STUDY?
Diets of altered nutrient content are known to have varying effects on appetite (i.e. how hungry you feel) but the reason why this happens is unknown. This study is looking at the effect of high-carbohydrate, high-fat and high-protein meals on digestion and appetite. We aim to investigate this by measuring how hungry a person is after ingesting these meals. This may be important in terms of controlling body weight and therefore preventing obesity. The study will also form part of a postgraduate research project (PhD).

WHY HAVE I BEEN INVITED?
You have been invited to take part in this study because you meet the following criteria:

- You are aged 18-60 years
- You have a body mass index (BMI) between 18.5-30 kg/m^2

If any of the following apply to you then unfortunately you are unsuitable to take part in this study:

- Treatment with any medication that is may affect your appetite and/or blood clotting
- Bleeding disorders and anticoagulant treatment
- Inborn errors of metabolism
- Abnormal liver function tests
- Current pregnancy or breast feeding (as determined by a pregnancy test)
- Having a baby within the last year
- Oesophageal stricture
- Significant long-term disease or history of clinically significant disease of any type, in particular liver, kidney, or heart disease, any form of diabetes mellitus or psychiatric illness
- History of cancer, excluding skin cancer
- History of severe or multiple allergies, severe adverse drug reaction or low white blood cells
- Lactose/milk protein intolerance
- Smokers
- Regular drinkers of more than three units of alcohol daily (1 unit = 300mL beer, 1 glass wine, 1 measure spirit)
- Anyone who has taken nutrient supplements either continuously or intermittently in the preceding 12 months

- History of illicit drug use or previous heavy alcohol use
- Regular intake of over-the-counter medication (other than the occasional paracetamol/aspirin)
- Blood donation within the 12 week period before the study starts
- Participation in a research study in the past 3 months

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

WHAT WILL HAPPEN TO ME IF I TAKE PART?

If you agree to take part in this study, you will first be invited to attend a health screening with one of the researchers involved in this study. This will involve a blood test (no more than 20mL blood, equivalent to just over one tablespoon of blood) and a pregnancy test (if female). You will be asked to complete several short questionnaires about your eating habits, personality and mood and asked details about your past medical history. You will also be asked to taste a number of different meals which will be used later on in the study.

As long as these medical checks are satisfactory and you are still happy to participate, you will then be enrolled onto the study.

Number of visits

All the screening and study visits will take part in the Clinical Investigation Ward (NIHR/Wellcome Trust Imperial CRF) at Hammersmith Hospital.

There will be one screening visit lasting approximately one hour followed by a separate four day in-patient stay for the entire study. Some participants will be invited to take part in a further study which takes place over 3 separate mornings.

Study visits

You will be asked to have nothing to eat and only water to drink from 21:00 on the evening before each of the study mornings and to abstain from alcohol and strenuous exercise for 24 hours before and during the study.

During the study you will be seated or lying on a couch and you may read or watch television.

When you arrive you will have a small plastic cannula tube inserted into a vein in one arm. A vein is the type of blood vessel commonly used for taking blood samples. You may feel some discomfort whilst the cannula is being inserted. After the cannula tube has been inserted this will be used to take blood samples. During each study day up to 104 mL or 7 tablespoons of blood will be taken over a 3-4 hour period. Blood samples will be used to measure the levels of hormones which control hunger in your body, as well as for routine tests, levels of nutrients and paracetamol levels. With your consent, some of your blood may be kept for future studies that have been granted suitable ethical approval. The total amount of blood taken over all your visits (including screening) will not be more than 436 mL. You will be asked to empty your bladder at the start of each study morning, and then collect all your urine over the course of the study (3 hour period). If you are female, you will be asked to provide us with a urine sample so that we can perform a pregnancy test.

Following the cannula insertion, you will have a tube placed through your nose by a trained medical professional. Some people may have a separate tube placed through the mouth. The ends of these tubes will lie within the stomach and the duodenum (the first part of your small intestine), allowing us to measure the contents of the gut at the same time as the blood samples. The tube will be placed whilst you are awake. It is not painful, but can result in transient discomfort which can be minimised with sips of water or sucking on an ice cube. The tubes will remain in place for the duration of the study (4 days).

Small samples of gut contents will be removed over the 3 hour period and measured for a number of factors including the breakdown of the various nutrients. You will feel no discomfort while the samples are being taken via the tube.

At the start of each study morning, you will be asked to consume a meal of varying nutrient content along with a dose of paracetamol (this allows measurement of how fast your stomach is emptying its contents). This will be followed by blood sampling through the cannula (as described above) and sampling through the tubes (as described above). Just before each sample is taken you will be asked to fill in a chart describing how hungry you feel. At the end of the study morning, you will be given access to a buffet meal (chosen on your screening day) and asked to eat until you are comfortably full. After the buffet meal, the cannula will be removed. You will be provided with all meals and snacks until 21.00, when you will be asked to fast again (nothing to eat or drink but water) till the next morning.

Some people will be asked to take part in a separate study which will involve having the meals but without the tubes. During this study, they will receive paracetamol with the meal and have blood tests taken over the course of a morning. This study will consist of three separate study mornings and will not involve an overnight stay. Over the course of this study, no more than 90mL blood in total will be taken over the three study days. Blood samples will be used to measure levels of paracetamol, which will allow measurement of how quickly your stomach is emptying.

WHAT DO I HAVE TO DO?

To take part in this study you must be willing to do the following things:

1. Attend the hospital for a four day study visit.
2. Have nothing to eat and only water to drink from 21:00 on the night before each study morning.
3. Refrain from doing strenuous exercise and drinking alcohol for 24 hours before and during the study.

