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Effects of gut hormones, glucagon-like peptide-1 and desacyl ghrelin, on eating behaviour in obesity and ex-smokers

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To Dad

ABSTRACT

Introduction: Unhealthy eating behaviour is more prevalent in obesity and contributes to weight regain after dieting. Smoking cessation weight gain, a common reason for relapse to cigarettes, also has adverse health consequences. Gut hormones, such as GLP-1 and desacyl ghrelin (DAG), reduce appetite and weight in obesity and Prader-Willi syndrome. GLP-1 and ghrelin signalling systems modulate central reward networks for food and nicotine in preclinical and human studies. However, the impact of GLP-1 and DAG on neurocircuitry involved in eating behaviour in obesity and ex-smokers remains unclear therefore further insight is needed to guide clinical use of gut hormones in prevention of weight gain during dieting and smoking cessation.

Aims: Here, the effects of acute administration of GLP-1 analogue, Exenatide or DAG was explored in dieting adults with obesity, or in abstinent nicotine-dependence (double blind randomised placebo controlled cross-over design), on food cue responsivity using fMRI in reward-processing regions, food intake, food reward and appetite.

Results: In dieting group with obesity, both Exenatide and DAG increased BOLD signal to high-energy (HE) food pictures in prefrontal cortex regions, implicated in inhibitory control. In contrast, in ex-smokers, both Exenatide and DAG decreased BOLD signal to HE food pictures in the mesolimbic reward-processing regions and prefrontal cortex, suggesting a reduction in anticipatory food reward with a concomitant decrease in executive control. With Exenatide, there was also a reduction in HE food appeal, food intake and appetite ratings in both groups. With DAG, there was no overall effect on HE food appeal, food intake or appetite ratings in both groups.

Conclusion: These findings are in accord with the possibility that Exenatide and DAG could be used in prevention of smoking cessation weight gain. This experimental medicine study has provided pilot data for a larger clinical study to trial these gut hormones as potential therapies in smoking cessation.

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Lastly, I would like to dedicate this PhD thesis to my father, Dr Y.S. Ling. He has always been my source of inspiration and his strive for excellence has moulded me into the person I am today. For this, I am forever grateful.

DECLARATION OF ORIGINALITY

The majority of the work described in this thesis was undertaken by the author. All collaboration and assistance is detailed below:

1. Dose-finding phase was performed by Dr Tony Goldstone, Dr Sri Akavarapu, Dr Nienke Pannekoek and Miss Barbara Kobson.
2. Clinical study visits involving Exenatide and DAG infusions were performed in collaboration with Dr Tony Goldstone, Dr Sri Akavarapu, Dr Katherine Herlinger, Dr Moaz Al-Lababidi, Dr Liam Nestor, Dr Nienke Pannekoek, Dr Federica Vanelli, Miss Barbara Kobson, Miss Felicity Aiano, Miss Silvia Moreira, Mr Jake Dagen, Miss Natalie Ertl, Miss Silvia Canizares and Miss Pallavi Chhibba.
3. Preparation and testing of the DAG peptide were performed with the assistance of Dr James Minnion.

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ABBREVIATIONS

5HT2c	5-hydroxytryptamine-2c
AAT	Approach avoidance task
ACC	Anterior cingulate cortex
AG	Acyl ghrelin
AgRP	Agouti-related peptide
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARC	Arcuate nucleus
AUC	Area under curve
AUDIT	Alcohol Use Disorders Identification Test
BAS/BIS	Behavioural Activation System / Behavioural Inhibition System
BDI-II	Beck Depression Inventory
BES	Binge Eating Scale
BMI	Body mass index
BOLD	Blood oxygen level dependent
CART	Cocaine-and Amphetamine-regulated transcript
CB-1	Cannabinoid
CNS	Central nervous system
D2	Dopamine (receptor)
DAG	Desacyl ghrelin
DEBQ	Dutch Eating Behaviour Questionnaire
dlPFC	Dorsolateral prefrontal cortex
DSM-5	Diagnostic and Statistical Manual of Mental Disorders (5th edition)
EMA	European Medicines Agency
FDA	Food and Drug Administration
FDR	False discovery rate
FEAT	FMRI Expert Analysis Tool
FOV	Field of view
fMRI	Functional magnetic resonance imaging
fMRIB	Functional magnetic resonance imaging of the brain
fROI	Functional region of interest
FSL	FMRIB Software Library
FTND	Fagerstrom Test for Nicotine Dependence
GABA	Gamma aminobutyric acid
GH	Growth hormone
GHADD	Gut Hormones in ADDiction
GHSR1a	Growth hormone secretagogue receptor-1a
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1R	Glucagon-like peptide-1 receptor
GLM	General linear model
GRAPPA	Generalised autocalibrating partial parallel acquisition
HE	High-energy
HOMA-IR	Homeostatic Model Assessment-Insulin Resistance

IFG	Inferior frontal gyrus
IHD	Ischaemic heart disease
LEAP2	Liver-expressed antimicrobial peptide 2
LSD	Least significant difference
MFG	Middle frontal gyrus
MPRAGE	Magnetisation prepared – rapid gradient echo
NAcc	Nucleus accumbens
nAChR	Nicotinic acetylcholine receptor
NIHR	National Institute for Health Research
NPY	Neuropeptide-Y
NTS	Nucleus of solitary tract
OFC	Orbitofrontal cortex
PANAS	Positive affect and Negative Affect Schedule
PFC	Prefrontal cortex
PFS	Power of Food Scale
PHG	Parahippocampal gyrus
POMC	Pro-opiomelanocortin
POMS-2	Profile of Mood States
PPG	Pre-proglucagon
PRT	Progressive ratio task
PSS	Perceived Stress Scale
PWS	Prader-Willi syndrome
PYY	Peptide YY
REE	Resting energy expenditure
RF	Radiofrequency
RR	Relative risk
RT	Reaction time
RYGB	Roux-en-Y gastric bypass
SEM	Standard error of mean
SFG	Superior frontal gyrus
SMA	Supplementary motor area
SPSS	Statistical package for the social sciences
SSAI	Spielberger State Anxiety Inventory
STAI	Spielberger Trait Anxiety Inventory
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TE	Time to echo
TFEQ	Three Factor Eating Questionnaire
TR	Repetition time
UK	United Kingdom
UPPS-P	Urgency, Premeditation, Perseverance, Sensation seeking, and Positive urgency
US	United States of America
VAS	Visual analogue scale
vCA1	Ventral hippocampal field CA1
VTA	Ventral tegmental area
WTAR	Wechsler Test of Adult Reading
YFAS	Yale Food Addiction Scale

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CHAPTER 1:

GENERAL INTRODUCTION

1.1 OBESITY

1.1.1 Introduction

Obesity is a major public health problem and leads to type 2 diabetes mellitus (T2DM), cardiovascular, psychiatric, musculoskeletal and oncological comorbidities. Obesity is defined as a body mass index (BMI) of 30 kg/m^2 or higher. Over the years, the increasing prevalence of obesity and associated illnesses pose a huge strain on health services and society. Alarmingly, according to the National Health System (NHS) publication of statistics on obesity in England, 29% of adults and 20% of Year 6 children were obese in 2018. 10,660 hospital admissions were directly attributable to obesity, with an additional 711,000 in which obesity contributed to the hospital admission, a rise of 15% from the previous year [1]. If the prevalence of obesity continues to rise at the current rate, close to half the adult population in the world would be overweight or obese by 2030 [2]. The NHS spends at least £5.1 billion annually on the cost of obesity and related comorbidities, however this is likely to be an underestimate as the data was based on 2004-2005 and had not taken in account the increasing prevalence [3]. A more recent report from London School of Economics estimated in the UK the cost of treatment for T2DM, a significant comorbidity of obesity, in 2012 to be £11.9 billion [4]. The overall cost to society, including the cost of lost economic productivity and the investment required to prevent and tackle obesity, was even greater and was estimated to be in the region of £40 billion a year in 2012 [5].

A sedentary lifestyle and the abundance of affordable high-calorie food contributes to this worrying trend. The mainstay management of obesity is to optimise medical management with lifestyle improvements, medications and bariatric surgery. According to National Institute for Health and Care Excellence (NICE) guidelines on obesity management, healthcare professionals should support patients with obesity in their weight management by advising behavioural interventions, such as self-monitoring of weight, goal setting, slowing rate of eating, encouraging physical activity and low-calorie diets (800-1600kcal/day) [6]. In some, pharmacological treatment may be initiated but options are limited. Bariatric surgery is an effective treatment for obesity yielding good long-term post-operative results but is only available to a subset of patients with obesity who meet the NICE criteria. In view of drug safety, surgical risks and complications, lifestyle modification by improving diet and exercise is preferable. Undoubtedly, there are other barriers to the management of obesity. These

include the general lack of awareness of the impact of obesity, the perception within the medical community for the need to treat and the sparsity of effective treatment options [7].

1.1.2 Control of body weight

Energy intake is metabolised to fuel basal metabolism, thermogenesis and energy expenditure and any excess is stored as fat in adipose tissue. Steady-state body weight is regulated by homeostatic, environmental and behavioural processes [8]. These neurohormonal homeostatic signals involve several nuclei with the hypothalamus, the brainstem, and corticolimbic system. There are various central neuropeptides, including orexigenic neuropeptide Y and agouti-related peptide (NPY/AgRP), and anorexigenic pro-opiomelanocortin and cocaine-and-amphetamine-regulated transcript (POMC/CART) neurons, and peripheral hormones, including orexigenic acyl ghrelin (AG), and anorexigenic glucagon-like peptide-1 (GLP-1), peptide YY (PYY), insulin and leptin, which all convey information about food intake and energy stores between the brain and peripheral tissues [8]. Interplay of these neuropeptides and hormones bring about changes in appetite and satiety.

Additionally, environmental factors influence energy intake and impact food-related reward, mood, emotions and memory. The increased availability of energy-dense foods and a high-stress society coupled with decreases in physical activity, stimulates food intake and contributes to obesity [9].

Although some people are able to lose substantial amounts of weight in the short-term, the main challenge is to maintain weight loss over a long term [10, 11]. Less than 10% are able to maintain a significant weight loss for 5 years or more [12]. This remains elusive due to a myriad of metabolic, neural and behavioural reasons [13, 14]. There are persistent physiological adaptations that encourage weight regain to compensate for a negative energy balance. For instance, resting energy expenditure decreases following weight loss which in turn contributes to weight regain [15]. Orexigenic stimuli, such as increase in plasma acyl ghrelin (AG) and decrease in plasma leptin, promote appetite to increase intake and replenish energy stores following weight loss [8, 16]. Having a more sedentary lifestyle, greater daily screen time and unhealthy eating behaviours, including disinhibited eating and stress eating,

are associated with weight regain [11, 17]. On the other hand, successful weight maintenance is associated with greater initial weight loss, achieving a goal weight, having a physically active lifestyle and regular meal patterns, as well as control of over-eating and better ability to handle stress [17]. Understanding these compensatory mechanisms in individuals who are dieting could help target behavioural and pharmacological strategies to prevent weight regain and predict their efficacy [18-20].

1.1.3 Regulation of food intake

Food intake is generally influenced by the energy homeostatic processes as well as hedonic consumption. The regulation of food intake through homeostasis is mediated by endocrine hormones and gut-brain signalling activated via the autonomic nervous system [21]. For instance, changes in the concentrations of nutrients and gut hormones, including anorexogenic hormones GLP-1 and PYY, and orexigenic hormone AG, as well as inputs from vagal nerves sensing gastric distension, all contribute to feeling satiated after food intake [22]. Other endocrine hormones such as leptin from adipose tissues and insulin from the pancreas inform the brain about energy homeostasis in the longer term [23].

In the brain, signals from the vagal afferents are transmitted to the nucleus of the solitary tract (NTS) in the brainstem before projecting to the hypothalamus where information from the peripheral hormones are integrated and processed [24]. An important area of the hypothalamus that is implicated in appetite control is the arcuate nucleus (ARC). There are two discrete sets of neurons in the ARC: NPY/AgRP expressing neurons and POMC/CART expressing neurons. Stimulation of POMC/CART neurons results in anorexigenic effects while NPY/AgRP neurons exerts orexigenic effects. The blood brain barrier is more permeable around the ARC therefore allowing rapid detection of and response to the fluctuations in nutrients and hormone signals in circulating blood by ARC [25].

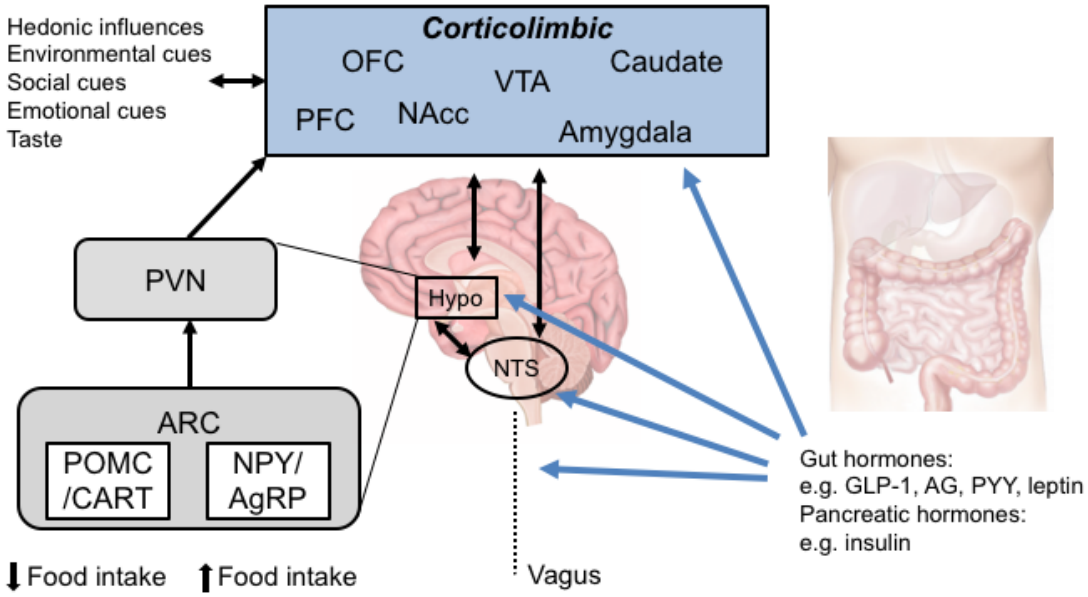


Figure 1.1. Regulation of food intake by gut-brain axis.

Actions of gut and pancreatic hormones (blue arrows) directly on NTS, hypothalamus, corticolimbic system and indirectly via vagus nerve. Interactions between various appetite-regulating brain areas (black arrows). Figure adapted from [26] and [27]. Abbreviations: AG, acyl ghrelin; ARC, arcuate nucleus; GLP-1, glucagon-like peptide-1; Hypo, hypothalamus; NAcc, nucleus accumbens; NPY/AgRP, neuropeptide Y/agouti-related peptide; NTS, nucleus of solitary tract; OFC, orbitofrontal cortex; POMC/CART, pro-opiomelanocortin/cocaine and amphetamine-regulated transcript; PFC, prefrontal cortex; PVN, paraventricular nucleus; PYY, peptide YY; VTA, ventral tegmental area.

On the other hand, homeostatic feeding can be overridden by other signals, for instance through neural circuits in the mesocorticolimbic system. They are responsible for encoding the incentive value of food cues and motivating reward-seeking behaviours [28, 29]. An increased salience to food or enhanced motivation to eat is referred to as food reward [30]. Several neurohormonal systems in the brain, including the dopaminergic, opioid, serotonin and cannabinoid pathways, have a major role in driving food reward [31]. These reward-related brain regions, including NAcc, caudate, putamen, insula, OFC, amygdala, and hippocampus, integrate various environmental and emotional factors to regulate hedonic eating which can be triggered by visual food cues and stress [32]. Furthermore, the striatum (NAcc, caudate and putamen) is involved in the anticipation of not only food-, as well as monetary- and drug-related rewards [33, 34]. Clinical studies have demonstrated that stress, negative emotions and hunger can modulate the food reward brain responses, for instance in the striatum, and contribute to overeating and obesity [35].

1.1.4 Pharmacological treatment options

Understanding the mechanisms that regulate food intake can provide guidance for developing drug therapies for obesity. Currently, the only pharmacological options available in the UK are Orlistat, a lipase inhibitor, which prevents gastrointestinal absorption of dietary fats [36], and glucagon-like peptide-1 (GLP-1) analogues, such as Exenatide and Liraglutide, which induce satiety, stimulate insulin secretion and improve insulin sensitivity [37-39] (See section 1.7 for further discussion). The clinical benefit in terms of weight loss and prevention of weight regain with orlistat, compared to placebo, is significant but minimal [36, 40]. With GLP-1 analogues, the mean weight loss with GLP-1 analogues is 4-11% greater than with placebo [41], making it more efficacious. However, the adherence to these treatment agents is limited by their gastrointestinal side effects. Commonly, these include nausea, vomiting, bloating and diarrhoea. In addition, another limitation of the widespread use of GLP-1 analogues is that all of them have to be administered via a subcutaneous injection, with the exception of the newer oral semaglutide.

In the US, the combination bupropion-naltrexone drug is also licensed for weight loss. Bupropion, a dopamine and noradrenaline reuptake inhibitor, is used to treat smoking cessation while naltrexone, an opioid receptor antagonist, is used in alcohol addiction treatment. The side effects include headache, blurred vision, dizziness and depression, most of which are likely to be mediated by central brain receptors.

The serotonin system is involved in rewarding and aversive processing, hedonic experience as well as mood [42]. Lorcaserin, a 5-HT2c receptor agonist, was previously licensed for weight loss. It exerted its anorexogenic effects by binding to serotonin receptors in the hypothalamus. It had marginal efficacy according to Food and Drug Administration (FDA) and had raised concerns with European Medicines Agency (EMA) regarding a possible long-term increased risk of depression and cancer [43]. Another anti-obesity drug which was removed from the US and European markets was Sibutramine, a serotonin and noradrenaline uptake inhibitor. This was a result of an increased risk of cardiovascular disease and death in treated patients [44]. Previously, Rimonabant, a cannabinoid (CB-1) receptor antagonist, had been licensed as a weight loss medication before being suspended due to risk of severe psychiatric problems, such as depression [45]. However, all three withdrawn medications were thought

to action their weight loss effects through the central nervous system (CNS), indicating the potential to influence food intake and satiety by targeting brain pathways involved in food intake.

1.1.5 Overlap between obesity and addiction

Judging from the limited pharmacological treatment options available, there is a clear need for novel anti-obesity therapies. As mentioned, anti-addiction medications, such as bupropion and naltrexone, are used as weight loss medication. This is in line with the idea that there are links between natural rewards from food and addictive substances through involvement of similar brain pathways, particularly the mesocorticolimbic dopaminergic system [46, 47]. Neuroimaging studies have demonstrated similar impairments in dopaminergic pathways between obesity and drug addiction [48]. There is an overlap of behavioural traits such as attentional bias toward relevant cues, impulsivity and increased stress responsivity, that are enriched in obesity as well as substance addiction.

The connection between obesity, food reward and addiction is supported by observations that obesity is also associated with an increased risk of taking up smoking and smoking frequency [49]. In the UK Biobank study, associations of BMI with measures of cigarette exposure appeared to be primarily driven by single nucleotide polymorphisms clustering in neuronal pathways, suggesting a common biological basis for addictive behaviours [48-50]. Arguably, there are also other factors that would contribute to this phenomenon. For instance, smoking could be used as a weight reduction strategy.

Another interesting observation is that a significant minority of patients who have undergone Roux-en-Y gastric bypass bariatric surgery develop new-onset alcohol use disorders, but not generally in those following laparoscopic adjustable gastric banding [51, 52]. This could arise from “addiction transfer” where one form of addiction (i.e. food) is substituted for another new addiction (i.e. alcohol). Taken together, these support the theory that there is overlap of brain pathways that modulate addictive behaviours to different rewards. Hence, by targeting these brain neural networks, new potential therapies for obesity and other addictions could be developed.

1.2 FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI) IN FOOD REWARD

Neuroimaging techniques have greatly facilitated the investigation of underlying neural circuits involved in appetite and reward processing. The greater insight into the brain mechanism underpinning obesity and addiction offers an important opportunity to develop new therapies, including gut hormones, for these diseases. Increasingly, it is possible to examine the brain response in reward processing regions to food-related cues and influence of addictive behaviour traits on food cue reactivity using non-invasive functional MRI (fMRI) in humans [53]. With obesity, there is a considerable body of evidence from fMRI studies demonstrating a dysregulated reward and motivation system that contributes to unhealthy eating behaviour and weight regain [54-56]. Therefore, through the use of fMRI, the effects of novel treatments on modulating brain reward pathways in various participant groups can be rapidly evaluated and can be an indicator of its potential clinical efficacy [57, 58].

1.2.1 BOLD signal in fMRI

During an MRI brain scan, a magnetic field aligns the protons longitudinally in water molecules in the body. An external radio frequency (RF) pulse is delivered which disrupts the alignment of the protons by transference of energy ('resonance'). A new transverse MRI signal is induced through protons rotating in sync, which can be recorded. Once the RF pulse is switched off, all the protons 'relaxes' and resume longitudinal alignment. During this relaxation period, the longitudinal magnetisation increases back to original (T1 time constant) and transverse magnetisation concomitantly decreases to null (T2 time constant). The number of protons in the various brain tissues determines the difference in T1 and T2. By selecting a suitable TR (time to repeat) and TE (time to echo), the repeated RF waves (pulse sequence) can demonstrate the difference in MRI signal intensity between the brain tissues thereby creating T1- or T2-weighted images of the whole brain across time [59].

In addition, fMRI blood oxygen level dependent (BOLD) signal adds a dynamic dimension to the MRI brain images. Simply, the BOLD signal measures regional cerebral blood flow in the brain, which is indirectly indicative of regional neural activity and energy demand (Figure 1.1). Typically, after a stimulus (e.g. food picture), there is approximately a 2 sec delay in the onset of a haemodynamic response, which is the time taken for blood to pass through the arteries

to capillaries and veins [60]. Thereafter, the BOLD signal reaches a plateau after 6-12 sec before returning to baseline. This BOLD signal enhancement usually reflects an increase in cerebral blood flow that oversupplies the brain region with oxygenated blood [60]. In general, the increase in BOLD signal during fMRI task is presumed to be an increased functionality of the brain region through this neurovascular coupling.