WHAT ARE WE TESTING?

We are measuring levels of hormones which influence appetite, other markers of appetite and metabolism, levels of nutrients in the bloodstream, as well as other routine blood tests. Food samples will be removed from your stomach and duodenum after eating to test for how different meals are broken down during digestion. Urine samples will be tested for levels of the breakdown products from the meal.

WHAT ARE THE SIDE EFFECTS OF TAKING PART?

The nutrients consumed over the course of the study are frequently used as dietary supplements. They are generally not found to be linked to any serious side effects.

WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART?

Insertion of the cannula into your arm on each of the study days may cause minor discomfort or superficial bruising. Serious risks associated with the insertion of the tubes are very rare and almost negligible. These risks include bleeding, perforation or damage to the base of the skull. Minor discomfort of the back of the throat does occur in the majority of patients and may result transiently in a sore mouth, thirst, swallowing difficulties or hoarseness.

It should be noted that the health screenings performed as part of this study cannot be viewed as a comprehensive health check. However, vary rarely, unexpected observations may be detected which may need further investigation. In this event, you will be informed and a report will be sent to your GP, who will arrange further tests and coordinate your further care.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

Taking part in the study will provide no direct benefit for you. The information that we get from this study will help us to better understand normal appetite regulation and may help us to better

treat future patients who suffer from obesity. If any of the screening questionnaires or blood tests reveal any medical problems (e.g. depression, diabetes, kidney or liver problems), your GP will be informed so that they can coordinate your further care, arrange any further tests, and refer you on to Hospital Doctors if necessary.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?
All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will be kept in a secure location at Imperial College London.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?
Sometimes during the course of a research project, new information becomes available about the study. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study.

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?
Once the study has finished, the results of the study can be made available to you and/or your GP should you wish. If you have any problems immediately following the study, then you should contact one of the research doctors on the numbers provided below.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?
The results are likely to be published in the year following the study. Your confidentiality will be ensured at all times and you will not be identified in any publication. At the end of the study, the results of the study can be made available to you and/or your GP should you wish.

WHO IS ORGANISING AND FUNDING THE RESEARCH?
This study is being organised by the Section of Investigative Medicine, Imperial College London and is being funded by Imperial NIHR Biomedical Research Centre (BRC).

WHO HAS REVIEWED THE STUDY?
This study has been reviewed by the NRES Committee London - Bloomsbury.

WILL I BE REIMBURSED FOR MY TRAVEL EXPENSES/TIME?
You will receive a fixed payment to cover expenses including travel costs. This will be £10 for the screening visit and £500 for the four day study. If you are invited to take part in the further study over three separate mornings, you will be paid £50 per study morning.

WHAT IF SOMETHING GOES WRONG?
Imperial College London holds insurance polices which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you wish to complain for any reason, you should approach the PALS office (PALS address: PALS manager, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, Telephone: 020 3313 3322, Fax: 020 3313 0087.

If you are harmed due to someone's negligence, then you may have grounds for legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect on the way you have been treated during the course of this study then you should immediately inform the Chief Investigator (Professor Gary Frost, Section of Investigative Medicine, 6th Floor Commonwealth Building, Hammersmith Hospital, Imperial College London, Du Cane Road, London, W12 0NN, U.K. Tel: 020 3313 8037). The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

CONTACT FOR FURTHER INFORMATION

If you have any questions regarding the study please contact the researcher (Dr. Anjali Amin) on 020 8383 4843 during working hours. The hospital switchboard (020 8383 1000) has home and mobile phone numbers for all the researchers involved in the study and can contact them at any time outside normal working hours. If you agree to take part in the study, you will also be given the contact details of the researchers.

If you experience any problems during this study, you may withdraw at any stage.

Thank you for taking the time to read this.

Imperial College London

Version 3.0, 15/LO/1060 2/2/16
Section of Investigative Medicine
6th Floor Commonwealth Building
Imperial College London
Hammersmith Hospital Campus, Du Cane Road
London W12 0NN