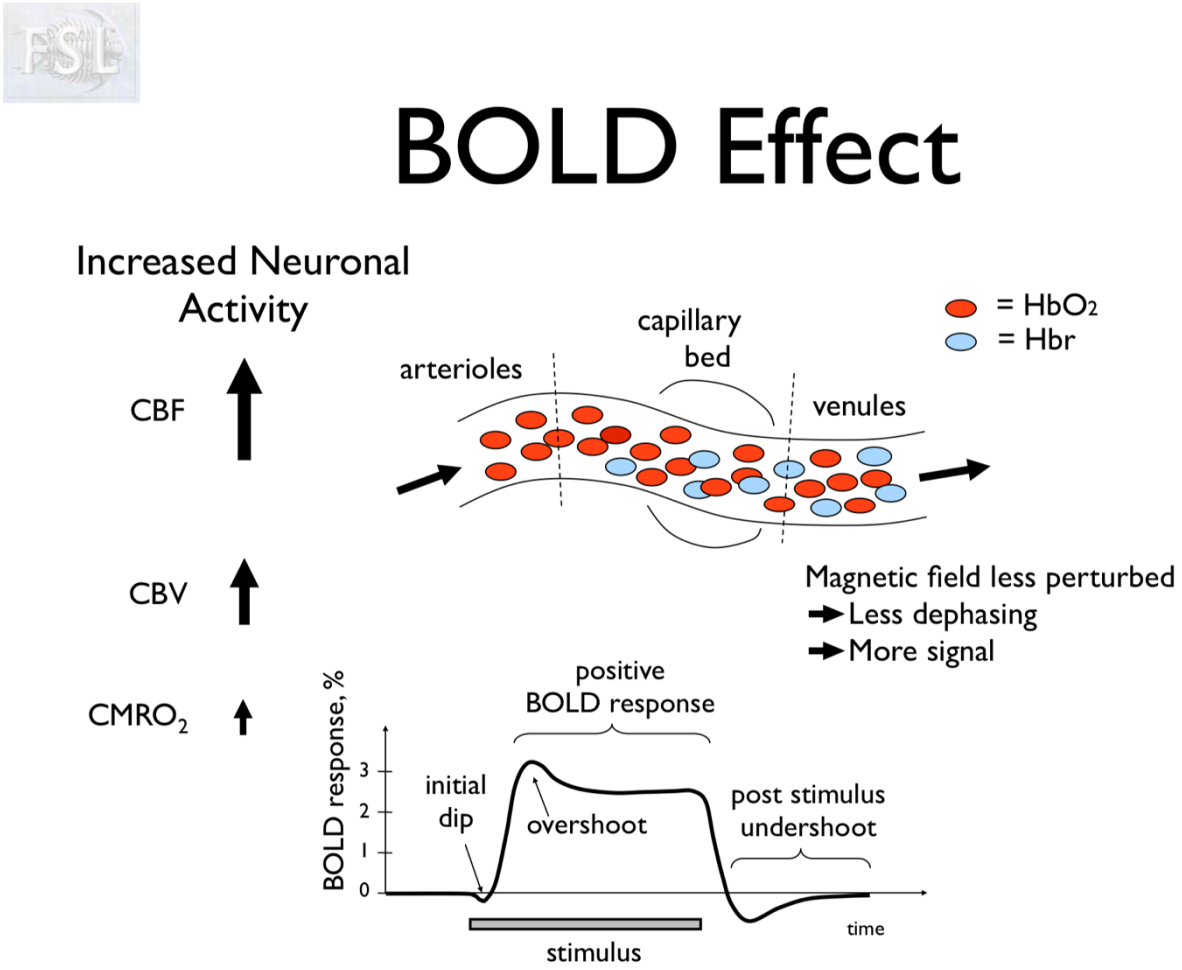


Figure 1.2 fMRI BOLD signal response.
Increased neural activity leads to increases in regional cerebral blood flow and decreases to the deoxy-haemoglobin (paramagnetic) to oxy-haemoglobin (diamagnetic) ratio. This results in less perturbance to magnetic field and higher BOLD signal. Abbreviations: BOLD, blood oxygen level dependent; CBF, cerebral blood flow; CBV, cerebral blood volume; $CMRO_2$, cerebral metabolic rate of oxygen; HBO_2, oxy-haemoglobin; Hbr, deoxy-haemoglobin. Figure taken from fMRI of the Brain (FMRIB) Software Library (FSL) [61].

Many of the fMRI studies are task-based subtraction analyses. For instance, in a high-energy (HE) food picture task, the BOLD signal when viewing HE food picture is contrasted against the BOLD signal when viewing neutral pictures in an individual and this difference in BOLD

signal across individuals within a group forms the group brain activation pattern [23]. This can be related to the salience and cravings for food depending on the brain regions involved in the particular task. For instance, when viewing images of HE foods, reward- and emotion-processing brain regions such as the nucleus accumbens (NAcc), caudate, insula, orbitofrontal cortex and amygdala are activated [62]. Alterations in the reward processing of food cues in these brain regions may contribute to dysregulated food intake and predispose to obesity. Another advantage of utilising fMRI is the ability to combining quantitative BOLD signal changes in the task with hormonal blood analyses to draw links between the gut-brain axis. Indeed, fMRI neuroimaging is a non-invasive way of bridging the gap between large body of animal studies in the realm of obesity and addiction research with human studies.

1.2.2 Regions of Interest (ROI) in Food reward

There are a few important mesocorticolimbic brain areas implicated in food reward processing but exactly how they contribute to drive behaviour remains to be fully understood (Figure 1.2). Altered reward sensitivity to food cues in the NAcc, caudate and putamen contributes to overeating and predisposes to obesity and eating disorders [63]. By overriding hypothalamic inputs, frontostriatal regions, implicated in emotional and cognitive control, can also disrupt the homeostatic energy balance and give rise to aberrant eating behaviours [56]. Therefore, a better understanding of how these neural correlates are modulated with gut hormones in the context of obesity and abstinent smokers may help inform potential new therapies in obesity and smoking cessation.

Nucleus Accumbens

The NAcc, along with the olfactory tubercle, forms the ventral striatum and extends dorsolaterally into the putamen and dorsomedially into the caudate [64]. The NAcc receives dopaminergic projections from the ventral tegmental area (VTA) as well as glutamatergic projections from the amygdala, prefrontal cortex and hippocampus [65]. Dopamine is an essential neurotransmitter in the brain that is associated with reward behaviour and craving [66]. The main efferent neurons project to the basal ganglia, pallidal complex, lateral habenular nucleus, lateral hypothalamus and amygdala [65]. Importantly, NAcc with its network to other brain structures, is frequently implicated in feeding behaviour [67],

motivation [68], reward sensitivity [69], learning [70], impulsivity [71] and risk-taking behaviours [72].

Caudate and Putamen

The dorsal striatum consists of the caudate and putamen and, together with the ventral striatum, they form an integral part of the subcortical basal ganglia. The dorsal striatum receives dopaminergic inputs from substantia nigra and other areas including orbitofrontal cortex, somatosensory and motor regions. It plays an important role in decision-making [73] and impulse control [74]. The caudate is thought to use the working memory to anticipate outcomes from certain actions and guide goal-directed actions in the future while the putamen is associated with formation of habitual decisions regardless of outcomes [75, 76]. There can be a bidirectional transition from goal-directed behaviour to habitual behaviour and beyond that, addictive and compulsive behaviour ensues [77].

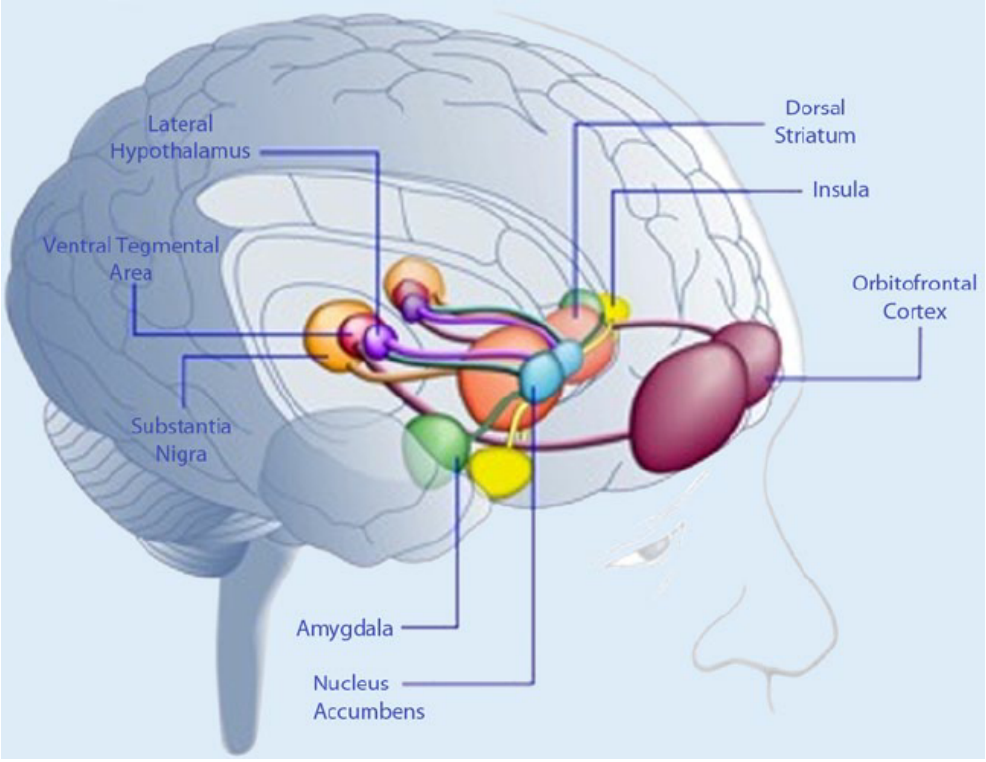


Figure 1.3. Brain regions implicated in food reward processing.
The nucleus accumbens and dorsal striatum regulates the motivation and incentive properties of food mediated through dopaminergic inputs from the ventral tegmental area and substantia nigra. The orbitofrontal cortex and amygdala encode information relating to saliency of food reward. The insula processes information related to hedonic value of food. Figure taken from [62]

Prefrontal cortex

The prefrontal cortex (PFC) lies in the frontal lobe of the brain and can be divided into various functional regions including orbitofrontal cortex (OFC) and dorsolateral PFC (dlPFC). The OFC is a locus for inhibitory control [78, 79], cost-benefit decision-making for reward [80] and encodes subjective value to rewards [81]. It plays a role in predicting specific outcomes that follow behavioural choices [82]. Lesions to this area typically result in behavioural rigidity and impairment of conditioned stimulus response [83, 84].

On the other hand, dlPFC purportedly controls behaviour on the basis of reward value [85] and decision-making specifically in the context of an unknown outcome or risk-taking behaviour [86, 87]. It is often implicated in regulating emotions. Lesions to this area caused short-term memory deficits [88].

Hippocampus

The hippocampus, an important component of the limbic system, is located in the medial temporal lobe. It is implicated in the formation of long-term memory and spatial memory and encodes information about our surroundings that facilitates flexible cognitive processes. Neurons from the hippocampus project into the NAcc [89] and PFC [90]. Lesions to this area bilaterally results in anterograde amnesia [91]. In contrast, reward-based learning was associated with neural activity in the hippocampus which ensued in a goal-directed response [92]. Unsurprisingly, there was hippocampal activation in response to food pictures during fMRI, indicating its role in modulating eating behaviour [93].

Amygdala

Considered part of the limbic system, the amygdala is located deep and medially in the temporal lobes of the brain. Classically it is involved in aversive learning and Pavlovian fear conditioning [94]. It is also associated with emotion and motivation and is essential for processing rewarding stimuli with regards to encoding valency and saliency [95]. It consists of glutamatergic neurons and receives inputs from sensory cortex and thalamus about the environment. Projections from the amygdala then connects with cortical regions, including the OFC, hippocampus, the striatum, especially NAcc, to subsequently mediate behaviour [96,

97]. Lesions to the amygdala not only prevent fear conditioning but also Pavlovian appetitive conditioning to rewards [98].

Insula (insular cortex)

The insula is situated in the cerebral cortex located deep within the lateral sulcus and the anterior part in particular forms part of the limbic system. It has a role in emotional perception and the interpretation of internal and external cues to elicit a behavioural response [99, 100]. It is closely connected with the amygdala, somatosensory cortex, OFC and the thalamus [101]. In human fMRI studies, the decreased activation of the anterior insula to food cues in obesity suggested reduced response to the anticipation of food such that it may predispose to overeating [55].

Anterior cingulate cortex

The anterior cingulate cortex (ACC) surrounds the frontal part of the corpus callosum and forms connections with the PFC and limbic system via dopaminergic and serotonergic pathways [102, 103]. It is thought to have an important role in the regulating emotions [103, 104], decision-making [105], error detection [106] and cost-benefit analysis of rewards [107].

1.2.3 Use and limitations of fMRI in drug development

In the past 20 years, the use of fMRI in drug development has enhanced the process of developing novel therapeutic drugs for some diseases. fMRI is a safe and non-invasive method of investigating drug effects on brain mechanisms, and can have utility in confirming CNS actions of manipulations and potential drugs, especially useful in early go-no go decisions in the drug development pathway [108]. However, there are certain limitations of utilising fMRI in the pharmacological development of de-novo agents. Firstly, there are difficulties in designing a highly reproducible fMRI paradigm to test a particular condition or pharmacological agent. Secondly, fMRI BOLD signals can be easily affected by artefacts, non-specific changes in neurovascular coupling induced by the drug, resulting in incorrect conclusions. Similarly, different methods of analysing fMRI results can lead to disparate and inaccurate results. Finally, directly relating changes in BOLD signal to clinically relevant outcomes can be problematic. These issues create a challenge to a more widespread use of fMRI in drug development [109].

1.3 EATING BEHAVIOUR

Eating behaviour encompasses all aspects of the relationship with food, including, food choice, food preference and hedonic response that influence food intake [110]. It is dictated by a complex interaction between genetic, physiological, environmental, psychological, cultural and socioeconomical factors [111, 112]. For instance, eating behaviour will be strongly influenced by the availability of food due to costs or accessibility [113], and changes in lifestyle with reduction in meal preparation time and cooking skills [114]. While some of these factors may not be easily modifiable, improvements in eating behaviour could significantly aid weight management in the overweight and obese population and mitigate the rising trend of obesity.

Measuring different facets of eating behaviour can be done through the use of questionnaires relating to appetite, preference and intake, the provision of test meals to study food intake and behavioural tasks assessing hedonic value of food. However, there are limitations to these methods. The key one being that in research studies the experimental situation does not simulate the usual environment, food choices or portions sizes people are used to in reality, so eating behaviour observed over a short term may differ with the norm [111].

1.4 EATING BEHAVIOUR IN OBESITY

1.4.1 Behavioural traits associated with obesity

Certain behavioural traits are more commonly found in obesity and can influence food choices and BMI. Generally, obesity is associated with an increased motivational drive to eat, an attention bias towards food images and food approach bias [115, 116], enhanced reward responses to food cues and impaired food-related inhibitory processes [63], thus increasing susceptibility to overeating. Attentional bias to food cues has been suggested to be associated with higher BMI and hunger although some studies contradicted this [117-119]. Similarly, an automatic approach bias to food cues is associated with self-reported restraint, external eating, emotional eating scores and obesity [115, 120-123]. Taken further, modifying this approach-avoidance bias to unhealthy food cues has been explored as a potential behavioural therapy for obesity [124, 125]. After receiving training to avoid unhealthy food in the Approach-Avoidance Task (AAT), participants with obesity demonstrated lower BOLD fMRI

activation in the right angular gyrus, which has a role in processing social cues and resolution of stimulus-response conflicts, but not in any reward-processing areas [126]. This suggests that brain responses to food cues can also be altered with behavioural interventions.

Unhealthy eating behaviours, such as emotional eating and restrained eating, were more prevalent in obesity and contributed weight loss failure after dieting [127]. Emotional eating, an overconsumption of food in response to negative emotions, is positively associated with BMI [128]. Indeed, emotional eating alters brain responses and is associated with increased BOLD activation in the insula to food cues in lean and obesity and in the amygdala, OFC and insula in people with T2DM [129]. In addition, impulsivity is associated with overeating, eating disorders and weight status and can result from impaired executive functioning [130-132]. Impulsivity specifically towards food, is increased in obesity, especially in those with Binge Eating Disorder [133]. Greater impulsivity is also associated with decreased caudate BOLD response to consumption of palatable food [134].

Food addiction, as measured using the Yale Food Addiction Scale (YFAS), is positively correlated to BMI and females are at a higher risk of food addiction [135]. Food addiction scores decreases following bariatric surgery [136]. Females with moderate-to-severe YFAS food addiction exhibited enhanced superior frontal gyrus BOLD responses (cue-induced craving) to food cues compared to weight-matched controls [137]. Furthermore, stress, whether psychological or physical, is intricately linked to the development and maintenance of obesity by the loss of self-regulation towards unhealthy food choices and increase in stress-induced eating [138]. Psychosocial stress and personality is also associated with enhanced response in the ventromedial PFC and amygdala, leading to an increased intake of palatable foods and weight gain in stress-reactive people [139]. Enhancing cognitive dietary restraint through interventional strategies is suggested to be a method to prevent weight regain by limiting food intake and overcoming the homeostatic changes that enhances appetite and decreases metabolic rate [8, 140].

1.4.2 fMRI studies relating to food in obesity

In response to visual food cues, participants with obesity, with or without T2DM, demonstrate increased brain activation in the appetite- and reward-related regions (NAcc, insula, amygdala

and orbitofrontal cortex (OFC)) when compared to lean subjects usually in the fasted condition [141-144], suggesting a possible reason for excessive eating due to altered reward processing in the brain. High-calorie food compared to low-calorie or neutral images also invoke a significantly larger BOLD reactivity in similar reward brain regions, including striatum, frontal, OFC and visual cortex [142, 145].

After a meal, there was also increased BOLD activation in response to food cues in obesity compared to lean subjects in brain regions implicated in decision making (OFC and caudate), reward anticipation (putamen, ACC and OFC) and emotional processing (insula, caudate and amygdala) [146-149]. The disparity between postprandial neural processing of food cues in obesity and lean subjects may drive hedonic eating behaviours and dysregulate executive control.

On the other hand, there are studies that demonstrated postprandial attenuated activations in other brain regions in obesity, such as the hypothalamus and prefrontal cortex (PFC) [150, 151]. PFC plays an essential role in inhibitory control of actions following visual cues and promotes satiation. While some studies report a greater increase in PFC activation from fasted to satiated state in obesity compared to lean subjects [152], others have shown the opposite [151, 153]. For instance, Jastreboff et al found a reduced perfusion in the dlPFC and medial ACC in obesity compared to lean [153]. Specifically, blunted postprandial dlPFC activation to food cues may be associated with overconsumption and weight gain in obesity [151, 154]. In the genetic obesity PWS, there is delayed meal termination, early return of hunger after a meal and seeking and hoarding food. This is also associated with an increased BOLD response in the ventromedial PFC to food cues compared to lean [155]. It was speculated that the differences observed was due to functional differences of PFC between participants with obesity and lean participants and this appeared to be reversible [154, 156].

Following glucose ingestion, participants with obesity showed enhanced BOLD response to food pictures in the putamen and occipital cortex (visual) compared to lean controls [149]. People with obesity, compared to lean, also showed greater activation in the putamen and less activation in the caudate, insula and OFC on consumption of palatable food [134, 157]. In another study, whilst there was no difference in postprandial food cue-induced BOLD

responses between obesity with T2DM and lean, there was more pronounced reduction in food cue-induced BOLD response after a meal in insula and OFC in obesity with T2DM [158]. It is unsurprising to note the differences in activations of various brain regions as there is a complex network involving the hypothalamus, mesolimbic and prefrontal regions following a meal. Nevertheless, if satiety signals are impaired after meals, the salience towards food cues remain high and may be susceptible to overeating and weight gain.

1.4.3 Factors influencing food cue reactivity when dieting

People who are actively dieting will generally display high dietary restraint, that is they are actively changing dietary habits by reducing consumption of food, particularly HE foods, in an attempt to lose weight. This may influence food cue reactivity, though the literature is rather contradictory as to the direction of the associations of questionnaire based measures of dietary restraint with fMRI measures of food cue reponsiveness. This may be related to nutriitonal state in the studies, dietary restraint questionnaire used, fMRI paradigm and statistcial analysis used, and other characteristics of the particular cohort with obesity [159-162] [163].

Functional MRI food cue reactivity can also be influenced by exercise. High intensity exercise, perhaps by altering gut hormone concentrations (including a decrease in plasma orexigenic AG and an increase in PYY concentrations), suppressed hunger and desire to eat. High intensity exercise is associated with not only with increased BOLD response to high-calorie food cues in dlPFC and suppressed BOLD response in OFC and hippocampus, but also increased BOLD response to to low-calorie food cues in insula and putamen and suppressed in OFC [164]. This suggests exercise promoted inhibition towards high-calorie food and enhanced salience to low-calorie foods. Consequently, in a dieting group with obesity the amount of high intensity exercise one undertakes could contribute to the variability observed in food cue reactivity. The type of weight management programme, including the amount of and form of exercise, should be taken into consideration when interpreting results from adults with obesity actively trying to lose weight.

Another factor that could influence food cue reactivity is energy intake though the associations may be bidirectional. Greater anticipatory responses to food cues in the anterior

cingulate cortex and striatum, regions responsible for attention and reward processing, have been associated with higher total energy intake at test meals [34].

Conversely, a reduced energy intake and removal of specific foods from the diet may be associated with altered food reward responses as could be predicted in a dieting group. For example, cross-sectionally frequent ice cream consumption is associated with reduced striatal responseto receipt of an ice-cream based milkshake [165]. This suggests down-regulation of taste responses with increased intake of a specific food which might reverse after a reduction in intake of that food, as would occur during dieting. However this can be complicated as artificial sweteners may mimic true sugar in also being negatively associated with brain responses to sucrose taste [166]. Associations bewteen habitual consumption and anticipatory food cue reactivity may be in the opposite direction to that using food taste. In lean participants, there was a positive correlation between added sugar intake and striatal response to food cues after glucose, but not water, ingestion [167].

In addition, glucose and appetitive hormones, including leptin, insulin and AG, act on the CNS system to modulate brain activity in response to food cues [93, 149, 174, 175]. For instance, different levels of plasma glucose mediates both stimulatory and inhibitory control in response to visual food cues; mild hypoglycaemia activates reward limbic-striatal regions while euglycaemia activates medial PFC, suggesting enhanced inhibitory control [175]. Dieting and associated weight loss will also result in long term changes of such hormone levels that in turn alter food cue reactivity [8], particularly falls in plasma leptin and increases in plasma AG [174, 176, 177]. Such appetitive hormone changes may also produce differences in food cue reactivity between adults with obesity who are or who are not dieting.

1.4.4 Changes following weight loss

Recent weight loss in those with obesity who are dieting may also alter food cue reactivity [142, 168]. After weight loss in obesity, there was a decrease in BOLD signal to high energy foods in several regions including the superior temporal gyrus, middle frontal gyrus, cingulate gyrus and lentiform nucleus [142]. However the direction and location of changes in BOLD signal from fMRI studies is quite varied, that again likely depends on the degree and duration of weight loss, fMRI paradigms and analysis, and cohort demographics [169]. For instance,

different diet regimes could also influence food cue reactivity. Total meal replacement, compared to calorie restriction, increased food cue reactivity in the dorsolateral prefrontal cortex, orbitofrontal cortex, nucleus accumbens and insula [170]. Differences in food cue reactivity have also been seen cross-sectionally in successful versus unsuccessful weight loss in obesity, and current versus past dieters [171]. Successful weight loss maintenance demonstrated a greater food cue reactivity in the frontal region and middle temporal region compared to obesity [171]. Food cue reactivity in prefrontal regions [56, 172] and striatum [173] were thought to contribute to weight loss and maintenance and may even predict outcome of weight loss interventions.

There is an increase in neuronal response to food cues after weight loss in brain regions implicated in emotional, executive and sensory responses to food while there are decreases in brain networks relating to emotional and cognitive control of food intake as well as integration of motor planning [56]. The areas which demonstrate an increase in response to food cues include the limbic system, such as brainstem, parahippocampal gyrus (PHG) and globus pallidus, and executive and decision-making regions, including PFC and frontoparietal regions [174]. Weight loss also led to a decrease in brain activity in hypothalamus, amygdala, fusiform gyrus, precentral gyrus and ventromedial PFC, which are areas implicated in appetite control, emotional control, cognitive control and motor planning respectively [56, 174]. After weight loss through a calorie-controlled diet, participants with obesity showed decreased dlPFC activation and increased PHG / fusiform activation in response to visual and auditory food cues [178]. The attentuation of dlPFC activation suggests a decreased behavioural inhibition towards that of food intake [179] while the increased PHG/fusiform activation implicates an increased attention and salience to food cues [55].

As a consequence, neural changes following weight loss could predict a phenotype of greater responsiveness to food with decreased control of food intake [174]. Moreover, this increased salience to food cues predicts future weight gain [180, 181]. Conversely, the decrease in dorsal striatal brain responses [caudate, putamen and pallidum] after dieting also predicted the success of weight loss maintenance in the longer term [173]. Successful weight loss following a diet correlated with an increase in BOLD activation to food cues in regions relating to cognitive control, including dlPFC, inferior frontal gyrus (IFG), dorsal ACC, inferior parietal

lobe and caudate, and satiety-induced attenuation of brain activation in OFC during receipt of food-related reward [56]. On a similar note, food cue responsitivity in the NAcc and hypothalamus can also predict weight loss at 12 months following sleeve gastrectomy [182] while impulse control in dlPFC can predict weight regain after dieting [183]. On top of that, there seems to be legacy effects of participation in a behavioural weight loss intervention (Look AHEAD study) for people with obesity and T2DM in which intensive lifestyle intervention resulted in a reduced reward-related activity and enhanced attention or visual processing in response to high-calorie food 10 years later [172].