Participant Consent Form

STUDY TITLE: ASSESSMENT OF DIGESTION AND METABOLISM FOLLOWING ALTERED MACRONUTRIENT INTAKE

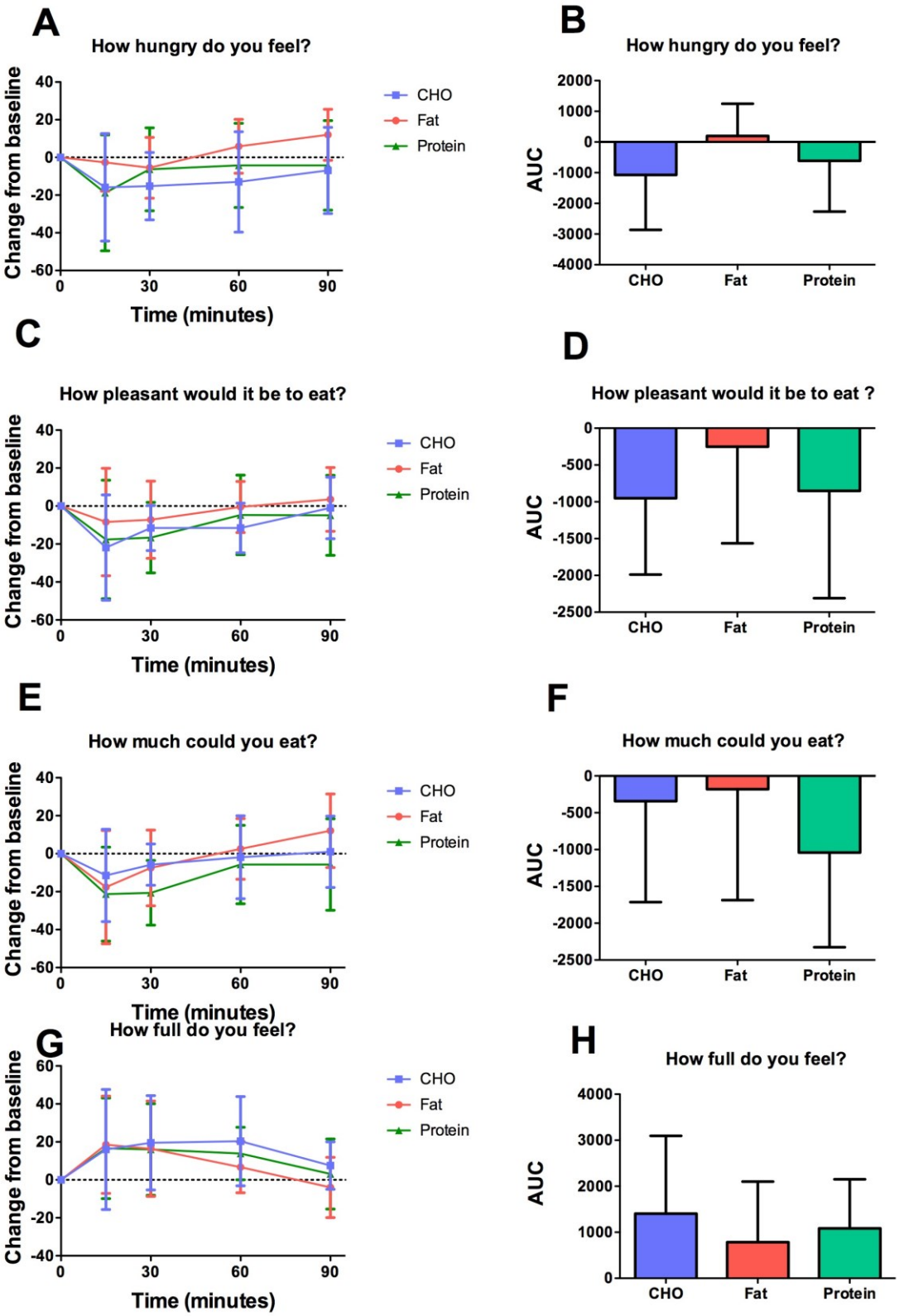
Name of Chief Investigator: Professor Gary Frost

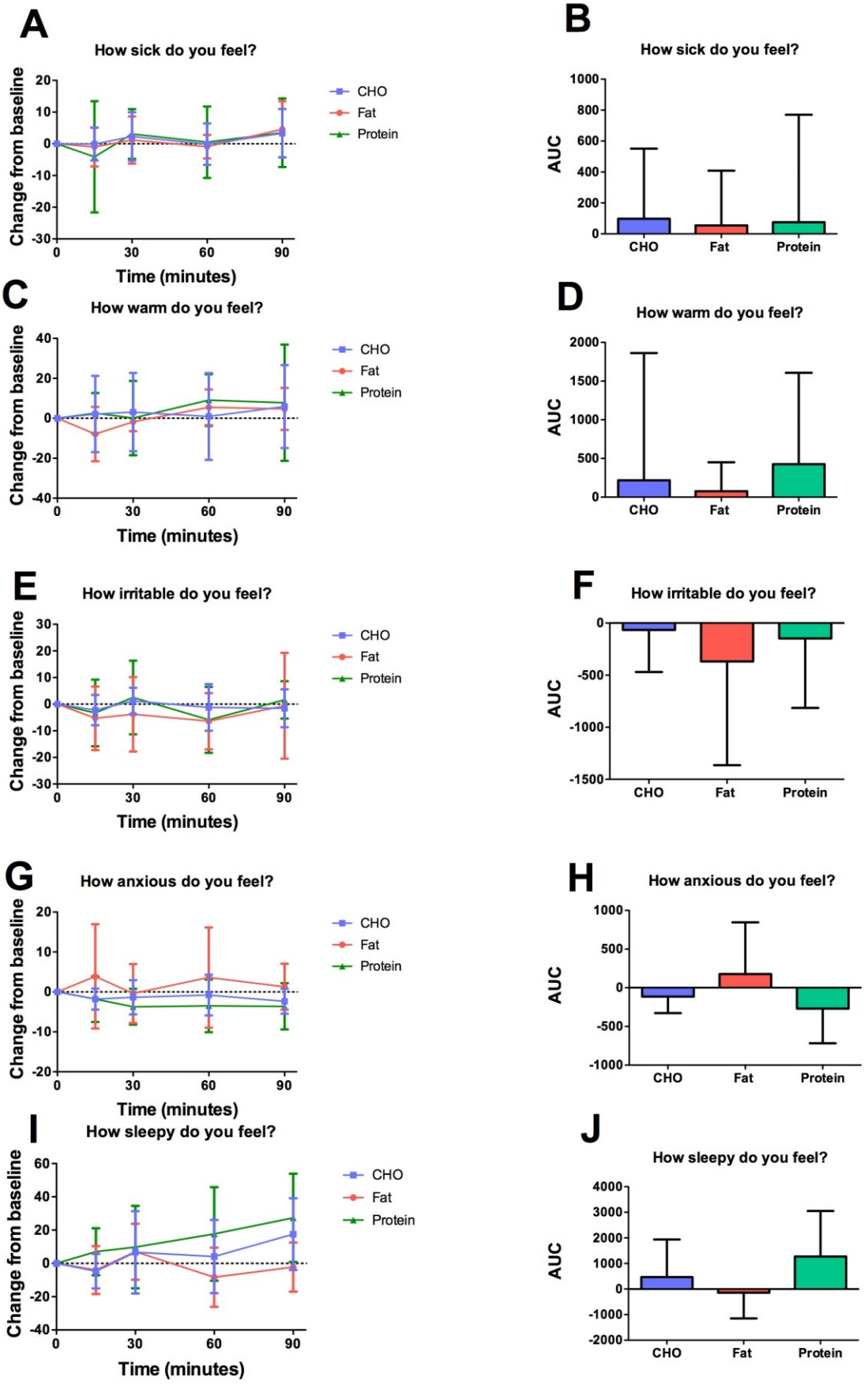
Please initial each statement:

1. I confirm that I have read and understand the participant information sheet for the above Study (Version 4.0). ☐
2. I have had the opportunity to ask questions and discuss this study. ☐
3. All my questions have been answered fully. ☐
4. I have received enough information about the study. ☐
5. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
6. I understand that sections of any of my medical notes may be looked at by responsible individuals from Imperial College London and Imperial College Healthcare NHS Trust or from regulatory authorities where it is relevant to my taking part in this research. ☐
7. I give permission for these individuals to access my records that are relevant to this research. ☐
8. I give permission for my test results to be used for this research and future ethically approved research by responsible individuals from Imperial College and Hammersmith Hospital so long as they do not contain identifying personal information. ☐
9. I give permission for my General Practitioner to be informed of my participation in this study and the results of any medical tests from my study visits. ☐
10. I give permission for any blood samples that I give during this study, to be used in future studies which have been granted suitable ethical approval. ☐
11. The compensation arrangements have been discussed with me. ☐
12. I agree to take part in the above study. ☐
13. OPTIONAL: I agree to be contacted again by the Investigators to participate in future research. ☐

Name of Subject (block capitals)	Signature	Date
Name of Person taking consent (if different from Chief Investigator)	Signature	Date

APPENDIX 6 – VAS 0-90 MINUTES (UPPER GI METABOLOMICS STUDY)




A
How sick do you feel?
Change from baseline
Time (minutes)
CHO
Fat
Protein
B
How sick do you feel?
AUC
CHO
Fat
Protein
C
How warm do you feel?
Change from baseline
Time (minutes)
CHO
Fat
Protein
D
How warm do you feel?
AUC
CHO
Fat
Protein
E
How irritable do you feel?
Change from baseline
Time (minutes)
CHO
Fat
Protein
F
How irritable do you feel?
AUC
CHO
Fat
Protein
G
How anxious do you feel?
Change from baseline
Time (minutes)
CHO
Fat
Protein
H
How anxious do you feel?
AUC
CHO
Fat
Protein
I
How sleepy do you feel?
Change from baseline
Time (minutes)
CHO
Fat
Protein
J
How sleepy do you feel?
AUC
CHO
Fat
Protein

Introduction

A study was performed whereby a group of 10 participants received three different treatments on three separate days. Measurements of hormone release over the course of 180 minutes post-treatment were made on each day. The aim of the analysis was to compare the hormone release values between the three treatments.

This first report details an analysis of the GLP-1 outcome.

Methods

GLP-1 measurements were made over the course of 180 minutes on each of the three days the participants were in the study. The outcome values for the analysis were assumed to be the change in GLP-1 from the baseline (time 0). Outcome values from the baseline time period (where all changes are zero) were omitted from the analysis.

The analysis was performed by modelling the response over time for each treatment group. To allow for the repeat measurements over time, the analysis was performed using multilevel linear regression. Three-level models were used with individual measurements nested within a measurement of participant/days, which in turn were contained within participants. Treatment group was considered to be a fixed effect. To allow a flexible curved relationship between time and the outcome variable, linear, squared and cubic terms for time were included if found to improve the fit of the model.

The differing relationships over time between treatments was determined by examining the size of the interaction between treatment and time. A significant interaction would imply that the changes in outcome over time varied between treatment groups. If no significant interaction was found, these terms were omitted from the model, and a constant difference between groups over time was assumed.

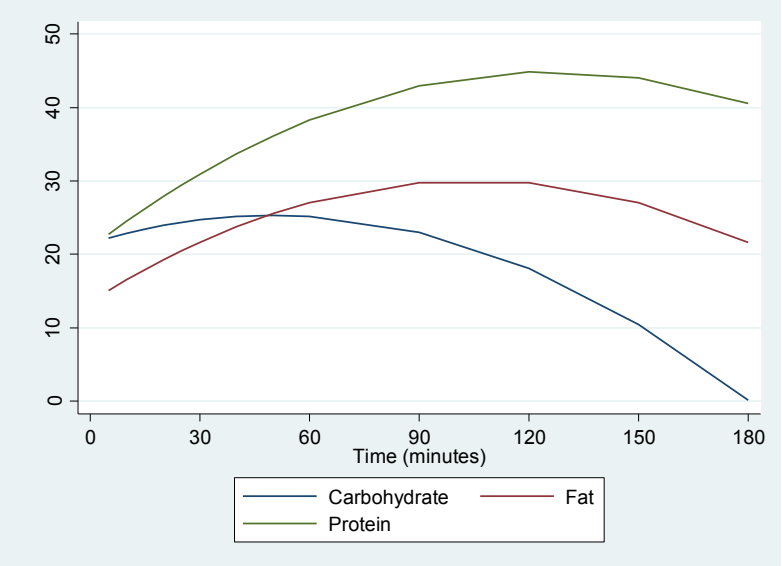
If a significant interaction was observed, this would suggest the treatment differences varied over time. If this was the case, the treatment differences were evaluated at two fixed timepoints, one half way through the measurement period (90 minutes) and one at the end of the measurement period (180 mins). An overall comparison between the three treatments was made, as well as a pairwise comparison between each pair of treatments. For the pairwise comparisons, the p-values were given a Bonferroni adjustment to allow for multiple statistical comparisons.