1.5 NICOTINE DEPENDENCE

1.5.1 Introduction

Smoking is another major public health concern and associated with many respiratory, cardiovascular and oncological comorbidities. It is also the leading cause of preventable deaths in the UK. In 2016-2017, there were 484,700 hospital admissions and 77,900 deaths attributable to smoking [184]. The prevalence of smoking in the UK in 2018 has declined over the past several years to 14.7%, roughly 7.2 million people, in conjunction with a small rise of prevalence of e-cigarettes use to 6.3%, approximately 3.2 million people [185]. This could be a result of increasing public awareness of the health risks associated with smoking, provision of smoking cessation services, and regulation and taxation of cigarettes [186]. Despite this, smoking still costs the NHS an estimated £2.5 billion a year and another £8.5 billion annually to the wider society [186].

1.5.2 Weight gain after smoking cessation

Stopping smoking is challenging despite behavioural support and pharmacological options, such as bupropion, varenicline and nicotine replacement therapy [187]. Quit attempts at NHS Stop Smoking services have declined over the past few years and anecdotal evidence suggest that people may be using e-cigarettes instead as a way to quit [184]. Certainly, providing behavioural support in addition to smoking cessation medications will increase successful quit rates [188].

In spite of this, relapse after smoking abstinence is common, around 80% at one year [189, 190]. Even after being abstinent for a year, relapse was estimated to be more than 35% within

a decade and risk of relapse increases with mental health problems and having partners who smoke [191]. The relapse after smoking cessation is comparable to the weight regain seen after dieting in obesity. It is worth noting that common barriers to successful smoking cessation included weight gain after cessation and stress [192, 193]. In fact, the mean weight gain was between 4.8 and 8.8 kg after quitting smoking and around 13% of ex-smokes gain at least 10kg [194, 195], leading to a short-term increased risk of T2DM. This risk of T2DM was proportional to the weight gain after cessation but importantly, those who have successfully quit smoking had a reduced mortality rate in spite of the weight gain [196]. An increased appetite and reduced energy expenditure is thought to contribute to the weight gain [197]. Therefore, it is important to explore new therapeutic strategies that could improve eating behaviour after smoking cessation and mitigate weight gain which hinders successful quit attempts.

1.5.3 Central reward system in nicotine dependence

Increasingly, nicotine and other substance addictions, are seen as a brain disease due to the underlying changes in neurobiology that results in impulsive, compulsive and addictive behaviours, similar to those seen in obesity. These neural changes are characterised by the desensitisation of reward circuits, the increasing strength of conditioned responses and stress reactivity and the weakening of the brain regions involved in executive functions [198]. Nicotine, as with food and other drugs of abuse, can activate brain circuitry involved in motivation, learning and behavioural reinforcement [46]. Greater neural reactivity to smoking cues, for instance in the amygdala, thalamus, insula and ACC, can predict decreased success at smoking cessation [199, 200]. Nicotine dependence, either through smoking cigarettes or vaping, is largely mediated through the mesolimbic dopaminergic system and nicotinic acetylcholine receptors (nAChRs) signalling. Nicotine activates the reward pathway through phasic increases in dopamine release that triggers associative learning and anticipatory response to a stimuli [201]. It is this dopamine receptor signalling that increases the motivational salience of nicotine and mediate reward-seeking behaviour [202-205]. However, chronic use of nicotine decreases levels of dopamine receptors and dopamine release [205, 206] although this smoking-related deficits in dopamine synthesis normalises three months after smoking cessation [207].

Even though the primary targets for nicotine are nAChRs, with the many different subunits, the effects are diverse and underexplored. It is thought α4, β2, α6 and possibly α7, but not β3, subunits mediate the rewarding effects of nicotine [208, 209]. In addition, α4 and β2, not α6, subunits are necessary for the transition from tonic phasic firing of dopaminergic neurons in the ventral tegmental area (VTA) that is crucial for reinforcement [210]. Importantly, α4 and α6 subunits are required for efficient dopamine release in the NAcc. Varenicline, an α4β2 nAChR partial agonist, is used in promoting smoking cessation. It decreases nicotine-induced change in D2 receptor binding in the thalamus, midbrain, putamen and NAcc and also significantly suppressed dopamine release in the NAcc [211]. This supports the hypothesis that nAChR blockade works by dampening the mesolimbic reward system. Mecamylamine, an unselective nicotinic antagonist, blocked the AG-induced increase in locomotor activity and NAcc dopamine release in rodents, raising the possibility that nAChR are involved in mediating the reward-seeking behavioural effects of AG [212].

1.5.4 Food reward in nicotine dependence

An overlapping pattern in brain activation to food and drug cues in addicts supports the idea of a common reward processing pathway [213]. In a meta-analysis of fMRI studies, food and smoking cues were associated with increased BOLD response in similar brain regions, namely the left amygdala, bilateral OFC, striatum and bilateral insula, the latter only with high smoking craving levels [214]. In addition, smoking cues elicited greater response than food cues in OFC and supplementary motor area (SMA), suggesting a greater salience towards smoking cues and reliance on habitual behaviour with smoking [215]. Furthermore, severe nicotine dependence is associated with reduced response to secondary reinforcers such as money, rather than enhanced response to cigarette cues [216]. Under short term deprivation, the cigarette-seeking responses to cigarette cue in current smokers surpassed food-seeking responses to food cues, suggesting that cigarette cues may have a stronger effect in eliciting reward-seeking behaviours than alternative non-drug food cues [217]. The attenuated striatal activation in smokers to food cues may be a consequence of decreased sensitivity of the dopaminergic reward system from prolonged nicotine stimulation [218, 219] possibly mediated by dopamine D2 receptor polymorphism [220] and may explain weight gain after smoking cessation.

Smokers score higher on measures of disinhibited eating compared to non-smokers [221] and those with elevated dietary restraint turned to cigarettes to prevent food intake [222]. Such restrained eating predicted increases in post-cessation food intake [223]. In smokers who demonstrated high cognitive restraint and disinhibited eating, there was higher leptin concentrations than those who scored low, suggesting an underlying hormonal factor associated with unhealthy eating behaviours [224]. Leptin is an adipokine which signals energy homeostasis to the brain by circulating at levels proportional to the amount of body fat as well as to acute changes in caloric intake [225, 226].

Generally, smokers weigh less than non-smokers [221], but report more frequent cravings for and higher consumption of high-fat food and fast-food fats [227]. On top of this, smokers have less healthy diets than non-smokers, consuming more energy from alcohol and saturated fats, less energy from vegetable protein and carbohydrates and less dietary fibre and minerals [228, 229]. Interestingly, heavy smokers weighed more than light or moderate smokers [230]. Indeed, smokers with obesity had more concerns about gaining weight after smoking cessation and had less confidence in their ability to maintain their weight without smoking [221, 231]. The negative health consequences of smoking are more severe especially among the smokers with obesity and therefore more attention should be given to mitigate any weight gain from smoking cessation to prevent relapses [232].

The anorectic effects of nicotine is well documented in preclinical and clinical studies demonstrating reduction in food intake following nicotine administration [233-235]. It is believed that nicotine stimulates the anorexigenic POMC neurons of the hypothalamic arcuate nucleus (ARC) to decrease food intake [236, 237]. The activation of nAChRs neurons in the ARC and ventromedial hypothalamus suppressed food intake [238]. Even though nicotine activates GLP-1 neurons in the nucleus of solitary tract (NTS), responding for food rewards was similarly reduced in wildtype controls and GLP-1R knockout mice after nicotine administration, suggesting that GLP-1 may not be involved in anorectic effects of nicotine [239]. An enhanced energy expenditure also contributes to the weight loss effect of cigarette smoke exposure [240]. On top of that, nicotine can cause lipolysis, stimulate the sympathetic nervous system and directly stimulate melanocortin receptor 4 (MC4-R), leading to a

reduction in food intake and leptin levels [197]. Acute administration of nicotine in never-smokers reduced subjective appetite and decreased food-cue reactivity in hypothalamus and basal ganglia, implicated in regulation of food intake [241]. In line with this, nicotine also increased the modulatory effects of AG and leptin on food cue reactivity in the ventromedial PFC and amygdala, resulting in a reduction in appetite [242]. Current smokers also displayed a greater BOLD response in the hypothalamus to consumption of palatable food compared to non-smokers [243].

1.5.5 Eating behaviour during nicotine abstinence

Following nicotine withdrawal, rats consumed a higher proportion of total food intake from sucrose than chow as compared to baseline, suggesting that a history of nicotine intake changed dietary preferences [244]. As there are discrepancies in the delivery method and doses of nicotine in various studies, it is postulated that larger nicotine doses may increase the extent of weight gain during withdrawal [244].

Following smoking cessation in humans, the amount of calorie intake increased as well as the frequency of snacking although the intake of lipids and carbohydrates remain unchanged. Predictors of higher weight gain post smoking cessation were female sex, younger age at smoking initiation, higher consumption of cigarettes, depression symptoms, lower socioeconomic status and weight concerns [245, 246]. It was suggested that individuals at higher risk of addiction may experience weight gain resulting from a rebound of appetitive processes that were temporarily suppressed during active addiction [247].

Apart from changes in food consumption, decreased resting metabolic rate, reduced physical activity and increased lipoprotein lipase activity have been cited as mechanisms of weight gain [248]. Through increases in appetite and food reward, smoking cessation weight gain may also attenuate health benefits [249], hinder attempts at abstinence [250, 251] and promote relapse [195]. Weight gain after first year of successful smoking cessation is the greatest [195, 252] and being overweight or obese increases the risk of weight gain substantially [194]. Improving eating behaviour and preventing weight gain after smoking cessation would negate associated morbidity from increased risk of T2DM and importantly help prevent smoking relapses [253, 254].

1.6 GUT HORMONES: GLUCAGON-LIKE PEPTIDE-1 (GLP-1)

Hormonal changes affecting hunger and satiety can influence food intake and eating behaviour and increase saliency of food cues. Recently, there is accumulating evidence to suggest that the appetitive gut hormones, such as glucagon-like peptide-1 (GLP-1) and ghrelin, can modulate reward processing via central actions on the mesocorticolimbic system and in turn affect hedonistic reward eating [141], and even reduce the intake of addictive substances [255, 256]. The ability to modulate reward value of food and alter eating behaviour raises the possibility of a therapeutic role for these gut hormones in the treatment of obesity and weight gain during smoking cessation.

1.6.1 Physiology of peripheral GLP-1

GLP-1 is an anorexigenic peptide hormone that is released post-prandially by the L-cells of the distal small intestine and activates GLP-1 receptors (GLP-1R) that are found on vagal afferent nerve of the gastrointestinal tract [257, 258]. Its role in regulating glucose metabolism and food intake is well-recognised. GLP-1 increases insulin secretion in a glucose-dependent manner, improves insulin sensitivity and increases satiety by delaying gastric emptying and hypothalamic-brainstem actions to reduce appetite and food intake [259]. In line with this, GLP-1 analogue administration results in reduced appetite, food intake and body weight [260-263]. This observed reduction in appetite and food intake is partially mediated through effects on the brain [264, 265]. At present, GLP-1 agonists have been developed successfully for the treatment of T2DM and more recently licensed for obesity.

Whilst GLP-1 analogues, such as Liraglutide and Exenatide, decrease appetite and induce weight loss, it is worth noting that there are inherent differences in test subjects in preclinical and clinical studies that result in varying degree of GLP-1R-driven responses. For instance, Exenatide had a significant reduction in food intake and subsequent weight loss only in a subset of participants with obesity [263]. Changes in other gut hormones, including gastric inhibitory polypeptide (GIP) and leptin, emotional eating and genetic variants were postulated to contribute to the variable response to Exenatide [129, 226, 266]. Consistent with this, Dickson et al (2012) observed that after giving Exenatide to ad libitum fed rats, a group still showed a high motivation to work for sucrose (high responders) while another had a much lower motivation to work (low responders) compared to the condition when they

were fasted [267]. Understanding the mechanism of action of GLP-1 and factors which modulate GLP-1R responses can help to better target a treatment population for maximal effect.

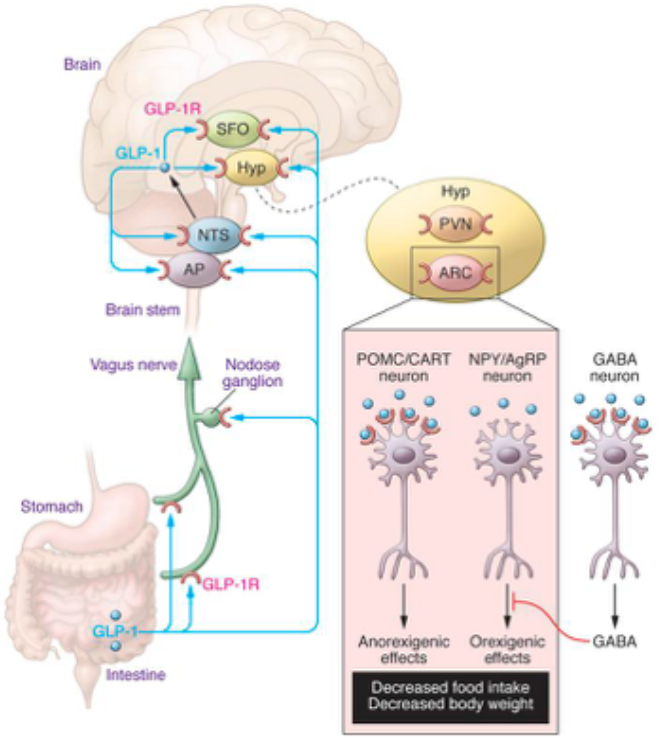


Figure 1.4. GLP-1 analogues reduce food intake via vagal afferents and actions on hypothalamus.
GLP-1 analogues directly activate POMC/CART neurons and indirectly inhibits NPY/AgRP neurons in the hypothalamus resulting in reduction of food intake. They transmit signals via the vagus nerve, NTS and AP to the same effect. Figure taken from [268] Abbreviations: AP, area postrema; ARC, arcuate nucleus; GABA, gamma aminobutyric acid; Hyp, hypothalamus; NPY/AgRP, neuropeptide Y/agouti-related peptide; POMC/CART, pro-opiomelanocortin/cocaine and amphetamine-regulated transcript; PVN, paraventricular nucleus; SFO, subfornical organ

1.6.2 Physiology of central GLP-1

Unlike many other gut hormones, GLP-1 can be synthesised in the brain by pre-proglucagon (PPG) neurons in the NTS, which innervates the hypothalamus and mesolimbic regions, namely the VTA and the NAcc [269]. GLP-1R are highly expressed in the hypothalamus and locally in the VTA, NAcc and amygdala [270, 271] and is necessary for uptake of GLP-1 and its analogues [272]. GLP-1 reduces and increases neuronal activity in the paraventricular nucleus and ventromedial hypothalamic nucleus respectively [273]. Activation of GLP-1R in the hypothalamic ARC reduced food intake by stimulating POMC/CART neurons and indirectly inhibiting NPY/AgRP neurons via GABA-dependent signalling [272] (Figure 1.4). Stimulation of

the NTS GLP-1 producing neurons and lateral dorsal tegmental nucleus had a similar effect on food intake [274] and reduced body weight [269], indicating a role of central GLP-1 system in regulating food intake. However, whilst inhibition of these NTS GLP-1 producing neurons had no significant effect on ad libitum feeding or body weight of mice, it increased post-fast refeeding intake and blocked stress-induced hypophagia. This suggests that the endogenous role of these PPG neurons is in regulating satiation during stress and after large meals, instead of primary intake [275].

Many studies have also explored the role of mesolimbic GLP-1R activation on food intake. Selective VTA application of GLP-1 analogues results in decreased intake of chow and palatable food and reduction of body weight [267]. However, when given a choice of chow and high-fat diet, intra-VTA Exenatide selectively reduced high-fat intake but unexpectedly increase chow intake in ad libitum fed rats [269]. Similarly, endogenous GLP-1 also suppress high-fat food intake by reducing activation of mesolimbic dopamine neurons [276]. Preclinical studies activating GLP-1R in NAcc have shown similar findings as that of VTA [30]. Consistent with this, activation of NTS GLP-1 neurons reduces the excitatory dopaminergic signal within the VTA and preferentially reduce palatable food intake and suppress food reward by suppressing mesolimbic dopamine signalling [277]. The induced suppression of food-reward behaviour as evident from inhibiting conditioned place preference and progressive ratio operant-conditioning for sucrose reward is seen after both central and peripheral administration of Exenatide [278].

Another brain region where GLP-1 acts to inhibit food intake and impulsive operant responding for sucrose is the ventral hippocampal field CA1 (vCA1). Knocking out GLP-1R on vCA1 neurons increases motivation for palatable food whilst disruption of the vCA1 projections to medial PFC ablates the food intake and body weight reduction [90]. On the other hand, central GLP-1R activation can also result in an elevated dopamine turnover in the amygdala where D2 receptor signalling partly mediates its anorexic, but not food reward, effect [278]. This demonstrates a role of GLP-1 signalling in regulating higher-order cognitive aspects of feeding behaviour [90]. Taken together, there are likely to be several mechanisms by which central GLP-1 signalling impacts food intake and food reward behaviour.

1.6.3 GLP-1 and GLP-1 analogues in fMRI clinical studies

GLP-1 or its analogues attenuate hedonic reward responses and attention-related responses to food cues in areas such as the insula, putamen, OFC and amygdala in lean, in obesity and people with obesity and T2DM [141, 158, 271] (Table 1.1). On the other hand, GLP-1 and its analogues also increases the brain responses on consumption of palatable food in brain reward areas, such as the insula, putamen and amygdala. Taken together, GLP-1 analogues may help to prevent overeating by reducing food cravings and increasing consummatory food reward. The rise in GLP-1 following a meal is correlated to the reduction in brain activation to food cues and can be prevented by blocking GLP-1R in subjects with obesity. Consistent with this, response to food-cue in the OFC, that is implicated in reward encoding, is also negatively correlated with the increase in GLP-1 levels following oral glucose tolerance test [279].

The attenuation in brain responses to food cues in a fasted state may be a result of the changes in hormone levels induced by GLP-1 analogues. In a short-term study using Liraglutide as treatment for T2DM, Liraglutide had decreased leptin levels and increased GIP concentrations [226]. This change in fasting leptin levels correlated negatively with activation of the midbrain (including VTA), dlPFC and sensorimotor-related motor cortex, and correlated positively with attention-related parietal cortex, cognitive control-related thalamus and pre-SMA. The change in GIP levels also correlate inversely with activation of the insula in the food cue fMRI task [226].

After 10 days of treatment with Liraglutide in people with T2DM, there was a decreased activation to food cues in insula when fasted and in putamen when satiated compared to insulin. Over time however, this effect diminishes and no significant differences in brain activations to food cues was noted after 12 weeks of treatment with either insulin glargine or Liraglutide [280]. This was corroborated in another study in which no changes in brain activation to food cues were noted after 5 weeks of treatment with liraglutide when compared to placebo [281]. Interestingly, when corrected for BMI and weight, there was greater activation in the right OFC to food cues on liraglutide, indicating possibly a counter-regulatory mechanism to prevent further weight loss [281]. Despite evidence from preclinical studies demonstrating the importance of VTA as focus of interest for GLP-1 signalling, human fMRI studies have focused on brain areas targeted by dopamine neurons

(NAcc, caudate, putamen) rather than on dopaminergic nuclei, such as VTA in brainstem. This is because creating reliable anatomical templates of VTA is technically challenging due to the difficulty in demarcating the region [282].

Author	Subjects	Study	Nacc	Caudate	Putamen	OFC	Insula	Amygdala	comments
fMRI BOLD - GLP1R analogues									
van	lean	fMRI to food cues (Exenatide)			→	↓	↓		
Bloemendaal	obese	fMRI to food cues (Exenatide)			↓	↓	↓		
et al	T2DM	fMRI to food cues (Exenatide)				↓	↓	↓	
van	lean	fMRI to palatable food - anticipation (Exenatide)				↓			
Bloemendaal	obese	fMRI to palatable food - anticipation (Exenatide)							
et al	T2DM	fMRI to palatable food - anticipation (Exenatide)			↓		↓	↓	
	lean	fMRI to palatable food - consummatory (Exenatide)		↑					
	obese	fMRI to palatable food - consummatory (Exenatide)				↑			
	T2DM	fMRI to palatable food - consummatory (Exenatide)			↑		↑	↑	
Farr et al	T2DM	fMRI to food cues (Liraglutide)			↓		↓		**↓ in inferior parietal cortex**
ten Kulve et al	T2DM	fMRI to food cues at Day 10 (Liraglutide)			**↓fed**		**↓fasted**	**↓fed**	
		fMRI to food cues at Week 12 (Liraglutide)			→		→	→	
ten Kulve et al	T2DM	fMRI to food cues at Day 10 (Liraglutide)		↑			↑		
		fMRI to food cues at Week 12 (Liraglutide)		→			→	↓	
Binda et al	obese	fMRI to food cues in cortical areas (Exenatide)							**↓ in high-level visual cortex and early visual areas**
Farr et al	obese	fMRI to food cues at Week 5 (Liraglutide)					↑		
fMRI BOLD - Endogenous GLP-1									
de Silva et al	lean	fMRI to food cues (Exendin 9-39)	→	→	→	→	↓	→	
Heni et al	lean	fMRI to food cues (OGTT)				↓	↓		**correlate negatively with GLP-1**
	obese					↓	↓		
ten Kulve et al	lean	fMRI to food cues (Exendin 9-39)					↓		
	T2DM	fMRI to food cues (Exendin 9-39)		↓		↓	↓		
ten Kulve et al	lean	fMRI to palatable food - consummatory (Exendin 9-39)					↓		
	T2DM	fMRI to palatable food - consummatory (Exendin 9-39)				→	→		
Dorton et al	lean	fMRI to food cues (OGTT)		↓	↓				**correlate negatively with GLP-1**

Table 1.1. Summary of clinical studies assessing effects of GLP-1 and GLP-1 analogues on BOLD signal to food cues.

Abbreviations: fMRI, functional magnetic resonance imaging; NAcc, nucleus accumbens; OFC, orbitofrontal cortex; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.

1.6.4 GLP-1 in patients with bariatric surgery

Research in patients following different types of bariatric surgery has provided important insights to the role of gut hormones in food reward. Patients with obesity after Roux-en-Y Gastric Bypass (RYGB) surgery, associated with increased GLP-1 levels have reduced food reward-hedonic responses and reduced brain reward system activation during an fMRI food cue evaluation task than after gastric banding, an effect that is reversed by acute suppression of GLP-1 and the other anorexigenic gut hormone PYY [283]. After RYBG, the increase in postprandial GLP-1 concentrations correlated with a decrease in the inferior temporal gyrus and right middle occipital gyrus in addition to an increase in the right medial prefrontal gyrus during a food cue fMRI task [178]. This suggests GLP-1 has a role in mediating these regions implicated in attention and inhibition during satiety.

1.6.5 GLP-1 and GLP-1 analogues in nicotine reward

The link between food and drug reward is increasingly recognised via actions on mesolimbic reward pathway and GLP-1 is considered to play a central role in the development of food reward and drug addictions [30, 66, 284]. In preclinical studies, GLP-1 agonists decrease both consumption and the rewarding value of alcohol, nicotine and psychostimulants (cocaine and amphetamine) and the drug-induced accumbal dopamine release [285-287]. In nicotine reward, Exendin-4 (Exenatide) abolished nicotine-induced locomotor stimulation, accumbal dopamine release and expression of conditioned place preference in mice [288]. The stimulation of GLP-1 neurons in the NTS decreased nicotine intake in mice as did stimulation of the GLP-1R in the medial habenular projections to interpeduncular nucleus [239]. Taken together, this suggests that the physiological role of endogenous GLP-1 extends beyond metabolic and food intake to include regulation of nicotine-induced reward, indicating a potential therapeutic target for smoking cessation and prevention of weight gain from smoking cessation. Following this, clinical studies examining the therapeutic value of GLP-1 analogues in addiction are now emerging [289].

1.7 GUT HORMONES: GHRELIN PEPTIDES – ACYL-GHRELIN AND DESACYL GHRELIN

1.7.1 Physiology of ghrelin system

Ghrelin is a stomach-derived peptide hormone, existing as 2 forms: predominantly des-acyl ghrelin (DAG) and a lesser proportion of acyl-ghrelin (AG). DAG is acylated by the enzyme ghrelin O-acyltransferase (GOAT) on its third serine residue with an octanyl group pertinent to its binding with growth hormone secretagogue receptor 1a (GHSR1a) [290-293]. GHSR1a is a 7-transmembrane G-protein coupled receptor widely expressed in various tissues, with the highest expression in the CNS [294]. GHSR1a is densely expressed in the hypothalamus and NTS, pointing to a role in feeding and energy homeostasis [295]. In a recent discovery, liver-expressed antimicrobial peptide 2 (LEAP2), an endogenous antagonist of GHSR, was found to be suppressed by fasting and blocked major effects of AG in vivo including food intake [296]. Both AG and DAG peptides have actions on the CNS and their transport across the blood-brain barrier can occur independently of GHSR [297].

1.7.2 Actions of AG on food intake

AG has a role in energy balance, regulating glucose metabolism, enhancing gut motility and mediates stress response [293, 298-300]. In addition, plasma AG, increased during fasting, scheduled mealtimes and weight loss, induces food intake and appetite through its actions on the GHSR1a [301, 302]. AG stimulates food intake via NPY/AgRP neurons and inhibits POMC neurons in the hypothalamic ARC [303-305]. Furthermore, AG administration in the lateral hypothalamic area and VTA not only increased food intake and but also motivated behaviour for sucrose [306]. This is consistent with human studies in which obese and lean subjects enhanced appetite and food intake after AG administration [302, 307]. AG was positively correlated with caloric intake, succumbing to food cravings and negatively correlated with insulin resistance, systolic blood pressure and heart rate although these correlations were not seen in obesity [308]. This may suggest a central resistance to ghrelin in obesity. On top of this, fasting AG levels and postprandial decline in AG levels were lower in obesity with binge eating than those without, suggesting a downregulation due to habitual overeating [309].

In preclinical studies, the role of AG in reward-based and stress-induced eating is mediated

though actions on mesolimbic dopaminergic circuitry, cholinergic systems, opioid and GABA signalling [310-317]. AG stimulates cholinergic projections from the laterodorsal tegmental area (LDTg) to the VTA [318], where it increases the expression of μ-opioid receptors and subsequently intake of sucrose and chow [319]. AG administration into VTA and NAcc promotes appetite and motivation to obtain palatable food by activating dopaminergic neurons and increasing dopamine release in NAcc [320, 321]. This observed AG-induced increase in food reward behaviour is abolished by AG, GHSR1a or dopamine antagonists and in GHSR1a knockout mice [322, 323]. It increases the reward value of high-fat diets in mice, as evidenced by conditioned place preference and operant conditioning tasks [324]. Furthermore, following consumption of pleasurable food, endogenous AG levels significantly increased in healthy satiated subjects [325]. Taken together, this suggests that AG may act to enhance and promote hedonic eating [319].

1.7.3 AG in fMRI studies relating to food

These findings are also corroborated in human fMRI studies. In healthy subjects, high levels of AG, both endogenous through fasting and exogenous, increase brain hedonic-reward activity in regions including amygdala, OFC, striatum and hippocampus, in response to food pictures and appear to enhance the salience of food cues [177]. Plasma level of AG positively correlate with activation in the PFC, amygdala and insula and negatively in subcortical areas [326]. The effects of AG on BOLD response to food cues in amygdala and OFC also correlate with hunger ratings [176, 327]. An attenuated suppression of plasma AG and increased insulin level after glucose ingestion was found in adolescents with obesity, relative to lean. This change in AG and insulin levels after glucose ingestion was associated with hypothalamic, thalamic and hippocampal food cue reactivity in obesity compared to lean [153]. Collectively, the obesity-related impaired prefrontal executive and enhanced hedonic responses to glucose consumption could be driven by gut hormonal changes and contribute to excessive intake and weight gain.

In subjects who are homozygous for the fat mass and obesity-associated gene (FTO) rs9939609 A allele, there are increased AG levels, attenuated postprandial appetite reduction and associated reduced BOLD response to food-related images within the hypothalamus, VTA and insula [328]. Subjects with homozygous AA genotype are predisposed to obesity and

demonstrate a reduced difference in BOLD response in the insula and putamen between high- and low-calorie food images in the fed compared to fasted state [328]. Following laparoscopic sleeve gastrectomy, patients have lower total ghrelin concentration and this was found to be associated with reduction in food cravings and BOLD signal to high-energy foods in the dlPFC [329]. Interestingly, nicotine enhances the modulatory effect of AG on food-cue reactivity particularly in the ventromedial PFC and the amygdala which may reduce appetite and contribute to its anorexic effect [242]. The finding that total ghrelin concentrations can predict risk of relapse in abstinent smokers also suggest that AG has a role in nicotine dependence [330].

1.7.4 Role of AG in drug reward

Indeed, AG has been implicated in the increased consumption and reward behaviour to nicotine, alcohol and other drugs of abuse, as well as food in preclinical studies [306, 317, 331, 332]. In alcohol use disorders, AG when administered intravenously increased alcohol craving in alcohol-dependent subjects who are currently drinking [333]. Plasma AG was also positively correlated to alcohol cravings and alcohol cue reactivity in NAcc in abstinent alcohol-dependent participants [334]. Conversely, GHSR1a antagonists have been reported to decrease consumption and reward-properties of morphine and alcohol in pre-clinical setting [335, 336].

AG acts via the GHSR-1a to exert its effects on food intake, body weight and glucose metabolism. Hence, the ability to antagonise the effects of AG presents as an attractive target for anti-obesity drugs [337]. One way to antagonise AG is to bind to circulating AG and rendering it ineffective at activating GHSR. By doing so, food intake and body weight gain in diet-induced obese mice were suppressed [338, 339]. Similarly, GHSR-1a antagonists reduce food intake, body weight and fat tissue mass in preclinical studies [340, 341]. The newly characterised endogenous GHSR antagonist, liver-enriched antimicrobial peptide-2 (LEAP2), increases with BMI and glucose, decreases with fasting and prevents AG activation of arcuate NPY neurons [342]. Administration of the N-terminal of LEAP2 also suppressed AG-induced food intake in mice [343]. Furthermore, inhibition of ghrelin-O-acyltransferase (GOAT), the enzyme which acylates DAG into AG, reduced food intake in rats by reducing meal frequency [344]. However, only a limited number of antagonists of GHSR and GOAT are currently

available for clinical studies. Disappointingly, an anti-ghrelin vaccine in obesity did not significantly reduce body weight after 16 weeks when compared to a control group [345].

1.7.5 Actions of DAG

DAG is now recognised in recent years to have biological functions after initially being regarded as an innate by-product of AG due to the lack of an acyl side chain required for full agonism of GHSR [290]. It does not function as an antagonist at the GHSR1a [305]. Although it has a very low affinity for GHSR1a, at supraphysiological concentrations, DAG can activate GHSR in vitro and regulates body adiposity and peripheral glucose metabolism through a CNS GHSR-dependent mechanism [346] (Table 1.2). DAG may directly counteract the effects of AG on glucose metabolism and food intake [347] and exert some anticonvulsant properties via the ghrelin pathway [348]. Transgenic mice overexpressing DAG exhibited lower body weights and smaller phenotypes [349].

Furthermore, it has other AG-independent effects [350], including stimulation of insulin release from INS-1E cells and inhibition of cell proliferation in human breast cancer and prostate cancer cell lines which do not express GHSR1a [351]. In addition, DAG has effects on cortical neuronal injury, processing emotional and anxiety-related behaviours, regulation of lipid metabolism and body temperature [346, 352-354]. Using DAG fluorescent tracer, DAG was found to bind to the ARC NPY neurons in high concentrations in wildtype and GHSR-deficient mice although AG and DAG were found to bind differentially to ARC neurons[347]. To date, the target receptor and pathway through which DAG exerts its actions remains unknown but there are likely to be additional yet undiscovered ghrelin receptors since both AG and DAG affect cells that do not express the GHSR-1a [355].

1.7.6 Role of DAG in food intake

The effect of DAG on food intake in pre-clinical studies is not consistent and, in some cases, contradictory [351] (Table 1.3). The reason for this remains unclear. Initial preclinical studies demonstrated an anorexigenic effect of peripheral DAG in fasted rats during light phase and non-fasted rats during dark phase [356]. This was also seen in fasted mice during light phase

Author	Peptide	Subjects	Study protocol	Duration	Fasting glucose	Fasting insulin	Postprandial glucose	Postprandial insulin	Mean glucose	GH	Cortisol	Appetite VAS	Food intake	Weight
Allas et al 2018	DAG analogue	47 PWS	Daily SC injection	14 days			↓	↓				↓		→
	DAG analogue	Subset with IGT / T2DM	Daily SC injection	14 days			↓	↓				↓		→
Allas et al 2016	DAG analogue	24 overweight	Daily SC injection + standard meals	14 days		→		→	→					↓
	DAG analogue	Subset with IGT	Daily SC injection + standard meals	14 days		→		→	↓					↓
	DAG analogue	27 T2DM	Daily SC injection + standard meals	14 days		→		→	(↓)					↓
Tong et al 2014	DAG	17 lean	IV GTT + test meal	3.5 hr infusion	→	→		→		→	→	→	→	
	AG	17 lean	IV GTT + test meal	3.5 hr infusion	↑	→		↓		↑	↑	→	**↑ (cf DAG)**	
	DAG + AG	17 lean	IV GTT + test meal	3.5 hr infusion	↑	→		↓		↑	↑	→	**↑ (cf DAG)**	
Ozcan et al 2014	DAG	8 T2DM	Overnight infusion + Fixed meal	15 hr infusion			↓	→						
Benso et al 2012	DAG	8 lean	Overnight infusion + Fixed meal	16 hr infusion			↓	↑		→	→			
Tong et al 2010	AG	12 lean	IV GTT	65 min infusion	→	→	↓	↓		↑	↑			
Kiewiet et al 2009	DAG	8 obese	Daily injections + Fixed meal	4 days	→	→	→	→		→				
	DAG + AG	8 obese	Daily injections + Fixed meal	4 days	→	↓	→	→		↑				
Gauna et al 2004	DAG	8 pit insufficiency	Fixed meal	Bolus	↑	→	↑	→						
	AG	8 pit insufficiency	Fixed meal	Bolus	↑	→	↑	→						
	DAG + AG	8 pit insufficiency	Fixed meal	Bolus	→	↓	↓	↓						
Broglio et al 2004	DAG	6 lean	Fasting	Bolus	→	→				→	→			
	AG	6 lean	Fasting	Bolus	↑	↓				↑	↑			
	DAG + AG	6 lean	Fasting	Bolus	→	→				↑	↑			
Broglio et al 2003	DAG	7 lean	Fasting	Bolus	→	→				→	→			
	AG	7 lean	Fasting	Bolus	↑	↓				↑	↑			

Table 1.2. Summary of clinical studies assessing the effects of DAG on metabolic parameters, hormone concentrations, appetite, food intake and weight.

Abbreviations: AG, acyl ghrelin; c.f, compared with; DAG, desacyl ghrelin; pit, pituitary; IGT, impaired glucose tolerance; IV GTT, intravenous glucose tolerance test; GH, growth hormone; PWS, Prader-Willi syndrome; SC, subcutaneous; T2DM, type 2 diabetes mellitus; VAS visual analogue scale.

Author	Peptide	Route	Subjects	Fasted	Phase	Chow Intake
Asakawa	AG	ICV	Male ddy mice	N	L	↑
et al	DAG	ICV	Male ddy mice	N	L	→ (trend to ↓)
	DAG	ICV or IP	Male ddy mice	Y	L	↓
	DAG	Endogenous	Transgenic DAG overexpressing male mice	N	weekly	↓
Chen	AG	IC	Male Wistar rats	Y or N	?	↑
et al	DAG	IC	Male Wistar rats	Y	?	↓
	DAG	IC	Male Wistar rats	N	?	→
Chen	AG	IP	Male Wistar rats	Y or N	L	↑
et al	DAG	IP	Male Wistar rats	Y	L	↓
	DAG	IP	Male Wistar rats	N	L	→
	DAG	IP	Male Wistar rats	N	D	↓
Neary	AG	IP	Male C57B16 mice	Y or N	?	↑
et al	DAG	IP	Male C57B16 mice	Y or N	?	→
Toshinai	DAG	ICV	Male Wistar Rats	N	L or D	↑
et al	AG	ICV or IV	Male Wistar Rats	N	L	↑
	DAG	IV	Male Wistar Rats	N	L	→
	DAG	ICV	Male ddy mice	N	L	↑
	AG	ICV	Male ddy mice	N	L	↑
	DAG	ICV or IP	Male ddy mice	Y	L	→
	DAG	IP	Male C57BL/6 mice	N	L	→
	AG	ICV	GHS-R deficient mice	N	L	→
	DAG	ICV	GHS-R deficient mice	N	L	↑
Matsuda	AG	ICV or IP	Carassius auratus Goldfish	NA	NA	↑
et al	DAG	ICV or IP	Carassius auratus Goldfish	NA	NA	→
Matsuda	AG	IP	Capsaicin-treated goldfish	NA	NA	↓ AG-induced intake
et al	DAG	ICV or IP	Carassius auratus Goldfish	NA	NA	→
	AG+DAG	ICV or IP	Carassius auratus Goldfish	NA	NA	↓ AG-induced intake
Inhoff	AG	IP	Male Sprague-Dawley Rats	N	L	↑
et al	DAG	IP	Male Sprague-Dawley Rats	N	L	→
	AG+DAG	IP	Male Sprague-Dawley Rats	N	L	→
	DAG	IP	Male Sprague-Dawley Rats	Y	L	→ (trend ↓)
Heppner	AG	ICV or SC	Male C57/BL6 mice	N	6 days	→
et al	DAG	ICV or SC	Male C57/BL6 mice	N	6 days	→ (↓ vs AG)
	DAG	ICV	GHSR deficient mice	N	6 days	→
Stevanovic	AG	ICV	Male Wistar rats	N	L	↑
et al	DAG	ICV	Male Wistar rats	N	L	→
	AG+DAG	ICV	Male Wistar rats	N	L	↓ AG-induced intake
Fernandez	DAG	ICV	C57BL6/J mice	Y	L or D	→
et al	AG+DAG	ICV	C57BL6/J mice	Y	L	→ AG-induced intake
	AG+DAG	SC AG + ICV DAG	C57BL6/J mice	Y	L	↓ AG-induced intake

Table 1.3. Summary of pre-clinical studies assessing effect of AG or DAG on food intake.

Abbreviations: AG, acyl ghrelin; D, dark phase; DAG desacyl ghrelin; GHSR, growth hormone stimulating receptor; ICV, intracerebroventricular; IP, intraperitoneal; L, light phase; N, no; NA, not applicable; SC subcutaneous, Y; yes. Trends in paracenteses.

after injecting DAG centrally and peripherally [357]. In contrast, another study reported that central administration of AG or DAG significantly induced feeding during both light and dark phases in rats [358]. Despite conducting similar experiments, other subsequent studies reported no effect of DAG on food intake [346, 347, 359-361]. DAG-induced reduction in food intake was associated with the activation of neurons in the paraventricular nucleus of the hypothalamus and was not mediated by the vagal afferents [362]. Furthermore, DAG bound to this subset of hypothalamic ARC cells, both NPY and non-NPY neurons, in a GHSR-independent manner [347] and suppressed AG induced neuronal activity in the hypothalamic ARC of rats thereby abolishing induction of food intake [360]. DAG only inhibited the orexigenic effect of peripherally-administered AG, but not centrally-administered AG [347]. Whilst peripherally-administered AG mainly activated the ARC, the difference with administering AG centrally is that it can access multiple other brain areas directly and does not require transport through the blood brain barrier. Therefore, this raises the possibility that DAG mediates the orexigenic effect of AG via ARC and prevents transport into the hypothalamus.

Until recently, there are only a few clinical studies examining the effects of exogenous DAG on food intake or body weight. In one study, Tong et al. reported a reduction of glucose and fructose consumption with a DAG infusion when compared to saline [363]. This result raises the possibility that DAG could influence the food reward pathway and in turn selectively reduce intake of sweet rewarding foods. The DAG analogue, AZP-531 (Livoletide), has been shown to be well-tolerated and effective in improving glycaemic control and producing weight loss in Phase 1 and 2a clinical trials for T2DM and obesity over 2 weeks duration [364]. A follow-on study using Livoletide in the genetic obesity PWS, went on to demonstrate an improvement in hyperphagia and appetite scores with a reduction in waist circumference and fat mass in 2 weeks [365].

At the time of the study grant application, DAG was the only available peptide for clinical use that could modulate the ghrelin system. Since then, there have been a limited number of GHSR1a antagonists or inverse agonists (e.g. PF-5190457), or GOAT inhibitors (GLWL-01), utilised in early phase clinical trials [366, 367]. However, they still have limited availability for clinical use in experimental medicine studies outside close collaboration with the pharmaceutical industry [368, 369]. In light of its ability to block AG effects on food intake, body weight and metabolism, DAG is thus a novel pharmacotherapeutic targets for human addictive and eating behaviours. No functional neuroimaging studies have previously been performed using DAG, or studies of its role

in addictive behaviours in humans.

1.8 RATIONALE FOR CURRENT STUDY

Obesity and smoking are common public health issues that predisposes to development of cardiovascular and respiratory diseases, type 2 diabetes mellitus, malignancies and other associated health conditions. Treating these associated, often chronic, comorbidities tend to have a significant economic consequence for the healthcare system and society at large. Therapeutic options for obesity and smoking cessation are limited. Moreover, weight regain after dieting in obesity and weight gain after smoking cessation is commonly seen. Weight gain after smoking cessation can hinder attempts at quitting and result in relapses. Appetitive gut hormones, such as GLP-1 and AG, can modulate neural circuitry involved in reward processing and affect food intake. Therefore, understanding the mechanisms by which central GLP-1- and ghrelin-signalling pathways can mediate brain responses to food cues and alter eating behaviours is crucial as these gut hormones could have therapeutic potential in the management of obesity to prevent weight regain and prevention of weight gain following smoking cessation.

1.9 OVERALL HYPOTHESES AND AIMS

Hypotheses:

In groups with obesity who are dieting, and recently abstinent smokers,

1. the GLP-1 analogue, Exenatide, will reduce high-energy (HE) food cue reactivity, food intake, appetite and food reward behaviour.
2. DAG will reduce HE food cue reactivity, food intake, appetite and food reward behaviour.

Aims:

Using a double-blind randomised placebo controlled cross-over study, to investigate in adults with obesity who are dieting, or in abstinent nicotine-dependence, the effects on eating behaviour of acute administration of

1. The GLP-1 analogue, Exenatide, or
2. desacyl ghrelin (DAG), using the following outcomes:
   (i) fMRI BOLD response during HE food picture evaluation task,
   (ii) food intake during *ad libitum* test meal,
   (iii) appetite visual analogue scale (VAS) ratings,
   (iv) motivation to eat using a progressive ratio task (PRT), and
   (v) attentional bias to food using an approach-avoidance task (AAT).

CHAPTER 2:

METHODS AND MATERIALS

This chapter provides an overview of the experimental design, participant selection and the outcome measures relevant in the following chapters of the thesis.

2.1 STUDY DESIGN

This "Gut Hormones in ADDiction" (GHADD) study was a UK Medical Research Council (MRC)-funded, double-blinded, randomised placebo-controlled, cross-over study using intravenous GLP-1 analogue, Exenatide or desacyl ghrelin (DAG) infusions in adults with obesity who are dieting, recently abstinent adult smokers and recently abstinent alcohol dependent adults [370]. It built on the Imperial College Cambridge Manchester (ICCAM) study platform to explore in depth the brain mechanisms underpinning reward and relapse circuitry so as to inform future drug development [57]. The ICCAM study used an experimental medicine platform utilising fMRI paradigms to evaluate new drugs for relapse prevention in alcohol and drug addiction. These included the Monetary Incentive Delay task which examines anticipatory responses to monetary reward as a secondary reinforcer, and Negative Emotional Reactivity task which examines stress responses towards unpleasant images [371]. Both of these fMRI tasks were used in the GHADD study.

However, for the GHADD study the ICCAM platform was built on to also include fMRI measure of cue reactivity to relevant salient cues (high-energy food, cigarettes, alcohol), and additional behavioural tasks outside the scanner. Specifically, this GHADD study examines the effects of acute administration of Exenatide or DAG on high-energy food, cigarette and favourite alcohol pictures in brain reward regions, and associated addictive and eating behaviour, including food intake during *ad libitum* test meal and food reward behaviour, including motivation to eat sweets using a progressive ratio task (PRT), and attentional bias to food (as well as alcohol and cigarette pictures) using an approach-avoidance task (AAT). However, for the purposes of this thesis, only the effects of Exenatide or DAG on eating behaviour and food-related tasks in the dieting group with obesity and ex-smokers will be discussed.

The study received ethical approval from the NHS Health Research Authority and Central Research Ethics Service Committee, London (reference 15/LO/1041) on 14th July 2015 and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. All participants gave written informed consent.

2.2 PEPTIDES

2.2.1 Pilot dose-finding phase

As mentioned in the acknowledgements, all work undertaken during the dose-finding pilot phase was performed by the principal investigator, Dr Tony Goldstone, Dr Sri Akavarapu (Clinical fellow), Dr Nienke Pannekoek (Research Fellow), Miss Barbara Kobson (Research nurse).

Healthy participants (n=4) were recruited for the pilot dose-finding phase to confirm that plasma levels of Exenatide and DAG were appropriately achieved. They were recruited via the Imperial College Healthcare NHS Trust website as well as Imperial College website and noticeboards. They were (i) male or female, (ii) aged between 18 to 60, (iii) no previous history of medical or psychological illness, including T1DM, T2DM and bariatric surgery, (iv) no current medical condition, or use of medications that would interfere with the study, (v) no history of nicotine, alcohol or drug dependence and (vi) no MRI contraindications. All participants provided informed written consent and the pilot phase was conducted according to the principles of the Declaration of Helsinki. They were not recruited in the main study. The demographics of the 4 healthy participants are shown in the Table 2.1.