Results

The multilevel regression analysis suggested that the best model to evaluate the relationship over time for the different treatments included linear and squared terms for time, along with an interaction term between group and the linear term for time only.

The interaction term between treatment and time was statistically significant ($p<0.001$). This suggests that the size of differences between the three treatments varied over the study period. In other words, there was not a constant treatment difference.

A graphical illustration of the fitted relationships for each of the three treatments is shown in the subsequent graph.



The graph suggests that in both the fat and protein groups there was an increase in GLP-1 after the start of treatment, with the values remaining above baseline for the whole study period. GLP-1 also increased after treatment after the carbohydrate meal, but return to baseline values after 180 minutes.

Due to the significant interaction between time and treatment, there was not a constant difference between the three treatment groups throughout the follow-up period. So, no single set of differences can be reported.

However, the treatment differences were quantified at two specific points in time, 90 and 180 minutes. The results of these analyses are summarised in the next table. The first figures shown the mean difference in GLP-1 between the fat and protein groups relative to the carbohydrate group, along with corresponding confidence intervals. A series of p-values are also reported. The first indicates the overall significance between the three treatments. Subsequently p-values from each of the pairwise treatment comparisons are reported.

Statistic	90 minutes	180 minutes
Mean diff (95% CI): Fat - Carb	7 (-4, 17)	21 (6, 37)
Mean diff (95% CI): Protein - Carb	20 (7, 34)	40 (26, 55)
Overall p-value (*)	**<0.001**	**<0.001**
Fat vs. Carbohydrate p-value (+)	0.63	**0.02**
Protein vs. Carbohydrate p-value (+)	**<0.001**	**<0.001**
Protein vs. Fat p-value (+)	**0.04**	**0.04**

(*) Significance of the overall difference between the Carbohydrate, Fat and Protein
(+) P-value given a Bonferroni adjustment to allow for multiple comparisons

The results suggested that at both the 90 and 180-minute timepoints there was a significant overall difference between the three treatments.

At 90-minutes there was a difference in GLP-1 between the protein and carbohydrate values, and also between the protein and fat values. At this timepoint, GLP-1 values after the protein treatment significantly higher than each of the other two treatments. On average, GLP-1 measurements after the protein treatment were 20-units higher than for carbohydrate, and 13-units higher than for fat. There was no significant difference between fat and carbohydrate groups at this timepoint.

At 180-mins, there was a significant difference between each pair of the three treatments. The protein group had significantly higher values than the other two groups, whilst the carbohydrate values were significantly lower than in each of the other two groups. The largest difference was between the protein and carbohydrate groups, with a mean difference of 40-units in GLP-1 between groups.

APPENDIX 8 – STATISTICIAN REPORTS FOR ANALYSIS OF GIP, INSULIN, GLUCOSE AND GLUCAGON (UPPER GI METABOLOMICS STUDY)

Introduction

A study was performed whereby a group of 10 participants received three different treatments on three separate days. Measurements of hormone release over the course of 180 minutes post-treatment were made on each day. The aim of the analysis was to compare the hormone release values between the three treatments.

This second report examines the differences between the meals for other study outcomes.

Methods

The statistical methods used for the outcomes in this report are very similar to those described in Report 1, and thus a full description is not given.

One slight amendment to the previously described methods involves the baseline value. For these additional outcomes, the outcome was also measured 15 minutes pre-treatment. For meals where the baseline (time 0) value was missing, the -15 minute value was used as the baseline value for which to calculate change from baseline. The time 0 value was used, as previously described, where this was measured.

Results

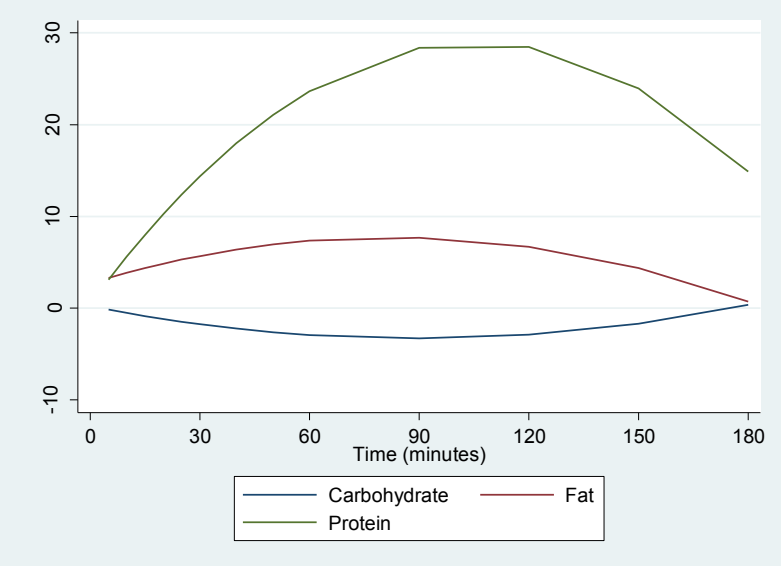
a) Glucagon

The first outcome analysed was changes in Glucagon over time.