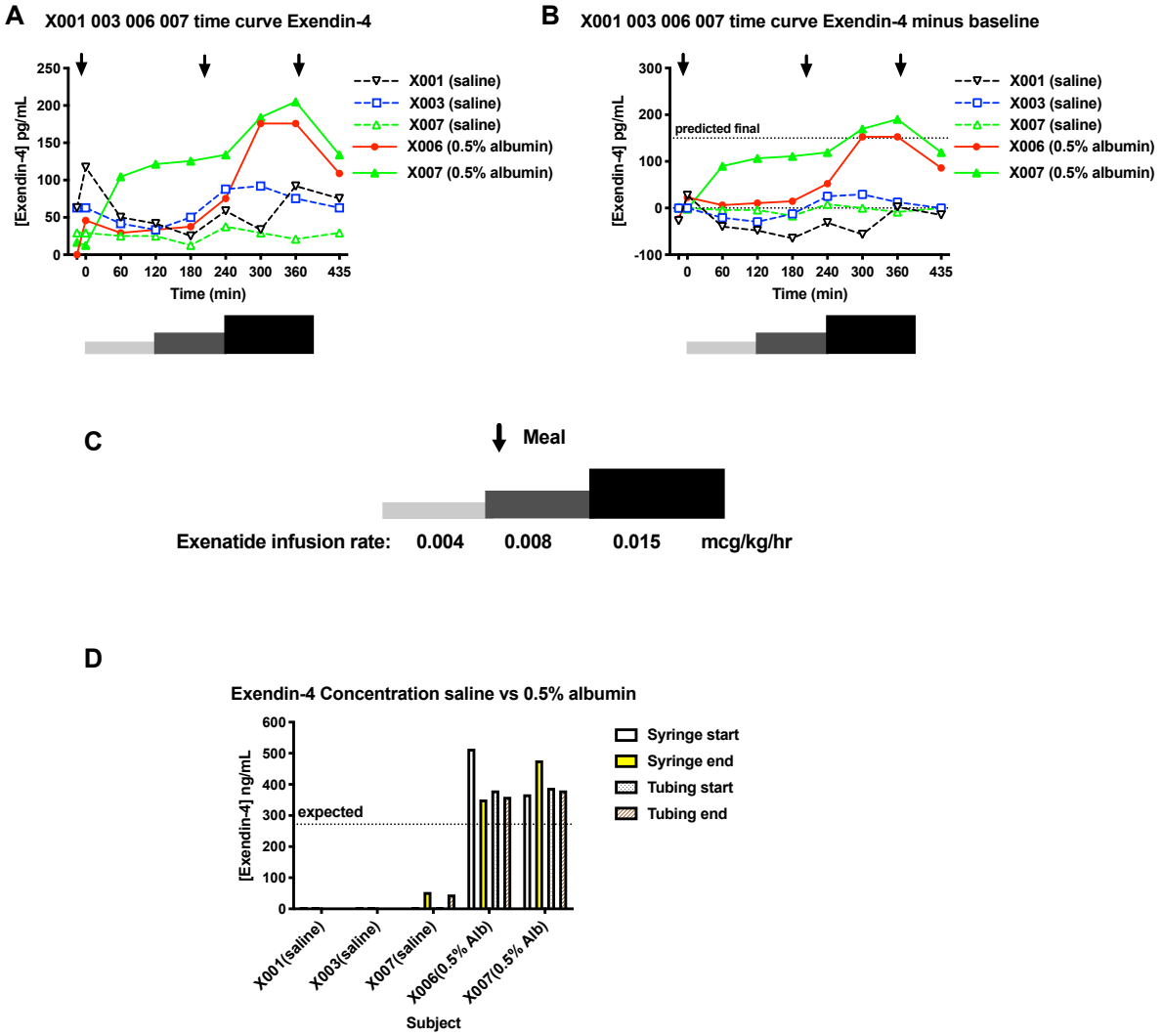
n	4	
Demographics		Range
Age (years)	29.0 ± 17.5	18.0 - 55.0
Male, n (%)	3 (75%)	
BMI (kg/m^2)	28.4 ± 3.2	24.9 ± 32.7

Table 2.1. Summary characteristics of healthy participants in dose-finding phase.
Data presented in mean ± SD.

After an overnight fast, the healthy participants attended NIHR-Wellcome Trust Imperial Clinical Research Facility, Hammersmith Hospital and were given either saline, DAG or Exenatide infusion on each of their visits for 6 hours via a cannula in one arm. Another cannula was inserted in the contralateral arm to take serial blood samples for measurement of plasma AG, DAG, Exenatide and GLP-1 concentrations throughout the infusion visits every 60 mins. Infusion rates were doubled every 2 hours over the 6-hour infusion to achieve the proposed maintenance infusion rates in the main study. Exenatide infusion was commenced at the initial rate of 0.004 mcg/kg/hr before doubling the rate every 2 hours to a final rate of 0.015mcg/kg/hr (Figure 2.1C). Similarly, DAG infusion was commenced at an initial rate of 1 mcg/kg/hr before doubling the rate every 2 hours to a final rate of 4 mcg/kg/hr.

No adverse events were reported in any participants. There was a problem with the commercial Exenatide assay initially used (enzyme immunoassay EK-070–94, Phoenix Pharmaceuticals), as the plasma matrix interfered with the assay giving high concentrations even during the saline infusion, despite sample dilution (results not shown). This was resolved by sending off the samples to the Holst laboratory, University of Copenhagen that uses a highly sensitive assay enabling greater sample dilution.

Using the assay from the Holst laboratory, Exenatide, when dissolved in saline, was undetectable in the plasma or tubing, despite pre-coating the syringe and infusion tubing with Gelofusin, likely



due

Figure 2.1. In dose-finding phase, Exenatide (Exendin-4) concentrations at Exenatide visits.
In healthy participants (n=4), comparisons of (A) absolute Exenatide concentrations (pg/mL) and (B) change from baseline of Exenatide concentrations (pg/mL) when Exenatide was dissolved in saline or 0.5% albumin. (C) Diagram of Exenatide infusion rate and timing of *ad libitum* meal. (D) Comparisons of Exenatide concentrations (ng/mL) in infusion samples taken from infusion syringe and tubings when Exenatide was dissolved in saline or 0.5% albumin. Figures were courtesy of Dr Tony Goldstone.

to peptide adherence to the plastic (Figure 2.1A,B,D). However, this issue was addressed by dissolving the Exenatide in 0.5% human albumin and priming the infusion tubing and syringe with 0.5% human albumin solution to minimise peptide adherence. Reassuringly, using 0.5% albumin did not adversely affect the infusion of DAG from measured plasma concentrations (Figure 2.2D).

Based on these results, the final maintenance doses of DAG (Clinalfa, Bachem) and Exenatide (Byetta, AstraZeneca) used in the main study were 4 mcg/kg/hour and 0.015 mcg/kg/hour respectively. These hormone infusion doses were developed from and similar to previously published human infusion studies (Tong et al, 2013; Tong et al, 2014; van Bloemendaal et al, 2014).

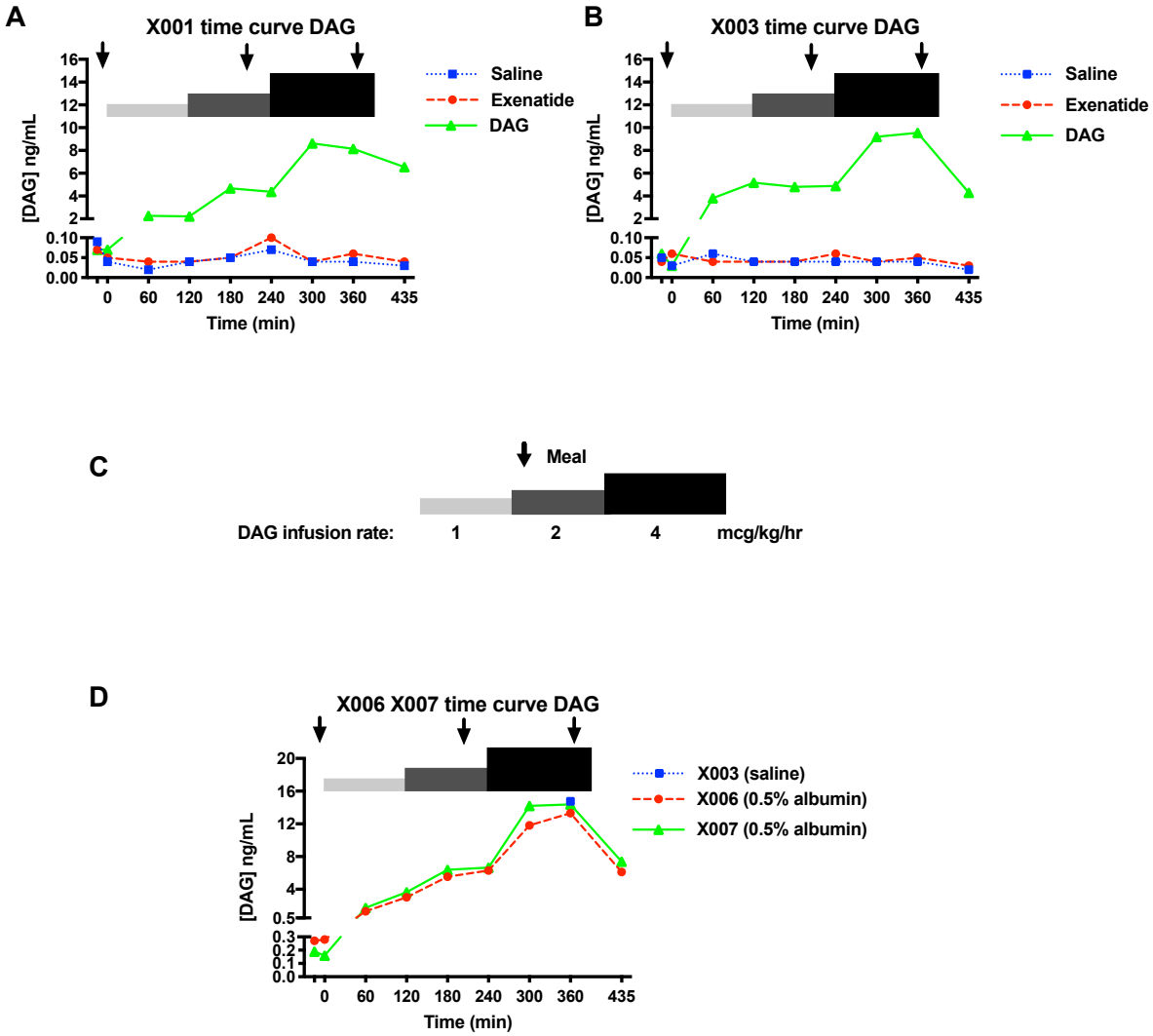


Figure 2.2. DAG concentrations during dose-finding phase.
Comparisons of (A-B) DAG concentrations (ng/mL) at saline, Exenatide and DAG visits in (n=2) healthy participants. (C) Diagram of DAG infusion rate and timing of *ad libitum* meal. (D) Comparisons of DAG concentrations (ng/mL) in (n=3) healthy participants when DAG was dissolved in saline (blue) or 0.5% albumin (red or green). Figures were courtesy of Dr Tony Goldstone.

2.2.2 Peptide infusion preparation for main study

Exenatide was the choice of GLP-1 analogue for this experiment as it has been shown in similar published studies to have an effect on food cue reactivity using fMRI allowing comparable intravenous infusion paradigms [141, 372]. These studies also provided a basis for the dosing schedule in this study. Exenatide has the shortest half-life of the available GLP-1 analogues. It can be eliminated from the body in a short time following cessation of the hormone infusion thereby minimising any side effects after departing from the study visit. Furthermore, Exenatide could be purchased cheaply as a pharmaceutical GMP-grade medicinal product, avoiding the need to purchase expensive GMP-grade native GLP-1 peptide. Furthermore, such purchased GLP-1 peptide would need to undergo expensive, time consuming individual vial preparation, quality control, safety and efficacy testing in mice, and sample vials endotoxin and peptide assays to confirm safety and appropriate peptide amounts (as needed for DAG). Exenatide (Byetta) was purchased from AstraZeneca, UK through Department of Pharmacy, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK. Exenatide (20mcg) was then diluted in 50ml of 0.5% albumin solution to make up the final Exenatide infusion.

GMP-grade desacyl ghrelin (DAG, human natural sequence) was purchased from Clinalfa, Bachem AG, Bubendorf, Switzerland. DAG peptide was dissolved in sterile 0.9% saline (Baxter) under aseptic conditions in a laminar flow cabinet, aliquoted into vials and freeze dried. Representative samples of DAG vials were sterile after culture for 7 days (Department of Microbiology, Hammersmith Hospital, London, UK) and endotoxin levels using Limulus Amoebocyte Lysate test were within safe range for human infusions. Another sample of representative DAG vial was also tested for peptide composition and purity using high-performance liquid chromatography to determine the correct dose of peptide to be administered. On the day of the study visit, a vial of DAG was then dissolved in 0.9% saline and diluted in 50 ml of 0.5% albumin solution (5% HAS diluted in 0.9% saline) to make up the final DAG infusion.

The peptide infusions were prepared on the morning of the study visits by the research nurses to ensure researchers were blinded.

2.3. PARTICIPANT SELECTION

Participants were recruited through advertising on social media platforms (Facebook), newspapers, University and Hospital websites and noticeboards (Recruitment will be discussed further in Chapter 3). A final n=25 in dieting group with obesity and n=25 ex-smokers were included in data analysis.

2.3.1 Inclusion criteria

Inclusion criteria for all participants were (i) aged 18 to 60 years, (ii) male or female, (iii) able to read, comprehend information in English and give written consent, (iv) healthy as determined by a medical physician based on a full history, physical examination, laboratory tests and cardiac monitoring.

Specifically, for the dieting group with obesity:

(i) BMI between 30 and 50 kg/m^2, (ii) actively dieting, (iii) never smoked (<100 cigarettes in lifetime).

Specifically, for the ex-smokers:

(i) previous nicotine-dependence as demonstrated by DSM-5 criteria, (ii) previously smoking at least 5 cigarettes daily and having first cigarette within an hour of waking, (ii) current stable abstinence from smoking for at least 6 weeks, (iv) current abstinence from nicotine replacement therapy for at least 2 weeks.

2.3.2 Exclusion criteria

Exclusion criteria for both groups included:

(i) significant cardiovascular, cerebrovascular, renal, hepatic, gastrointestinal, respiratory disease or neurological disease, or previous bariatric surgery, (ii) type 1 or 2 diabetes mellitus, (iii) current use of regular medications that interfere with study integrity or subject safety, (iv) history of psychiatric illness apart from previous depression, (v) previous substance addiction (apart from nicotine for ex-smokers) or problem gambling, (vi) positive drug or alcohol screens other than that explicable by other causes (such as recent use of opiate containing analgesic or consumption of poppy seeds for positive opiate screen), (vii) breath carbon monoxide reading of more than 10ppm, (viii) conditions which preclude safe MRI scanning, (ix) history of eating disorders, (x) vegetarianism, veganism, gluten or lactose-intolerant, (xi) current pregnancy or breast feeding or (xii) unwillingness or inability to follow the study protocol. Full criteria in Appendix 1.

The reason for selecting these groups of participants was to assess the effects of Exenatide or DAG on adults who may be susceptible to weight regain following diet or weight gain following smoking cessation. In particular, the group with obesity had to be dieting for at least 6 weeks to match the duration of nicotine abstinence in the ex-smokers. Moreover, dieting in the group with obesity may result in weight loss which will increase AG concentrations. This is of importance as there is a suggestion from emerging evidence in clinical trials in PWS, where AG concentrations are higher than in obesity, that DAG analogue (Livoletide) may improve hyperphagia. It could be possible that DAG functionally antagonises AG to a greater extent when AG concentrations are higher. Therefore, choosing a group with obesity who are dieting may accentuate any effects of DAG on eating behaviour.

Due to the cross-sectional design of the study, it was not possible to accurately record weight loss of the dieting participants, relying on self-reports, and therefore the success of their dieting could not be quantified or verified. Similarly, there were a variety of weight management programs that the dieting participants were reported to have adopted. These factors contribute to the heterogeneity of the dieting group with obesity and have to be taken into consideration when interpreting results.

2.3.4 Participant recruitment

A total of n=2,254 dieting participants with obesity and n=3,523 ex-smokers completed our online screening questionnaire, that consisted of questions regarding age, BMI, previous medical or psychiatric conditions and medications (Figure 3.1, which also includes the abstinent alcohol dependent group not analysed in this thesis). Only those who met the relevant inclusion/exclusion criteria were asked to leave their name and contact details for our study team to get in touch (Appendix 1 for inclusion/exclusion criteria). This step in the recruitment process minimised the workload considerably and allowed efforts to be focused in conducting more detailed telephone screenings with potential participants who would be more likely to be eligible.

After telephone screening, only n=144 in dieting group with obesity and n=216 in ex-smoker group were eligible to be invited for an in-person screening visit. Out of these, only n=45 in dieting group with obesity and n=43 in ex-smoker group actually attended the screening visit. Finally, n=36 in dieting group with obesity and n=34 in ex-smoker group were recruited for the study (Figure 3.1).

There were n=23 participants who withdrew from the study and so did not complete all the study visits. The reasons cited for drop-out were as follows:

- An incidental but significant meningioma was discovered at MRI scanning which required referral to a neurosurgical unit for further management (n=1)
- Significant nausea and vomiting at study visit and subsequent withdrawal (n=3)
- Vasovagal episode during cannulation at first study visit and subsequent withdrawal (n=1)
- Claustrophobia during the fMRI scan (n=1)
- Difficulty staying still throughout the fMRI scan (n=1)
- Transient heating effect of the MRI on metallic implant in forearm (n=1)
- Withdrawal from the study following screening visit or $1^{st}/2^{nd}$ study visits due to other work or family commitments (n=15)

The final sample included n=25 dieting participants with obesity and n=22 ex-smokers who completed all 3 study visits. In addition, several participants completed their saline and at least one peptide infusion visit which were also included in the relevant analysis: n=1 with obesity (DAG) and n=3 ex-smokers (1 Exenatide, 2 DAG). Thus, in total there was data available in the dieting group with obesity for n=25 Exenatide, n=26 for DAG, and for ex-smokers, n=23

Exenatide, n=24 DAG.

For the data analysis, n=1 dieting participant with obesity had to be completely excluded from data analysis due to excessive head motion on fMRI scans (average >0.5mm per volume relative motion) and difficulty performing study tasks. Another n=1 dieting participant with obesity had to be excluded from the fMRI BOLD signal data analysis due to excess signal dropout in the frontal lobe, but their data could be included in behavioural analyses.

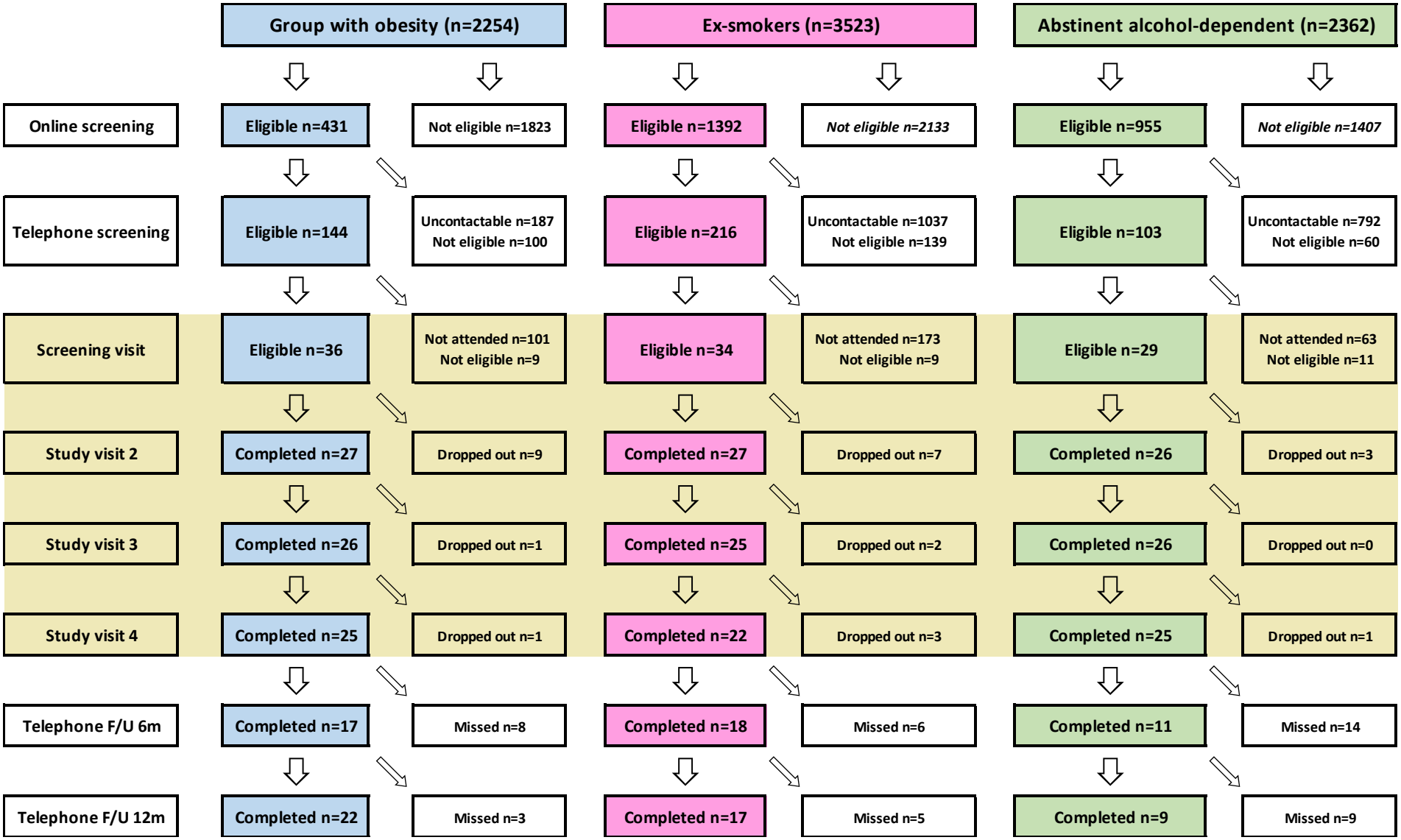


Figure 3.1. Recruitment flowchart in the Gut Hormones in Addiction study.

Screening and study visits are highlighted in yellow. Telephone follow-up (F/U) was performed for n=25 with obesity, n=25 ex-smokers included in analysis. Missed telephone F/U at 6m did not preclude F/U at 12m. F/U at 6 and 12 months are pending for n=1 and n=3 ex-smokers respectively. Abbreviations: F/U, follow-up.

2.4. STUDY PROTOCOL

2.4.1 Screening visit (Visit 1)

Interested potential participants filled out an initial online screening tool and left their contact details if they were potentially eligible. They were contacted and had a telephone screening interview to assess potential eligibility and willingness to participate in the study.

Following successful telephone screening, participants then underwent an in-person screening visit at the NIHR-Imperial Clinical Research Facility, Hammersmith Hospital, West London at which they provided written informed consent to participate in the study and were confirmed to have met the inclusion and exclusion criteria. All participants were deemed to be healthy and eligible at the screening visit by a study physician after undergoing medical history, physical examination, anthropometric measurement, routine haematology and biochemistry tests (including full blood count, renal function, liver function tests, clotting, glucose, thyroid function, HbA1c), electrocardiogram (ECG), and psychiatric evaluation (including a Mini International Neuropsychiatric Interview). They were breathalysed for carbon monoxide and alcohol to confirm abstinence from cigarettes and not intoxicated, and urine samples were analysed for a selection of drugs of abuse (cocaine, amphetamine, cannabis, benzodiazepines, tricyclic antidepressants, barbiturates, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), methadone, morphine). Current pregnancy was excluded in females using a urine human chorionic gonadotropin test.

They were also familiarised with study procedures and completed computer-based behavioural and cognitive measures. These included a progressive ratio task (PRT) and approach-avoidance task (AAT) amongst others. In addition, they viewed high-energy food, cigarette, favourite alcohol pictures and neutral object pictures that were going to be presented at the study visits to minimise any confounding effects on outcome measures arising from a novel stimulus. The following questionnaires, relevant to this PhD thesis, were also completed at the screening visit (Appendices 2-7):

1. Beck Depression Inventory II (BDI-II): to identify symptoms of depression. A score of 29 or more suggests severe current depression and therefore they would be excluded from the study.
2. Alcohol Use Disorders Identification Test (AUDIT): to screen for alcohol use disorders. A score of 20 or more suggests possible alcohol dependence and therefore corroborated with DSM

5 to exclude participants with severe alcohol use disorder.