The multilevel regression analysis suggested that the best model to evaluate the relationship over time for the different treatments included linear and squared terms for time, along with an interaction term between group and both the linear and squared terms for time.

The interaction term between treatment and time was statistically significant ($p<0.001$). This suggests that the size of differences between the three treatments varied over the study period. In other words, there was not a constant treatment difference.

A graphical illustration of the fitted relationships for each of the three treatments is shown in the subsequent graph.



The graph suggests relatively little changes in Glucagon over time for the fat and carbohydrate. However, for the protein group there was an increase in Glucagon values up to 90 minutes, with a slight decrease for the rest of the study period.

Due to the significant interaction between time and treatment, there was not a constant difference between the three treatment groups throughout the follow-up period. So, no single set of differences can be reported.

However, the treatment differences were quantified at two specific points in time, 90 and 180 minutes. The results of these analyses are summarised in the next table. The first figures shown the mean difference in Glucagon between the fat and protein groups relative to the carbohydrate group, along with corresponding confidence intervals. A series of p-values are also reported. The first indicates the overall significance between the three treatments. Subsequently p-values from each of the pairwise treatment comparisons are reported.

Statistic	90 minutes	180 minutes
Mean diff (95% CI): Fat - Carb	11 (4, 18)	0 (-8, 9)
Mean diff (95% CI): Protein - Carb	32 (25, 38)	15 (7, 22)
Overall p-value (*)	**<0.001**	**<0.001**
Fat vs. Carbohydrate p-value (+)	**0.006**	1.00
Protein vs. Carbohydrate p-value (+)	**<0.001**	**<0.001**
Protein vs. Fat p-value (+)	**0.04**	**0.003**

(*) Significance of the overall difference between the Carbohydrate, Fat and Protein
(+) P-value given a Bonferroni adjustment to allow for multiple comparisons

The results suggested that at both the 90 and 180-minute timepoints there was a significant overall difference between the three treatments.

At 90-minutes there was a difference in Glucagon between each pair of treatments. Glucagon values were significantly higher in protein groups than in each of the other two groups, whilst values were significantly lower in the carbohydrate group than in the other two groups. The largest difference was between the protein and carbohydrate groups, with a mean difference of 32-units in Glucagon between groups.

At 180-mins, outcomes values in the protein group were significantly higher than in the two other groups. Glucagon value in the protein group were, on average, 15-units higher than in the carbohydrate group. There was no significant difference in Glucagon between the fat and carbohydrate groups at this timepoint.

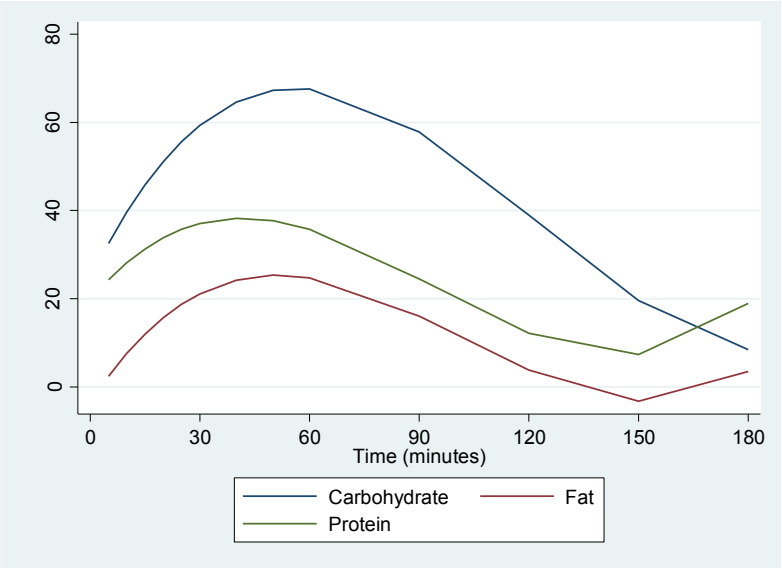
b) Insulin

Next the changes in insulin over time were evaluated in the three treatment groups.

The analysis suggested that the best model to evaluate the relationship over time for the different treatments included linear, squared and cubic terms for time, along with an interaction term between group and both the linear and squared terms for time.

The interaction term between treatment and time was statistically significant ($p<0.001$). This suggests that the size of differences between the three treatments varied over the study period. In other words, there was not a constant treatment difference.

A graphical illustration of the fitted relationships for each of the three treatments is shown in the subsequent graph.



The graph suggests that in all three groups there was an increase in Insulin from baseline up to around 50 minutes, after which there was a decrease for the rest of the study period. However, the increase and subsequent decrease was largest in the carbohydrate group.

Due to the significant interaction between time and treatment, there was not a constant difference between the three treatment groups throughout the follow-up period. So, no single set of differences can be reported.

However, the treatment differences were quantified at two specific points in time, 90 and 180 minutes. The results of these analyses are summarised in the next table. The first figures shown the mean difference in GLP-1 between the fat and protein groups relative to the carbohydrate group, along with corresponding confidence intervals. A series of p-values are also reported. The first indicates the overall significance between the three treatments. Subsequently p-values from each of the pairwise treatment comparisons are reported.

Statistic	90 minutes	180 minutes
Mean diff (95% CI): Fat - Carb	-42 (-53, -31)	-5 (-21, 12)
Mean diff (95% CI): Protein - Carb	-33 (-44, -22)	10 (-5, 28)
Overall p-value (*)	**<0.001**	0.18
Fat vs. Carbohydrate p-value (+)	**<0.006**	1.00
Protein vs. Carbohydrate p-value (+)	**<0.001**	0.62
Protein vs. Fat p-value (+)	0.43	0.22

(*) Significance of the overall difference between the Carbohydrate, Fat and Protein
(+) P-value given a Bonferroni adjustment to allow for multiple comparisons

The results suggested that at 90-minutes there was a difference in Insulin between both the fat and protein groups with the carbohydrate group, with outcomes values highest in the carbohydrate group. Values were, on average, 42-units lower for the fat treatment compared to carbohydrate, and 33-units lower in protein relative to carbohydrate. The Insulin values of protein and fat treatments did not differ at this timepoint.

At 180-mins, there was no overall significant difference between the three groups, or between any pair of groups.

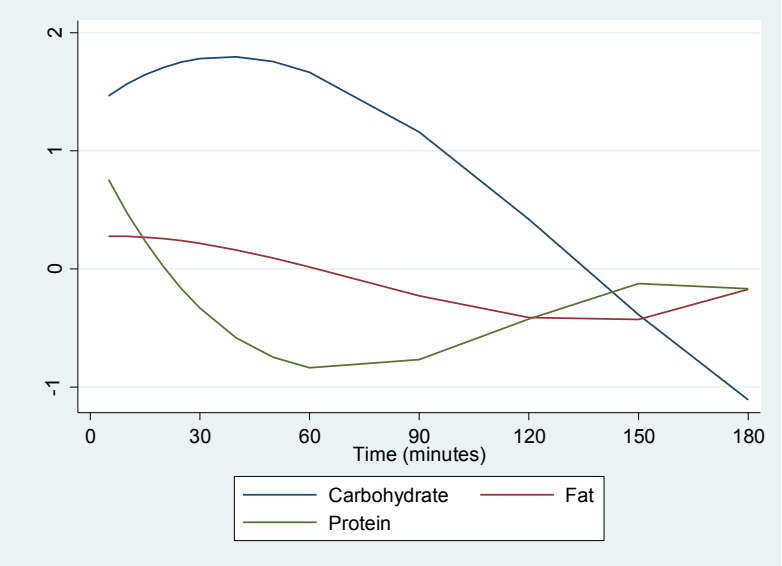
c) Glucose

The third outcome to be evaluated was levels of Glucose over time.

The multilevel regression analysis suggested that the best model to evaluate the relationship over time for the different treatments included linear, squared and cubic terms for time, along with an interaction term between group and all of the linear, squared and cubic terms for time.

The interaction term between treatment and time was statistically significant ($p<0.001$). This suggests that the size of differences between the three treatments varied over the study period. In other words, there was not a constant treatment difference.

A graphical illustration of the fitted relationships for each of the three treatments is shown in the subsequent graph.



The graph suggests that carbohydrate values rapidly increased from baseline, but fell to below their baseline by 180 minutes. The fat treatment also decreased values after an initial increase, whilst there was relatively little change over time in the fat group.

Due to the significant interaction between time and treatment, there was not a constant difference between the three treatment groups throughout the follow-up period. So, no single set of differences can be reported.

However, the treatment differences were quantified at two specific points in time, 90 and 180 minutes. The results of these analyses are summarised in the next table. The first figures shown the mean difference in GLP-1 between the fat and protein groups relative to the carbohydrate group, along with corresponding confidence intervals. A series of p-values are also reported. The first indicates the overall significance between the three treatments. Subsequently p-values from each of the pairwise treatment comparisons are reported.

Statistic	90 minutes	180 minutes
Mean diff (95% CI): Fat - Carb	-1.4 (-1.9, -0.8)	0.9 (0.1, 1.7)
Mean diff (95% CI): Protein - Carb	-1.9 (-2.5, -1.4)	0.9 (0.1, 1.7)
Overall p-value (*)	**<0.001**	**0.03**
Fat vs. Carbohydrate p-value (+)	**<0.001**	0.07
Protein vs. Carbohydrate p-value (+)	**<0.001**	0.05
Protein vs. Fat p-value (+)	0.14	1.00

(*) Significance of the overall difference between the Carbohydrate, Fat and Protein
(+) P-value given a Bonferroni adjustment to allow for multiple comparisons

At 90-minutes there was a significantly overall difference in Glucose between the three timepoints. Specific pairwise comparisons suggested that the carbohydrate group significantly varied from the two other groups, with significantly higher values in this group. The largest difference was a 1.9-unit difference between the protein and carbohydrate treatments. At this timepoint, Glucose values of the protein and fat groups did not significantly differ.

At 180-mins, there was a significant overall difference between the three groups. There was no strong evidence of a pairwise difference between any pair of the three groups. However, there was slight evidence, although not quite significant, that the Glucose values were higher in both the fat and protein treatment compared to the carbohydrate treatment.