Additional questionnaires were administered to provide additional descriptive variables to compare between groups in additional analyses both as factors that may differ or be similar between the obese and traditional addiction groups, and also factors that may influence the response to Exenatide or DAG within groups, for particular outcomes, such as negative emotional reactivity task, cigarette cue reactivity, and memory tasks:

3. Spielberger Trait Anxiety Inventory (STAI): to measure trait anxiety with a score of more than 40 indicating high anxiety.
4. Perceived Stress Scale (PSS): to measure perceived stress with a score of more than 26 indicating severe stress.
5. Fagerstrom Test for Nicotine Dependence: to assess nicotine dependence in ex-smokers. The higher the score, the more severe the nicotine dependence.
6. Wechsler Test of Adult Reading (WTAR): to measure intellectual functioning. Out of a maximum score of 50, a higher score suggests a higher intelligence quotient and memory performance.

2.4.2 Study visit (Visit 2-4)

After the screening visit, eligible participants attended at least three study visits, at least five days apart (to allow for washout of hormone effects), comparing the effects of three different infusions: 1) Placebo (0.5% human albumin solution and 0.9% normal saline, 0.1113ml/kg/hr); 2) Exenatide (Byetta, AstraZeneca, 0.015 mcg/kg/hr); 3) DAG (Clinalfa, Bachem, 4.00 mcg/kg/hr). n=3 participants had to return for a fourth study visit due to technical problems at a previous study visit. Randomisation of the participants to the order of DAG, Exenatide and placebo infusions was done using a sequential list. The final infusion doses of peptides were determined after an initial dose-finding phase to achieve circulating plasma DAG concentration of 14-18 ng/mL [363, 373] and plasma Exenatide concentrations of 110-150 pg/mL [141, 374, 375] (See section 2.2 for dose-finding phase).

At T=0min (~10:10am), infusions were commenced at 0.03 mcg/kg/hr and 8 mcg/kg/hr for Exenatide and DAG respectively for 30 mins initially (double-dose ramping phase) before halving them to maintenance rates. An equivalent maintenance rate was used for the saline administration. The double-dose ramping was intended to speed up the time taken to achieve steady state for both peptide infusions. The DAG infusion dose did not exceed doses of 10.0

mcg/kg/hr which was the maximum safely administered to participants with obesity and T2DM previously [376]. Similarly, the Exenatide infusion dose did not exceed the maximum safely administered dose of 0.04/kg/hr [377, 378].

The study protocol was identical at each of the three study visits (Figure 2.3). On each study visit, participants arrived at the Invicro Clinical Imaging, Hammersmith Hospital after an overnight fast from 2000h the previous evening. The participants were also requested to refrain from strenuous exercise or alcohol on the preceding day. After verbal consent, they were breathalysed for carbon monoxide and alcohol, had urine test for pregnancy and drugs and ECG recording. Height, weight and % body fat by bio-electrical impedance analysis (BC-418 or MC-780 P, Tanita) were measured at each study visit. Two cannulae were inserted in the antecubital veins of each arm for infusions and serial blood sampling.

Snacks consisting of two biscuits (McVities^{TM} plain digestive biscuits, providing 142 kcal, 40.6% (total energy intake) fat, 52.4% total carbohydrate (18.6g), 14.1% sugars, 6.2% protein) and a 116g fruit-flavoured jelly (Hartley's no added sugar jelly, providing 6 kcal, 0% fat, 100% total carbohydrate, 100% sugars, 0% protein) were given at T=-25 min and T=+220min. This was done to help prevent hypoglycaemia and minimise hunger so that participants were able to concentrate on performing the tasks, but without giving a large energy intake that would induce satiety.

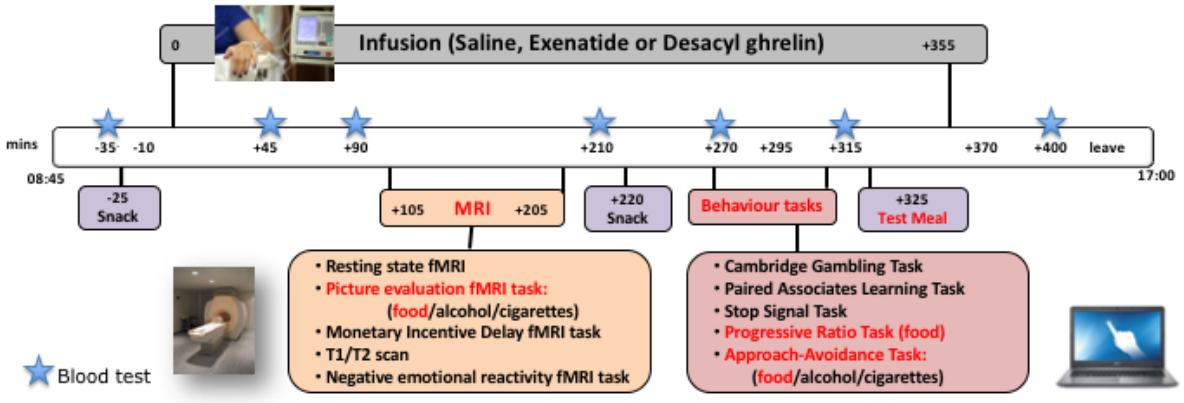


Figure 2.3. Study visit protocol

Timeline of study visit showing infusion, fMRI, behavioural tasks, snacks, test meal, and blood sampling (blue star). VAS ratings were performed at timepoints denoted in white bar. fMRI and behavioural tasks included in this thesis are in red.

Throughout the study visit, from T= -35mins till T=400min, participants completed serial subjective visual analogue scale (VAS) ratings (0-100mm) of hunger, appetite, nausea, stress,

food cravings on a tablet device. At the same time, they were monitored at various time points for blood pressure, pulse rate, capillary blood glucose measurements and venous blood sampling. The following questionnaires (relevant to this thesis) were completed at the start of the infusion relating to mood states, eating behaviour and personality traits (Appendices 8-18). Only the mood state questionnaires were repeated at each study visit to compare across visits, and so ensure that differences in mood were not a confound influencing results from different infusions. The remaining questionnaires were done once at a study visit as they were stable traits. These were again included to compare reward sensitivity and impulsivity between groups in additional analyses, both as factors that may differ or be similar between the obese and traditional addiction groups, and also factors that may influence the response to Exenatide or DAG within groups, for particular outcomes, such as the non-scanning stop signal task and monetary incentive delay fMRI task.

Mood state questionnaires:

1. Profile of Mood States (POMS-2) measuring fatigue, tension, vigour, depression, anger and confusion;
2. Positive affect and Negative Affect Schedule (PANAS) as a measure of positive and negative mood;
3. Spielberger State Anxiety Inventory (SSAI) measuring state anxiety;

Eating behaviour questionnaires - dietary restraint and food hedonics:

4. Dutch Eating Behavioural Questionnaire (DEBQ) measuring dietary restraint, emotional eating and external eating;
5. Three Factor Eating Questionnaire (TFEQ) measuring dietary restraint, disinhibited eating and susceptibility to overeating from hunger cues (TFEQ-hunger);
6. Power of Food Scale (PFS) measuring appetitive drive to consume palatable food;
7. Yale Food addiction Score (YFAS) measuring 'food addiction' traits;
8. Binge Eating Scale (BES) measuring binge eating symptoms;

Trait questionnaires - impulsivity, reward and punishment behaviour:

9. Barratt Impulsivity scale measuring impulsivity;
10. Urgency, Premeditation, Perseverance, Sensation seeking, and Positive urgency (UPPS-P) measuring five subscales of impulsivity;
11. Behavioural Activation System / Behavioural Inhibition System (BAS/BIS) measuring disposition to punishment and reward.

At around 12:00h, fMRI scans were performed between T=+115 min and +195 min including a practice run of the picture evaluation task followed by the actual task using an MRI-compatible 5-button box to rate pictures at T=+120 min. This fMRI picture evaluation task was based on protocols of previous studies [283, 379-382] (Figure 2.4). In this task, four types of colour photographs were presented in a block design split across two 10 mins runs: (i) 60 high energy food images (e.g., pizza, cake), (ii) 60 cigarette images (e.g., lit cigarettes, people holding a cigarette), (iii) 60 personalized alcohol images to two types of alcoholic drinks (e.g., beer & wine) and (iv) 60 matched control neutral images (containing hands, faces, objects).

These images were presented in blocks of 18 seconds each and separated by rest periods of 12 seconds (software: E-prime, Psychology Software Tool Inc, Pittsburgh, PA, USA). Each participant viewed a total of 60 images in each of the four categories across ten blocks by using a pseudorandom block order (randomized for each subject across study visits) with a randomized picture order within each block. Every image was displayed for 2500ms and followed by an interstimulus fixation cross interval of 500ms. This task had been successfully utilized in previous research [35, 381]. In order to minimize habituation effects across study visits, participants were familiarised with the images at the initial screening visit. The food images were selected to represent familiar foods that are typical to the modern Western diet and obtained from freely available websites. The food, cigarettes, alcohol and neutral object pictures were of similar luminosity and resolution.

Images were viewed via a mirror mounted above an 8 channel RF head coil which displayed images from a projector using the ePrime 2 software (Psychology Software Tool Inc., Pittsburgh, PA, USA).

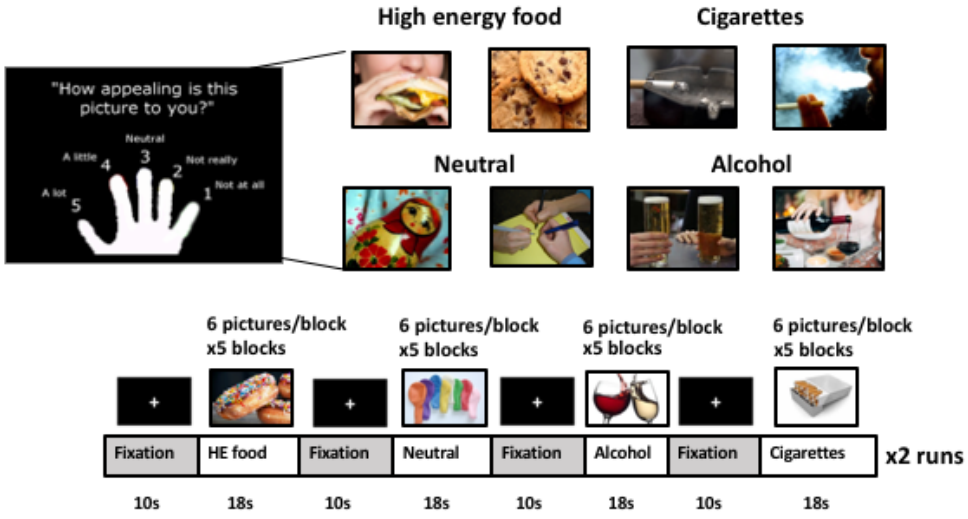


Figure 2.4. Diagram of fMRI picture evaluation task.
During the fMRI picture evaluation task, high-energy food, cigarette, alcohol and neutral pictures were evaluated using a 5-button box.

Then, behavioural tasks were performed (T=+230min to +315min) including a progressive ratio task (PRT) for M&M sweets [283, 383] and approach avoidance task (AAT) to HE food, cigarette, alcohol and neutral pictures [384]. A taste test of the test meal dishes was carried out prior to serving up the *ad libitum* test meal. The infusion was stopped shortly after the test meal at T=+355min and participants were monitored for any side effects for another hour before they were allowed to leave the unit.

2.4.3 Telephone follow-up sessions

Upon completion of the study visits, participants were followed up through telephone interview at 6 and 12 months after their first study visit. At the interview, they provided information about their weight and compliance to diet, cigarette, alcohol and drug use and any information regarding relapses if applicable. After the 12-months follow-up interview, participants sent a urine sample back in the post for detection of cotinine (nicotine breakdown product).

2.4.4 Participant expenses

To cover travel expenses and time, participants were given £25 for screening visits and £50 for each scanning or infusion visit, and a bonus of £50 for completion of all 3 study visits, up to a

total of £225, plus money won during the fMRI MID task at each study visit (maximum per visit £16.50). For some participants travelling from outside of London, extra reimbursement of their travel expenses was made. Payment for the screening visit was processed after that visit, but payment for study visits was processed after completion of all study visits. If participants did not complete all their study visits, they were paid pro rata.

2.5 FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI)

2.5.1 fMRI acquisition

Whole brain fMRI data was acquired on a 3 Tesla Siemens Verio MRI scanner at the Invicro Clinical Imaging, Hammersmith Hospital, West London, UK using a 32 channel radiofrequency head coil with T2* weighted gradient-echo echoplanar imaging (EPI) with an automated higher-order shim procedure: 54 ascending contiguous 3.0mm thick slices, 3.0 x 3.0 mm voxels, multiband 2, GRAPPA acceleration factor 2 repetition time (TR) 1500ms, echo time (TE) 30ms, 80^{o} flip angle; field of view (FOV) 192mm, slice acquisition angle parallel to anterior-posterior commissure line.

The number of volumes of each fMRI task were as follows: (i) resting state 320 in total over 8 mins, (ii) picture evaluation task 414 over 10.35 mins for each of the 2 runs, (iii) MID task 288 over 7.2mins for each of the 2 runs, and (iv) negative emotional reactivity 196 in total over 7.4 min.

High-resolution T1-weighted MPRAGE structural scans were also collected for image registration (TE 2.98 ms, TR 2300 ms, flip angle 9^{o}, FOV 256 mm, voxel dimensions 1.0 x 1.0 x 1.0 mm). Field maps were used to correct for geometric distortions caused by inhomogeneities in the magnetic field as follows (taken from FMRI Expert Analysis Tool (FEAT) analysis software output). Structural T2-weighted scans were acquired to identify any structural abnormalities.

2.5.2 fMRI preprocessing

The first 8 scans were discarded to allow for the BOLD signal to stabilise. fMRI data processing used the FEAT version 6.0 provided by Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) v5.0.10 (Oxford Centre for Functional MRI of the Brain; www.fmrib.ox.ac.uk/fsl).

The following pre-processing was applied:

(i) Motion correction using Motion Correction of the MRI Linear Image Registration Tool (MCFLIRT). Participants were excluded from the fMRI analysis if their average relative motion over the food evaluation was greater than 0.5mm/vol;

(ii) Field inhomogeneity correction: Field map-based EPI unwarping using PRELUDE and Functional MRI Utility for Geometrically Unwarping EPIs (FUGUE);

(iii) Non-brain removal using Brain Extraction Tool (BET);

(iv) Spatial smoothing using a Gaussian kernel of full width at half maximum (FWHM) 6.0mm;

(v) Global intensity normalisation: grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor;

(vi) Temporal smoothing: high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=45.0s);

(vii) Time-series statistical analysis was done using FMRIB's Improved Linear Model (FILM) with local autocorrelation correction including even onsets as explanatory variables within the context of the general linear model (GLM) on a voxel-by-voxel basis (stick functions convolved with the gamma haemodynamic response function) for the relevant contrast. Motion parameters and temporal derivatives of the event onsets were included as co-variates as part of the GLM to correct for motion and slice timing;

(viii) Registration to high resolution of T1 structural and/or standard space images was carried out using the Oxford Centre for Functional MRI Linear Image Registration Tool (FLIRT). Registration from high resolution structural to standard space was then further refined using the Oxford Centre for Functional MRI Linear Image Non-linear Registration Tool (FNIRT);

(ix) Fixed effect analysis of runs: For the food pictures, higher level analysis was performed using a fixed effect model to combine the two runs, by forcing the random effects variance to zero in the Oxford Centre for Functional MRI Local Analysis of Mixed Effects (FLAME) and in order to determine activation for the food>objects contrast.

2.5.3 Whole brain analysis

A whole brain analysis to determine the spatial extent of differences in the BOLD signal between study visits for food and neutral contrasts in the picture evaluation task was examined by 1-factor repeated-measures ANOVA within FSL with FEAT version 6.0 software. Whole brain mixed effects

analysis compared BOLD signal between the saline visits and Exenatide or DAG visits of each participant group (obese and ex-smokers), using unpaired t-tests with cluster threshold Z>2.3, corrected P<0.05.

2.5.4 Region of interest (ROI) analysis

Functional regions of interest (fROI) covered reward processing (nucleus accumbens (NAcc), caudate, putamen), emotional and motivational (orbitofrontal cortex (OFC), anterior insula, amygdala, hippocampus), and executive control [ventral anterior cingulate cortex (vACC) and dorsolateral prefrontal cortex (dlPFC)] regions in the brain (Figure 2.5, Table 2.2). These 9 fROI were determined from the average of a separate cohort of 42 lean healthy participants who underwent an identical fMRI paradigm without any infusion, using the food or alcohol > neutral contrast, voxel-wise false discovery rate (FDR) corrected P<0.05. They were chosen on the basis of previous similar fMRI studies looking at gut hormone effects of bariatric surgery [381] or AG administration in non-obese subjects [177]. Additional ROIs were not examined to avoid type 1 errors because no Bonferroni correction was made for multiple comparisons.

The fROI were obtained by masking the group activation maps with *a priori* anatomical ROI with fslmaths software within FSL. These were defined by the relevant bilateral ROIS from the cortical and subcortical structural Harvard FSL atlases with a threshold at 10% probability. The OFC fROI included regions in the OFC and frontal pole with $-32 < x < 32$, $y > 24$ and $z < -6$ because the analysis of functional activation in this region showed distinct bilateral clusters that overlapped anatomical Harvard atlas regions.

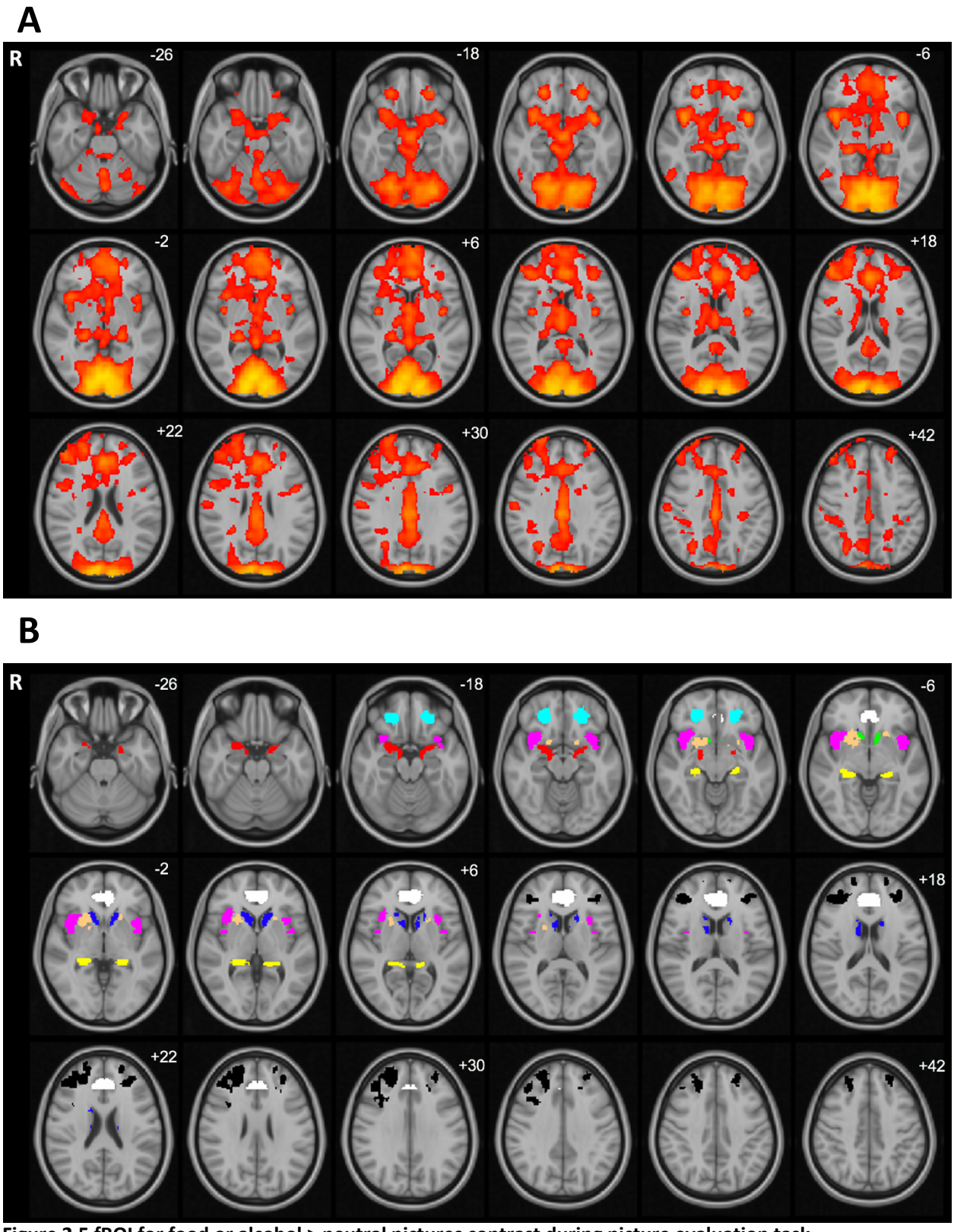


Figure 2.5 fROI for food or alcohol > neutral pictures contrast during picture evaluation task.

Group activation maps for (A) food or alcohol > neutral pictures in (n=43) healthy volunteers. (B) fROI: amygdala, red; caudate, dark blue; dorsolateral prefrontal cortex, black; hippocampus, yellow; insula, magenta; nucleus accumbens, green; OFC, light blue; putamen, beige; ventral anterior cingulate cortex, white.

Region	L/R/B	Voxels	Z	x	y	z
NAcc	L	44	5.86	-6	8	-4
	R	42	4.63	8	18	-4
Caudate	L	198	5.32	-6	10	0
	R	257	5.25	10	2	10
Putamen	L	58	4.29	-18	20	-4
	L	24	4.05	-14	6	-14
	R	422	6.02	16	4	-12
Amygdala	L	205	5.19	-14	-4	-14
	R	318	5.88	18	0	-12
OFC	L	348	7.16	-22	38	-14
	R	325	7.15	24	38	-14
Anterior insula	L	617	8.41	-38	8	-10
	R	831	8.41	38	8	-10
Hippocampus	L	187	6.69	-22	-28	-6
	R	272	6.67	22	-34	2
vACC	B	1569	8.29	0	36	20
dlPFC	L	500	5.85	-34	40	16
	L	81	4.64	-22	58	20
	R	1712	7.36	46	42	20

Table 2.2. fROI coordinates and voxels for food or alcohol > neutral during picture evaluation task.
Coordinates given in Montreal Neurological Institute (MNI) space.
Abbreviations: B, bilateral; dlPFC, dorsolateral prefrontal cortex; L, left; NAcc, nucleus accumbens; OFC, orbitofrontal cortex; R, right; vACC ventral anterior cingulate cortex.

2.6. OUTCOME VARIABLES

For the purpose of this thesis, only the outcome variables related to eating behaviour in the dieting group with obesity and ex-smokers were examined. Effects of Exenatide and DAG were examined by utilising within-group analysis for the outcome variables.

Any between-group analysis (effects of hormones in dieting obese vs. ex-smokers) has its limitations, notably because of the differences in BMI, recent weight loss/gain and previous nicotine dependence which may have led to changes in reward processing pathways in the brain. Furthermore the groups may not be exactly matched for age and sex. Any differences in effect of the peptide hormones between the groups could therefore arise from these confounding variables either acting alone (e.g. through floor or ceiling effects), or altering hormones responsivity. Thus cautious interpretation of between group results is warranted. Ideally, a comparator group should consist of healthy, non-obese, never smoked participants to be able to draw better and more accurate conclusions. However, the purpose of the GHADD study was to explore, as a high risk, high reward proof-of-concept experimental medicine study, the potential of Exenatide and DAG as therapeutic options in relapse prevention treatment for weight regain after dieting, smoking and alcohol cessation, as novel targets for addiction and obesity. In view of this, a clinically relevant patient participant group was considered to be a more efficient use of resources than a group of healthy controls. There were unfortunately insufficient financial, staff and time resources to recruit a 4th group of healthy controls.

2.6.1 Appeal ratings during HE food picture evaluation task

When participants were evaluating the pictures, the appeal ratings of each picture [where 5 = a lot (high appeal), 4 = a little, 3 = neutral, 2= not really, 1 = not at all (low appeal)] was recorded simultaneously. Their responses (HE food vs. neutral at Exenatide or DAG vs saline visits) were examined for any effect of the gut hormones. Reaction times, head motion during the fMRI task and response accuracy (the percentage of recorded picture evaluation responses averaged across four picture types for a study visit) were assessed for any confounding effects on outcome.