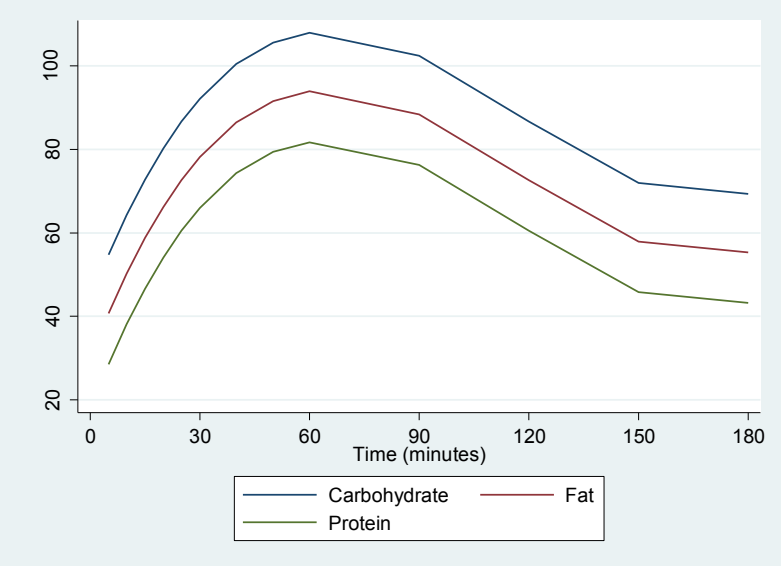
d) GIP

GIP was the final outcome assessed.

The multilevel regression analysis suggested that the best model to evaluate the relationship over time for the different treatments included linear, squared and cubic terms for time, along with an interaction term between group and all of the linear term for time only.

The interaction term between treatment and time was not found to be statistically significant (p=0.16). This suggests that the size of differences between the three treatments was fairly constant over the study period. As a result of the non-significant interaction, this term was removed from the final model, and a constant treatment difference assumed.

A graphical illustration of the fitted relationships for each of the three treatments is shown in the subsequent graph.



The graph suggests that for all treatments, the GIP values increased from baseline to 60 minutes, after which there was a slight reduction, although values at 180-minutes were above baseline values in all three treatment groups.

Due to the non-significant interaction between time and treatment, there was a constant difference between the three treatment groups throughout the follow-up period. Therefore, a single comparison between treatments using data from all timepoints can be made. The results of these analyses are summarised in the next table. The first figures shown the mean difference in GLP-1 between the fat and protein groups relative to the carbohydrate group, along with corresponding confidence intervals. A series of p-values are also reported. The first indicates the overall significance between the three treatments. Subsequently p-values from each of the pairwise treatment comparisons are reported.

Statistic	All timepoints
Mean diff (95% CI): Fat - Carb	-16 (-35, 1)
Mean diff (95% CI): Protein - Carb	-26 (-42, -10)
Overall p-value (*)	**0.006**
Fat vs. Carbohydrate p-value (+)	0.20
Protein vs. Carbohydrate p-value (+)	**0.003**
Protein vs. Fat p-value (+)	0.89

(*) Significance of the overall difference between the Carbohydrate, Fat and Protein
(+) P-value given a Bonferroni adjustment to allow for multiple comparisons

The results suggested that throughout the study period, there was a significant overall difference between the three treatments. However, there was only a specific difference between the protein and carbohydrate groups. GIP values in the protein group were significantly lower than in the carbohydrate group, by an average of 26-units. GIP values over the course of the study period were not significantly different in the fat treatment when compared to each of the other two treatments.

APPENDIX 9 – PRINCIPAL COMPONENT ANALYSIS

PCA is a statistical technique for determining patterns in data of high dimension. It is a multivariate technique that analyzes a data table in which observations are described by several inter-correlated quantitative dependent variables (Worley and Powers, 2013). PCA represents an unsupervised linear mixture model which aims to extract the important information from the table, to represent it as a set of new orthogonal variables called principal components, and to display the pattern of similarity of the observations and of the variables as points in maps, with an axis for each of the variables. These principal components represent vectors of metabolite contributions. All of the principal components are constructed such that they are pairwise orthogonal (decorrelated) to each other and ordered by the amount of variance they explain. A trajectory can be constructed to demonstrate whether there is clustering over time (in this case between the meals).

APPENDIX 10 – RELEVANT PUBLICATIONS DURING PHD

Alamshah A, Spreckley E, Norton M, Kinsey-Jones JS, **Amin A**, Ramgulam A, Cao Y, Johnson R, Saleh K, Akalestou E, Malik Z, Gonzalez-Abuin N, Jomard A, Amarsi R, Moolla A, Sargent PR, Gray GW, Bloom SR, Murphy KG (2017) L-phenylalanine modulates gut hormone release and glucose tolerance, and suppresses food intake through the calcium-sensing receptor in rodents. *Int J Obes* doi: 10.1038/ijo.2017.164

Alamshah A, McGavigan AK, Spreckley E, Kinsey-Jones JS, **Amin A**, Tough IR, O'Hara HC, Moolla A, Banks K, France R, Hyberg G, Norton M, Cheong W, Lehmann A, Bloom SR, Cox HM, Murphy KG (2016) L-arginine promotes gut hormone release and reduces food intake in rodents. *Diabetes Obes Metab* 18(5): 508-18

McGavigan AK, O'Hara HC, **Amin A**, Kinsey-Jones J, Spreckley E, Alamshah A, Agahi A, Banks K, France R, Hyberg G, Wong C, Bewick GA, Gardiner JV, Lehmann A, Martin NM, Ghatei MA, Bloom SR, Murphy KG (2015) L-cysteine suppresses ghrelin and reduces appetite in rodents and humans. *Int J Obes* 39(3): 447-55