2.6.2 High-energy food picture evaluation fMRI task

One of the main study aims was to examine effects of Exenatide and DAG on BOLD signal changes in whole brain and relevant fROI to HE food > neutral pictures during the HE food picture

evaluation task. For each group, a GLM was implemented using FEAT in FSL for the whole brain analysis using contrast saline > Exenatide or DAG and Exenatide or DAG > saline.

The median magnitude of bilateral BOLD signal within each a priori fROI was extracted for each individual participant separately for any HE food > neutral contrast using Featquery in FSL, to measure the differences in activation between saline visits and Exenatide or DAG visits in each participant group. Average BOLD activation of each of these food > neutral contrasts within each fROI was then compared between the hormone and saline visits outside FSL.

2.6.3 Ad libitum test meal and taste visual analogue scale (VAS)

The *ad libitum* meal consisted of a choice of low fat (LF) savoury soup (Sainsbury's Tomato and Basil), high fat (HF) savoury soup (Baxters cream of tomato with added double cream), LF sweet (Yeo Valley 0% fat vanilla yoghurt) and HF sweet (Haagen-Dazs vanilla ice cream)(Table 2.3). Each food type was served in excess. Firstly, each food type was tasted and rated using the Sussex Ingestion Pattern Monitoring rating scales for creaminess, sweetness, just right for creaminess or sweetness (using the intensity general Labelled Magnitude Scale, gLMS), liking and pleasantness, before participants were allowed to eat as much as they wished. They consumed their test meal in the quiet experiment room and were left alone while eating. They were not told that their food intake was measured. The remaining food was then weighed, and consumption was then recorded for further comparisons. If a participant expressed a dislike to the tomato soups, they were offered cream of chicken and chicken broth in replacement (n=1).

per 100g	Tomato & Basil Soup	Cream of Tomato Soup	Vanilla Yoghurt	Vanilla Ice Cream
Energy (kcal)	35.56	110.06	87.00	251.00
Fat (g)	0.98	7.87	0.00	17.00
of which saturates (g)	0.18	4.31	0.00	10.40
Carbohydrate (g)	5.07	6.45	15.50	20.20
of which sugars (g)	4.71	5.12	15.50	14.30
Fibre (g)	0.62	0.36	0.00	0.00
Protein (g)	1.24	1.18	5.90	4.20
Salt (g)	0.38	0.63	0.23	0.15
Amount served (g)	800	800	450	500
Amount served (kCal)	284.4	880.4	391.5	1255.0

Table 2.3. Nutritional information of each food dish in test meal.
Comparison of tomato & basil soup (savoury low-fat), cream of tomato soup (savoury high-fat), vanilla yogurt (sweet low-fat) and vanilla ice-cream (sweet high-fat).

2.6.4 Progressive Ratio Task

The progressive ratio task (PRT) assesses the effort expended to earn a food reward, in this case, an M&M crispy chocolate (in 100g (485kcal), 21g fat (189kcal), 69g carbohydrate (276kcal), 4.2g protein (16.8kcal); Mars UK Ltd) as an indication of food reward [383]. Using a computer mouse, participants earned 1 M&M each time they met the required rate of ratio responding for that trial. Thereafter, the required number of computer mouse clicks increased exponentially on each trial (i.e., 10, 20, 40, 80 etc.) until the participant voluntarily stopped responding (breakpoint). The participants familiarised themselves with the PRT on the screening visit.

On the study visits, the PRT was administered at around T=280 min in the experimental room. The same number of chocolates (n=20) were presented to each participant. The instructions to the participants appeared on the computer screen. They were told by the instructor, "Press the mouse key as little or as much as you like to earn the chocolates. You can only earn a piece each time. When you no longer want to continue, press the space bar and this is not a competition." Thereafter, the instructor left the room and the participants were left alone to complete the task. Upon completion of each ratio, a message box appeared on the screen stating, "You have earned food. Enjoy your reward and after you have swallowed it completely you may click on OK to continue with the programme." After having the M&M, the participants could choose to continue with the task or terminate it at any time by pressing the space bar. This task works on the assumption that when the effort to earn the chocolate is deemed greater than the rewarding value of the chocolate, participants will stop the task [383, 385]. The total number of chocolates consumed is then calculated and recorded. This was then correlated with the number of completed ratios from the computer software to ensure the participants had completed the task correctly.

2.6.5 Approach-Avoidance Task

The Approach-Avoidance Task (AAT) measures automatic approach tendencies (i.e. approach bias) towards high energy food-related cue images. Participants were instructed to either pull or push a joystick in response to the stimulus property around each image (black or white border). There was a total of 30 pull and 30 push trials in the task. This task has been successfully utilised in previous research [384]. As with the picture evaluation task, participants were familiarised with the images at the initial screening visit. Participants with less than 75% mean accuracy in

the task would be excluded from data analysis. An AAT-food bias score was calculated by subtracting median reaction time (RT) for pulling a picture from median RT for pushing a picture [384, 386]. A positive Push-Pull RT indicates an approach bias and conversely a negative one indicates an avoidance bias.

Participants performed the AAT at T=+300min and did a practice task at the screening visit as well as before each actual study visit AAT. They are presented with these instructions on the computer, "Pull the joystick towards you if a picture has a black border (until the image fills the screen). Push the joystick away from you if a picture has a white border (until the image shrinks and disappears). Pull and push as fast as you can.". They perform 2 runs of the AAT. In the first run, the black border denotes a pull trial whereas in the second run, the black border denotes a push trial. Within each run, the 30 different pictures from each category (high-energy food, alcohol, cigarettes and neutral objects) were presented in a randomised order. Therefore, 1 run consisted of 60 trials (15 pictures x 4 categories).

2.6.6 Visual analogue scale ratings of appetite, food craving and confounding factors

During the study visit, participants completed 10 serial visual analogue scale (VAS) ratings for hunger, prospective volume consumption, pleasantness to eat, fullness, 'desire to eat now', 'better to eat now', nausea, anxiety, sleepiness and stress (Figure 2.4; Appendix 20). VAS ratings were only analysed from time point 2 (T=-10min) to avoid initial stress at the beginning of the study visit, to T=+315 min to avoid variability from the *ad libitum* meal. Absolute VAS ratings, change from baseline (T=-10min), area under the curve ($AUC_{-10\text{-}315min}$) and delta area under the curve ($\Delta AUC_{-10\text{-}315min}$) calculated using the trapezoid rule, were examined for any effects of Exenatide or DAG.

The composite appetite score was calculated from the equation Appetite = [Hunger + Volume to eat + Pleasantness to eat + (100 – Fullness)]/4, in which participants rated their hunger, prospective volume consumption, pleasantness to eat and fullness on a visual analogue scale.

The composite food craving VAS rating was calculated from the sum of the VAS ratings of 'better to eat right now' and 'desire to eat right now', with a maximum score of 14.

2.6.7 Plasma glucose, serum insulin and other hormones

Participants had blood taken for assay of glucose, insulin, GH, prolactin, cortisol, Exenatide

(Exendin-4) and gut hormones (GLP-1, PYY, AG, DAG) at seven time points throughout each study visit (T=-35, +45, +90, +210, +270, +315, +400min)(Figure 2.4 for timings). GH, prolactin and cortisol were measured to allow examination of effects of these gut hormones on other hormones, and allow comparison to previous studies where GH and cortisol were shown to increase with acute GLP-1 analogues administration, but remain unchanged with DAG [141, 363, 387-389]. Missing data was usually attributed to difficulty with phlebotomy and grossly haemolysed blood samples.

Blood for gut hormone concentration was collected in a 10mL lithium heparin tube containing 10mg of 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF, Sigma-Aldrich, Merck UK) and aprotinin protease inhibitor (Nordic Pharma UK). All insulin and gut hormone samples were centrifuged at 4000 Hz for 10 minutes at 4^{o}C. Aliquots of separated plasma were immediately mixed with hydrochloric acid for subsequent assay of acyl ghrelin, and separate unacidified aliquots for assay of GLP-1, Exenatide and PYY. Serum samples were aliquoted and frozen at -80^{o}C until analysis. Samples were analysed using in-house and commercial immunoassays [177, 390]. Remaining plasma samples were analysed for metabolites and hormones at the Department of Biochemistry, Hammersmith Hospital, Imperial College Healthcare NHS Trust immediately upon collection. Plasma glucose and serum insulin were measured using either an Abbott Architect ci8200 analyser (Abbott Diagnostics, Maidenhead, UK) or an Axsym analyser (Abbott Diagnostics, Maidenhead, UK).

Analysis excluded blood samples after T=+315 to avoid variability from *ad libitum* meal. In addition, analysis excluded those visits in which there were no baseline sample or more than one missing value per participant's study visit between T=+45 and T=+315. Delta values from baseline (T=-35), and delta $AUC_{-35\text{-}315min}$ were calculated. Blood AUC data for a visit was calculated only if data from all six timepoints for the variable were available from each study visit. Specifically, a female participant from the dieting group with obesity had to be excluded from the cortisol analysis as she was on the combined oral contraceptive pill. Another female participant from the dieting group with obesity had to be excluded from the prolactin analysis as she was likely to have a microprolactinoma.

2.7 STATISTICAL POWER

The novelty of this experimental design precludes formal power analysis due to lack of directly comparable data. Therefore, the power to detect an effect of gut hormone treatments in fMRI BOLD response was based on an analysis of previous pilot data from studies of the effect of feeding in healthy controls on fMRI tasks, similar to those utilised in the GHADD study. This indicates that with a final n=24 per group and alpha 0.05 the power to detect the following reductions in % BOLD signal with food intake (estimated to be similar in effect size to the gut hormone infusions) are as follows:

i) Food picture evaluation task: 71% power for delta 0.135 with SD 0.251 in OFC during evaluation of high-energy food vs. neutral pictures.

ii) Negative emotional reactivity task: 86% power for delta 0.142 with SD 0.218 in OFC, and 84% power for delta 0.089 with SD 0.142 in dorsal striatum during viewing of unpleasant vs. neutral images;

iii) Monetary Incentive Delay task: 74% power for delta 0.172 with SD 0.311 in amygdala, and 73% power for delta 0.111 with SD 0.203 in striatum during anticipation of win vs. neutral monetary reward;

2.8 STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS v26 (IBM) and GraphPad PRISM v8.

Repeated Student t-test for normally distributed, Mann-Whitney test for non-normally distributed data or Chi-squared test was used to compare participant characteristics between groups (Chapter 3).

Comparison of difference in outcome variables between both groups at the Exenatide vs. saline visit were performed using a repeated measures ANOVA with post-hoc Fisher's Least Significant Difference (LSD) test with visit (saline, Exenatide/DAG) as within subject factor and group (obese, ex-smokers) as between subject factor. Other within subject factors include fROI, time, picture type, dish sweetness (savoury, sweet) and dish fat content (low fat, high fat). No corrections for multiple comparisons were needed as the main effects invariably compared only 2 variables each time (hormone vs. saline visit or dieting obese vs. ex-smokers).

Subsequent analysis on outcome variables was done with repeated measures ANCOVAs with visit order, plasma glucose and nausea ratings as covariates. All statistical tests were two-tailed with a 5% significance level. Cohen's d effect size was measured using G*Power application.

Correlational analysis was used to measure relationship between changes in BOLD signal to HE foods between Exenatide or DAG and saline visits to eating behavioural traits (using DEBQ, TFEQ and BES scores). A number of eating behaviour questionnaires were compared between the 2 groups. Correction for multiple corrections was not performed, and so some caution is needed in their interpretation, though it should be appreciated that these are not all independent measures of eating behavioural traits, since they either directly measure similar traits (e.g. different questionnaires for dietary restraint from DEBQ and TFEQ questionnaires), or are correlated as measure overlapping phenotypes (e.g. DEBQ-emotional eating, TFEQ-disinhibition eating, YFAS, BES all include eating in response to stress and/or loss of control; DEBQ-external eating and Power of Food both include overeating in response to hedonic properties of food).

CHAPTER 3:

PARTICIPANT CHARACTERISTICS

3.1 INTRODUCTION

A comparison between the characteristics and eating traits of the two participants groups relevant to this PhD; (i) group with obesity on a diet and (ii) abstinent nicotine-dependent group is presented in this chapter. As previously discussed (Section 1.3), eating behaviour encompasses many aspects of the relationship with food, including food choice, preference, hedonic responses and eating traits [110]. Some of these facets can be measured through fMRI neural responses to food cues, food intake at a test meal and food-related or personality-related questionnaires, including Three Factor Eating Questionnaire (TFEQ), Dutch Eating Behavioural Questionnaire (DEBQ) and Barratt Impulsivity Scale.

As previously discussed in Section 2.6, the between-group analysis between dieting group with obesity and ex-smokers has its limitations, notably because of the differences in BMI and previous nicotine dependence which may have led to changes in reward processing pathways in the brain. Therefore, a thorough comparison of the two participant groups is performed, including demographics, metabolic parameters, eating behavioural questionnaires, personality trait questionnaires. These differences in group characteristics are likely to influence the effects of gut hormones in the current study and therefore should be taken into consideration when interpreting the results of outcomes measures.

3.2 AIMS

To investigate the differences between dieting adults with obesity, and abstinent nicotine-dependent adults in:

1. demographic parameters, including age, sex, BMI, bio-electrical impedance analysis of body fat, and waist circumference,
2. metabolic parameters, including fasting glucose, insulin, Homeostatic Model Assessment-Insulin Resistance (HOMA-IR), lipids and metabolic syndrome score,
3. eating behaviour questionnaires, including Dutch Eating Behavioural Questionnaire (DEBQ)-emotional eating, Three Factor Eating Questionnaire (TFEQ)-hunger related eating, Binge eating scale (BES), Yale Food Addiction Scale (YFAS), TFEQ-external eating, TFEQ-disinhibited eating, TFEQ- and DEBQ-dietary restraint,

Secondary analyses will explore:

4. differences in impulsivity [Urgency Premeditation Perseverance Sensation-seeking Positive urgency Impulsive behavioural scale (UPPS-P), Barratt impulsivity scale], stress [Perceived Stress scale (PSS)], trait anxiety [State-Trait Anxiety Inventory (STAI)], reward and punishment [Behavioural Approach Scale Behavioural Inhibition Scale (BAS/BIS)], intellectual function and depression,
5. correlation between BMI and questionnaire-based eating behaviours in both groups,
6. correlation between nicotine dependence [as measured by Fagerstrom Test for Nicotine Dependence (FTND)], and duration of cigarette abstinence, with BMI and eating behaviours in the ex-smoker group.

3.3 RESULTS

3.3.1 Demographics and metabolic profile

Characteristics for participants from dieting group with obesity (obese) and ex-smokers included in data analysis in Table 3.1.

	Obese		Ex-smokers		Test statistic	P value
n	25		25			
Demographics		Range		Range		
Age (years)	42.4 ± 11.5	(24.0 - 60.0)	37.7 ± 9.4	(25.0 - 60.0)	t -1.65	0.11
Male, n (%)	7 (28.0%)		12 (48.0%)		γ 2.12	0.15
Caucasian, n (%)	16 (64.0%)		21 (84.0%)		γ 2.60	0.11
Height (m)	1.68 ± 0.08	(1.53 - 1.83)	1.72 ± 0.09	(1.53 - 1.88)	t 1.54	0.13
Weight (kg)	105.5 ± 17.4	(78.4 - 154.3)	75.5 ± 13.6	(49.7 - 101.0)	t -6.80	**<0.001*** **
BMI (kg/m^2)	37.1 ± 4.9	(29.5 - 46.1)	25.4 ± 3.6	(19.1 - 32.4)	t -9.74	**<0.001*** **
Max BMI (kg/m^2)	40.1 ± 5.4[a]	(30.3 - 49.4)	26.7 ± 4.4	(20.6 - 36.5)	t -9.47	**<0.001*** **
Metabolic parameters						
Body fat (%)	41.1 ± 6.4	(27.8 - 51.9)	26.8 ± 7.7	(15.0 - 42.7)	t -7.14	**<0.001*** ** [c] **<0.001 adj**
Truncal fat mass (kg)	22.2 ± 6.0	(9.55 - 37.3)	10.9 ± 4.9[a]	(4.2 - 19.9)	t -7.23	**<0.001*** ** [c] **<0.001 adj**
Truncal to total fat mass (%)	0.51 ± 0.07	(0.36 - 0.61)	0.53 ± 0.10[a]	(0.37 - 0.66)	t 0.99	0.33 [c] 0.57 adj
Waist (cm)	112.2 ± 14.1	(88.0 - 148.0)	89.9 ± 12.9	(69.0 - 112.0)	t -5.83	**<0.001*** **
Waist-hip ratio	0.89 ± 0.10	(0.76 - 1.08)	0.91 ± 0.12	(0.78 - 1.37)	t 0.61	0.54 [c] 0.93 adj
Systolic BP (mmHg)	128.8 ± 6.3	(114.0 - 143.0)	120.1 ± 11.6	(103.0 - 146.0)	t -3.29	**0.002****
Diastolic BP (mmHg)	76.8 ± 6.8	(65.0 - 88.0)	73.0 ± 8.5	(60.0 - 92.0)	t -1.76	0.084
Fasting glucose (mmol/L)	5.1 ± 0.5	(4.3 - 6.1)	4.8 ± 0.4	(4.1 - 5.7)	t -1.87	0.067
Fasting insulin (mU/L)	9.4 [5.8 - 12.7]	(2.3 - 47.3)	3.6 [2.4 - 6.5]	(1.4 - 12.3)	Z 3.70	**<0.001*** **
HOMA-IR	1.95 [1.27 - 2.82]	(0.46 - 10.96)	0.75 [0.49 - 1.37]	(0.30 - 2.74)	Z 3.85	**<0.001*** **
Fasting lipids						
Total cholesterol (mmol/L)	5.15 ± 0.97	(3.63 - 7.13)	4.81 ± 0.81	(3.60 - 6.33)	t -1.35	0.18
HDL (mmol/L)	1.17 ± 0.30	(0.73 - 2.09)	1.40 ± 0.39	(0.67 - 2.27)	t 2.29	**0.026***
LDL (mmol/L)	3.32 ± 0.83	(1.90 - 4.91)	2.92 ± 0.69	(2.01 - 4.56)	t -1.83	0.074
Triglycerides (mmol/L)	1.46 ± 0.66	(0.43 - 2.77)	1.15 ± 0.70	(0.49 - 3.07)	t -1.57	0.12
Metabolic syn score[b]	3.0 [1.0 - 4.0]	(0 - 5.0)	1.0 [0 - 2.0]	(0 - 5.0)	Z 3.31	**0.001****
Metabolic syn diagnosis[b], n (%)	13 (52.0%)		5 (20.0%)		γ 5.56	**0.018***
Liver function tests						
ALT [<35 U/L]	28.0 [17.7 - 40.3]	(10.3 - 163.0)	23.0 [17.8 - 26.8]	(10.7 - 67.7)	Z 1.71	0.088
AST [<41 U/L]	31.0 [26.3 - 36.3]	(24.0 - 89.0)	27.6 [25.4 - 30.1]	(19.0 - 48.5)	Z 1.62	0.11
GGT [<40 U/L]	25.7 [16.0 - 39.5]	(10.3 - 66.0)	16.7 [13.0 - 21.7]	(10.3 - 76.0)	Z 2.08	**0.038***

Table 3.1. Participant characteristics.

Data given as mean ± SD (range), or median [IQR] (range) if not normally distributed, or n (%). Statistics and P value given for between group comparison using Student t-test (t), Mann-Whitney test (Z) and chi-squared test (γ) respectively: *obese vs. ex-smokers: *P<0.05, **P<0.01, ***P<0.001. Weight, BMI, body fat percentage, truncal fat mass, blood pressure measurements, plasma glucose, serum insulin, HOMA-IR, HOMA-B, lipid profiles and liver function tests were averaged across the 2-3 study visits.

[a] n=24, [b] refers to NCEP ATP III classification, [c] represents P values adjusted for sex.

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; BP, blood pressure; GGT, Gamma-glutamyl Transferase; HDL, High-density lipoprotein; HOMA-B, Homeostatic Model Assessment-beta; HOMA-IR, Homeostatic Model Assessment-Insulin Resistance; LDL, Low-density lipoprotein; Metabolic syn, metabolic syndrome.

There were no significant differences in age, sex or ethnicity between groups. As expected, the dieting group with obesity had higher current weight, BMI and maximum lifetime BMI than the ex-smoker group (Table 3.1). Similarly, they had higher body fat percentage, truncal fat mass, waist circumference and systolic blood pressure than the ex-smokers (Table 3.1).

Regarding metabolic parameters, the dieting group with obesity had a significantly higher fasting serum insulin, reduced fasting serum HDL-cholesterol and elevated fasting LDL concentrations, as compared to ex-smoker group (Table 3.1). There was also higher serum GGT concentrations in dieting group with obesity compared to ex-smokers. This is likely to reflect the increased fatty deposits in the liver in the group with obesity.

As hypothesised, the dieting group with obesity had a higher homeostatic model assessment of insulin resistance (HOMA-IR), and higher metabolic syndrome score based on US National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) classification relative to the ex-smokers (Table 3.1).

HOMA-IR is used as a model to estimate insulin resistance [391]. It is calculated from this formula: HOMA-IR = glucose(mmol/L) * insulin (mU/L) / 22.5. The NCEP ATP III classification defines metabolic syndrome as having at least three of the following five abnormalities [392]:

1. Blood pressure: systolic $\geq$ 130 mmHg and/or diastolic $\geq$ 85 mmHg;
2. Serum triglycerides: $\geq$1.69 mmol/L;
3. Serum HDL cholesterol: <1.04mmol/L in men, or <1.29 mmol/L in women;
4. Waist circumference: >102 cm in men, or >88 cm in women;
5. Fasting blood glucose: $\geq$6.1mmol/L.

3.3.2 Eating behaviour and trait questionnaires

To test if the dieting group with obesity had unhealthier eating behaviour, or other traits related to overeating, eating behaviour questionnaire scores and trait questionnaires (such as perceived stress, trait anxiety, impulsivity, reward sensitivity) were compared between groups (Table 3.2).

As hypothesised, the dieting group with obesity, compared to the ex-smokers, had:

(i) greater dietary restraint indicated by higher DEBQ-restraint and TFEQ-restraint scores,

(ii) greater 'food addiction' trait indicated by higher YFAS score and prevalence of YFAS food

addiction diagnosis,

(iii) greater binge eating, and greater prevalence of moderate/severe binge eating, from higher BES questionnaire scores,

(iv) greater emotional eating indicated by higher DEBQ-emotional scores,

(v) greater uncontrolled eating indicated by higher TFEQ-disinhibition scores,

On the other hand, the ex-smoker group had:

(vi) a higher UPPS-sensation seeking score, indicating a greater tendency to seek out novel experiences as part of an impulsive trait;

However, there was no significant differences between the 2 groups in:

(vii) overeating due to hedonic qualities of food indicated by similar DEBQ-external eating score,

(viii) perceived stress (PSS),

(ix) trait anxiety (STAI),

(x) other impulsivity measures from other sub-scales from UPPS-P or Barratt impulsivity scale (BIS-11) questionnaires,

(xi) reward and punishment sensitivity (BAS-BIS scores).

	Obese		Ex-smokers		Test statistic	P value
n	25		25			
Eating Behaviour Questionnaires						
Dietary restraint						
DEBQ-Restraint (max 5)	3.03 ± 0.69	(1.50 - 4.20)	2.46 ± 0.89	(1.10 - 3.70)	t -2.54	**0.014***
TFEQ-Restraint (max 21)	11.0 ± 5.0[a]	(2.0 - 20.0)	7.9 ± 4.6	(1.0 - 17.0)	t -2.31	**0.025***
Food hedonics						
DEBQ-External (max 5)	3.20 ± 0.84	(1.70 - 5.00)	2.95 ± 0.65	(1.60 - 4.20)	t -1.19	0.24
Power of Food Scale (max 105)	58.2 ± 19.6[a]	(24.0 - 98.0)	47.9 ± 17.3	(24.0 - 91.0)	t -1.94	0.058
YFAS score (max 7)	2.0 [1.0 - 4.5]	(1.0 - 7.0)	1.0 [1.0 - 1.5]	(0.0 - 5.0)	Z 5.93	**<0.001*** **
Food addiction diagnosis	9 (36.0%)		3 (12.0%)		γ 3.95	**0.047***
Binge Eating Scale (max 46)	17.6 ± 10.1	(1.0 - 38.0)	7.8 ± 6.2[a]	(0.0 - 21.0)	t -4.13	**<0.001*** **
Moderate/Severe BES (score > 16)	13 (52.0%)		3 (12.5%)		γ 8.69	**0.003****
Other						
DEBQ-Emotional (max 5)	2.94 ± 1.13	(1.00 - 4.62)	2.09 ± 1.10	(1.00 - 4.62)	t -2.70	**0.010***
TFEQ-Disinhibition (max 16)	9.9 ± 4.1[a]	(3.0 - 16.0)	5.8 ± 3.5	(2.0 - 14.0)	t -3.82	**<0.001*** **
TFEQ-Hunger (max 14)	6.9 ± 3.9[a]	(1.0 - 14.0)	5.1 ± 3.3	(0.0 - 12.0)	t -1.69	0.097
Trait questionnaires						
PSS (max 40)	11.0 ± 7.0	(0.0 - 28.0)	10.0 ± 6.2	(0.0. - 20.0)	t -0.58	0.57
STAI trait (max 80)	33.2 ± 8.5	(21.0 - 53.0)	33.8 ± 11.2	(0.0 - 53.0)	t 0.20	0.84
Barratt impulsivity scale (max 120)	60.0 ± 10.4[a]	(39.0 - 79.0)	61.9 ± 9.8	(44.0 - 79.0)	t 0.67	0.51
UPPS-P						
Negative urgency (max 4)	2.32 ± 0.49	(1.33 - 3.17)	2.34 ± 0.53	(1.33 - 3.25)	t 0.14	0.89
Premeditation (max 4)	1.95 ± 0.41	(1.18 - 2.91)	2.04 ± 0.35	(1.36 - 2.73)	t 0.84	0.41
Perseverance (max 4)	1.94 ± 0.47	(1.00 - 2.90)	1.90 ± 0.38	(1.20 - 2.50)	t -0.33	0.74
Sensation seeking (max 4)	2.57 ± 0.46	(1.20 -3.42)	2.95 ± 0.66	(1.00 - 3.83)	t 2.41	**0.020***
Positive urgency (max 4)	1.75 ± 0.45	(1.07 - 2.79)	1.86 ± 0.45	(1.29 - 3.00)	t 0.84	0.40
Behavioural activation and inhibition scale						
BAS drive (max 16)	11.6 ± 2.6	(7.0 - 16.0)	11.3 ± 1.4[a]	(8.0 - 13.0)	t -0.24	0.81
BAS reward responsiveness (max 20)	16.9 ± 1.8	(14.0 - 20.0)	16.9 ± 1.6[a]	(15.0 - 20.0)	t 0.74	0.47
BAS fun-seeking (max 16)	11.7 ± 2.0	(7.0 - 16.0)	11.8 ± 2.3[a]	(7.0 - 16.0)	t 0.59	0.56
BIS (max 28)	20.4 ± 3.5	(13.0 - 27.0)	21.1 ± 2.9[a]	(17.0 - 26.0)	t 1.30	0.20

Table 3.2. Eating behaviour and trait questionnaires for participants.

Data given as mean ± SD (range), median [IQR] (range) if not normally distributed, or n (%). Statistics and P value given for between group comparison using Student t-test(t), Mann Whitney test (Z) and chi-squared test (γ) respectively: *obese vs. ex-smokers: *P<0.05, **P<0.01, ***P<0.001.

[a] n=24. Abbreviations: BAS, Behavioural Activation Scale; BES, Binge Eating Scale; BIS, Behavioural Inhibition Scale; DEBQ, Dutch Eating Behaviour Questionnaire; PSS, Perceived Stress Scale; STAI, State-Trait Anxiety Inventory; TFEQ, Three Factor Eating Questionnaire; UPPS-P Urgency, Premeditation, Perseverance, Sensation seeking, and Positive urgency; YFAS, Yale Food Addiction Scale.

3.3.3 Correlations of eating behaviour

3.3.3.1 Correlations with BMI in dieting group with obesity and ex-smokers

To examine the relationship between BMI and eating behaviours in both participant groups, a correlational analysis was performed between these variables separately in each group (Table 3.3).

In dieting group with obesity, there was a trend for a negative correlation between BMI and TFEQ-restraint, suggesting the higher the BMI, the lower the dietary restraint. However, no correlational relationship was seen between BMI and the DEBQ dietary restraint questionnaire, nor other unhealthy eating behaviours (Table 3.3, Figure 3.2A&B).

By contrast, in the ex-smokers (Table 3.3, Figure 3.3), there were moderate positive correlations between BMI and:

(i) DEBQ-restraint,

(ii) TFEQ-restraint (Figure 3.3A),

(iii) TFEQ-disinhibition (Figure 3.3B), and

(iv) TFEQ-hunger scores,

These correlational results in the ex-smokers indicates that the higher the BMI, the greater dietary restraint, increased uncontrolled eating and greater susceptibility to hunger cues.

OBESE	**DEBQ-emotional**	**DEBQ-external**	**DEBQ-restaint**	**TFEQ-restraint**	**TFEQ-hunger**	**TFEQ-disinhibition**	**BES**
BMI							
Correlation Coefficient	-0.02	-0.24	-0.03	-0.37	-0.007	0.11	-0.04
P value	0.93	0.25	0.88	0.073	0.98	0.62	0.83
EX-SMOKERS							
BMI							
Correlation Coefficient	0.07	0.18	0.53	0.45	0.48	0.47	0.40
P value	0.75	0.39	**0.006**	**0.026**	**0.015**	**0.018**	0.056

Table 3.3. Correlations between BMI and eating behaviour in dieting group with obesity and ex-smokers.
In ex-smokers, but not dieting group with obesity, BMI positively correlated with dietary restraint, TFEQ-hunger and TFEQ-disinhibition scores. Spearman correlation coefficients in obesity (n=25) and ex-smokers (n=25) between BMI and DEBQ, TFEQ and BES questionnaire scores. Abbreviations: BES, Binge Eating Scale; BMI, body mass index; DEBQ, Dutch Eating Behavioural Questionnaire; TFEQ, Three Factor Eating Questionnaire.

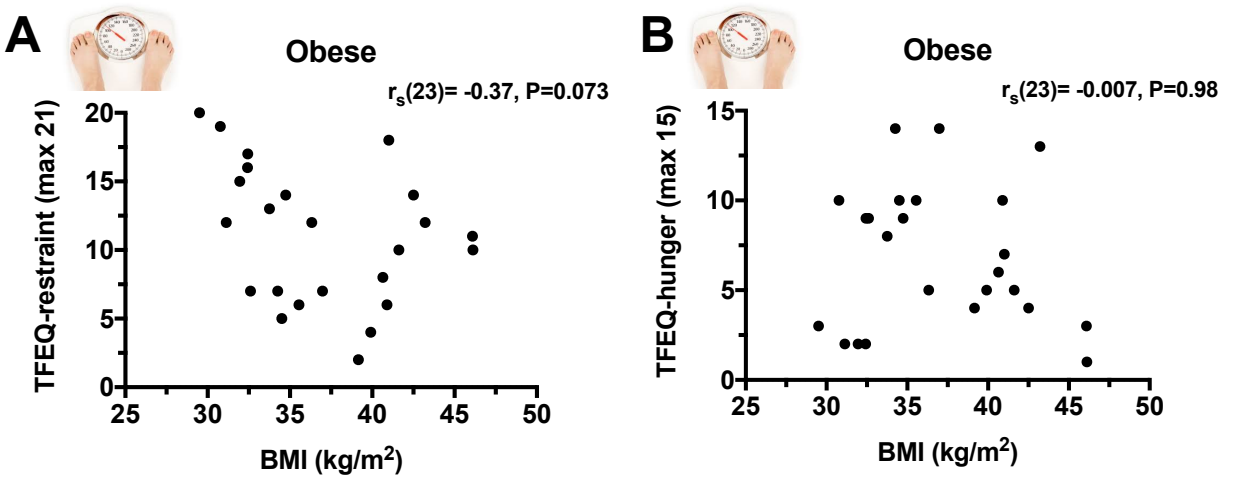


Figure 3.2. Correlation between BMI and dietary restraint and hunger-related eating in dieting group with obesity. Correlational analysis in obesity (n=25) between BMI and scores from (A) TFEQ-restraint and (B) TFEQ-hunger questionnaires, indicating a trend for a negative correlation between BMI and dietary restraint. r indicates Spearman correlation coefficient. Abbreviations: BMI, body mass index; TFEQ, Three Factor Eating Questionnaire.

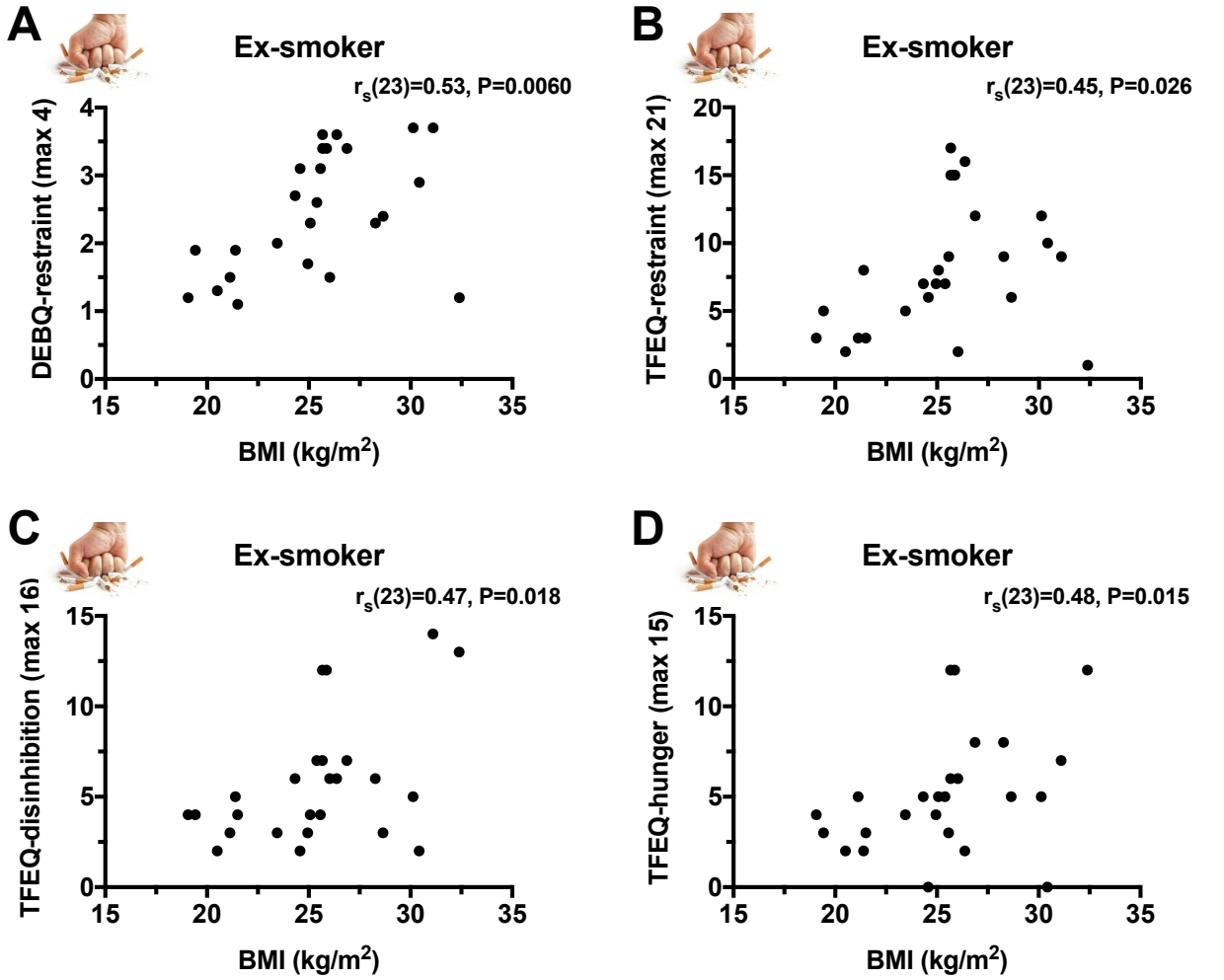


Figure 3.3. Correlation between BMI and dietary restraint, uncontrolled and hunger-related eating in ex-smokers. Correlational analysis in ex-smokers (n=25) between BMI and scores from (A) DEBQ-restraint, (B) TFEQ-restraint, (C) TFEQ-disinhibition and (D) TFEQ-hunger. Questionnaires, indicating positive correlations between BMI and dietary restraint and hunger-related over-eating**.** r indicates Spearman correlation coefficient. Abbreviations: BMI, body mass index; DEBQ, Dutch Eating Behavioural Questionnaire; TFEQ, Three Factor Eating Questionnaire.

3.3.3.2 Correlations with smoking variables in ex-smokers

To explore the relationship between severity of nicotine dependence and duration of cigarette abstinence with eating behaviours in ex-smokers, correlations were examined between retrospective FTND score and weeks since last smoking, with BMI and eating behaviour questionnaires (Tables 3.4 and 3.5).

There was a moderate positive significant correlation between BMI and FTND score, suggesting the higher the previous nicotine dependence, the higher the current BMI (Table 3.4, Figure 3.4A). Related to this, FTND also had a significant positive correlation with TFEQ-hunger scores (Table 3.4, Figure 3.4B). This indicates that the higher the previous nicotine dependence the higher the hunger-related overeating.

However, there were no significant correlations between duration of cigarette abstinence and BMI or any eating behaviour questionnaire (Table 3.4).

EX-SMOKERS	BMI	DEBQ-emotional	DEBQ-external	DEBQ-restaint	TFEQ-restraint	TFEQ-hunger	TFEQ-disinhibition	BES
FTND								
Correlation Coefficient	0.40	0.25	0.25	0.09	-0.006	0.41	0.32	0.40
P value	**0.048**	0.23	0.23	0.67	0.98	**0.040**	0.13	0.051
Duration of cigarette abstinence								
Correlation Coefficient	0.27	-0.04	-0.05	0.24	0.17	0.24	0.27	0.10
P value	0.19	0.84	0.82	0.25	0.41	0.25	0.19	0.66

Table 3.4. In ex-smokers, FTND positively correlated with BMI and TFEQ-hunger scores.
Spearman correlation coefficient in ex-smoker group (n=25) between FTND and duration of cigarette abstinence (weeks) with BMI, DEBQ, TFEQ and BES scores. Abbreviations: BES, Binge Eating Scale; BMI, body mass index; DEBQ, Dutch Eating Behavioural Questionnaire; FTND, Fagerstrom Test for Nicotine Dependence TFEQ, Three Factor Eating Questionnaire.

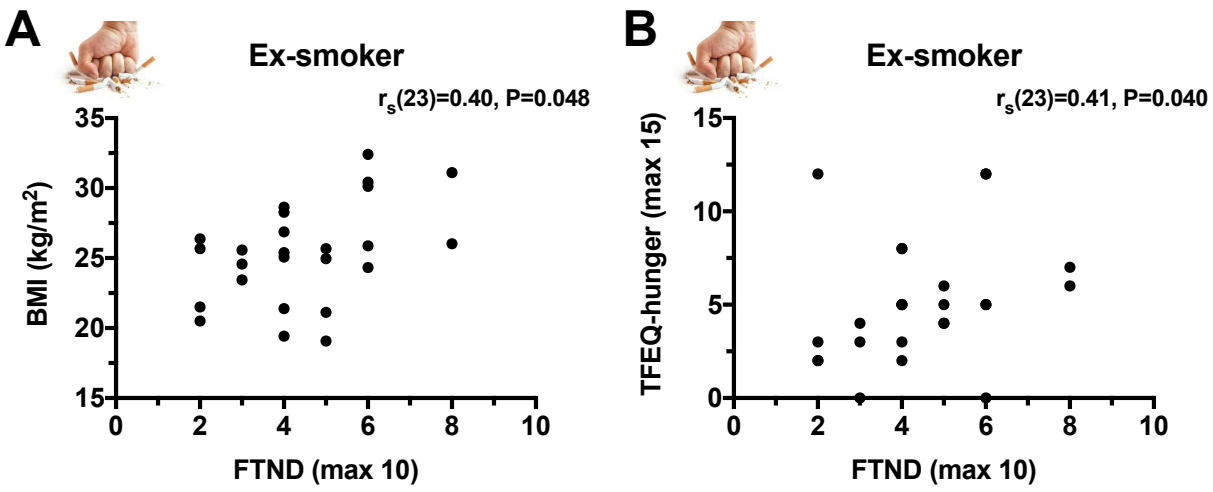


Figure 3.4. Correlation between severity of nicotine dependence and BMI and hunger-related eating in ex-smokers.

Correlational analysis in ex-smokers (n=25) between FTND and (A) BMI and (B) TFEQ-hunger questionnaire score, indicating positive correlations between severity of retrospective nicotine dependence and current BMI and hunger-related over-eating. r indicates Spearman correlation coefficient. Abbreviations: BMI, Body mass index; FTND, Fagerstrom Test for Nicotine Dependence; TFEQ, Three Factor Eating Questionnaire.

3.3.4 Other group characteristics

To test if there were any differences in other characteristics between the two groups, including those that may influence comparison of the main study outcomes, comparison was made between dieting group with obesity and ex-smokers for measures of intellectual function (education and reading level), psychiatric history (alcohol use, traits and current symptoms), and family history (Table 3.5).

There was no significant difference in years of education or reading level (from WTAR score) between groups (Table 3.5). Intellect could affect the ability to comprehend and perform fMRI and behavioural tasks, particularly those involving memory (not included in this thesis), thereby confounding interpretation of results.

The ex-smokers had significantly higher scores than dieting group with obesity for DSM-5 measures of alcohol use disorder (AUD) and AUDIT questionnaire, suggesting greater alcohol misuse in the ex-smokers (Table 3.5). However, no participants had severe AUD (all DSM 5 scores < 6) although the absolute difference in AUDIT scores between groups was large [effect size mean ± SEM 4.16 ± 1.08 , (95%CI 1.96, 6.36), P=0.001, Cohen's d=2.43]. Whilst this should not affect outcomes relating to high-energy food and food reward behaviours, this could impact and confound the interpretation of outcomes relating to alcohol cues.

There were no significant differences between the two groups in current depressive symptoms as measured from BDI-II questionnaire, or prevalence of previous diagnosis of depression (Table 3.5). Depression may lead to alterations in BOLD signal in various fMRI tasks as well as motivation to participate in behavioural tasks, therefore it is an important variable to compare between-groups.

However, there was an increased prevalence of obesity in first degree relatives in the dieting group with obesity compared to ex-smoker group (relative risk (RR) 2.67). Similarly, in ex-smokers, there was higher prevalence of a positive family history of not only smoking (RR 1.75), but also alcohol problems (RR 4.5), when compared to dieting group with obesity. This comparison was undertaken to better characterise the cohorts as it has been shown that people with positive family history of obesity [393] and smoking [394] have altered brain responses to cue reactivity.

	Obese		Ex-smokers		Test statistic	P value
n	25		25			
Intellect		Range		Range		
Education (years)	16.0 ± 4.3	(7.0 - 25.0)	16.4 ± 2.8	(8.0 - 20.0)	t 0.35	0.73
WTAR (max 50)	40.4 ± 8.5	(16.0 - 50.0)	43.6 ± 4.7	(33.0 - 49.0)	t 1.62	0.11
Medical history and substance use						
Abstinence from cigarettes (weeks)	n/a		20.7 [12.3 - 55.0]	(7.1 - 161.6)	n/a	n/a
FTND (max 10)	n/a		4.4 ± 1.7	(2.0 - 8.0)	n/a	n/a
DSM 5 for alcohol dep (max 11)	1.0 [0 - 1.0]	(0 - 3.0)	1.0 [0 - 2.0]	(0 - 5.0)	Z -2.15	**0.031***
AUDIT (max 40)	3.5 ± 1.9	(1.0 - 8.0)	7.7 ± 5.0	(0.0 - 18.0)	t 3.86	**0.001****
Previous depression, n (%)	3 (12.0%)		6 (24.0%)		γ 1.22	0.27
BDI-II (max 63)	3.0 [0.5 - 8.0]	(0.0 - 24.0)	4.0 [0.0 - 6.0]	(0.0 - 13.0)	Z 0.50	0.62
Family history						
IHD, n (%)	10 (40.0%)		6 (24.0%)		γ 1.47	0.23
DM, n (%)	11 (44.0%)		6 (24.0%)		γ 2.23	0.14
Obesity, n (%)	16 (64.0%)		6 (24.0%)		γ 8.11	**0.004****
Smoking, n (%)	12 (48.0%)		21 (84.0%)		γ 7.22	**0.007****
Alcohol problem, n (%)	2 (8.0%)		9 (36.0%)		γ 5.71	**0.017***
Drug problem, n (%)	1 (4.0%)		5 (20.0%)		γ 3.03	0.080

Table 3.5. Intellectual function, psychiatric and family history of participants.

Data given as mean ± SD (range), median [IQR] (range) if not normally distributed, or n (%). Statistics and P value given for between group comparison using Student t-test(t), Mann Whitney test (Z) and chi-squared test (γ) respectively: *obese vs. ex-smokers: *P<0.05, **P<0.01, ***P<0.001. DSM 5 for alcohol dependence (Appendix 19). Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; BDI-II, Beck Depression Inventory-II; dep, dependence; DM, Diabetes Mellitus; DSM 5, Diagnostic and Statistical Manual of Mental Disorders-5; FTND, Fagerstrom Test for Nicotine Dependence; IHD, Ischaemic heart disease; WTAR, Wechsler Test of Adult Reading.

3.4. DISCUSSION

This chapter presented the participant characteristics and a direct comparison between the dieting group with obesity and ex-smokers in terms of BMI, metabolic profile and eating behaviour.

Demographics

The dieting group with obesity has significantly higher weight, BMI, maximum lifetime BMI, body fat and waist circumference than ex-smokers. By virtue of the study design, the dieting group with obesity had to have a BMI above 30 kg/m^2 at screening visit. However, this criterion did not apply to ex-smokers as they were selected based on their previous nicotine dependence. Even though the ex-smoker had significantly lower BMI than the dieting group with obesity, it should be highlighted that the ex-smokers were by no means a 'lean' group as 15 out of the 25 were overweight (BMI $\geq$25 kg/m^2) including 4 with obesity (BMI $\geq$30kg/m^2).

It was important to explore the differences in BMI and other related demographic variables so as to account for any confounding variables. Notably, there were also no significant differences in age, sex or ethnicity between the two participant groups.

Metabolic parameters

The dieting group with obesity, compared to ex-smokers, had significantly higher fasting serum insulin, higher HOMA-IR index, higher prevalence of metabolic syndrome diagnoses (RR 2.6), reduced fasting serum HDL-cholesterol and elevated fasting LDL-cholesterol concentrations and tended to have a higher fasting plasma glucose concentration.

Different studies have proposed a range of cut-off values for diagnosis of insulin resistance depending on the presence of T2DM and other features of metabolic syndrome. There is great variability in the threshold levels but in general, approximately an index of 1.85 in men and 2.05 in women without T2DM is thought to indicate insulin resistance [395]. Based on this, there were n=13 dieting participants with obesity and n=4 ex-smokers with insulin resistance in the current study. The relative risk of insulin resistance in obesity is 8.7 times that in ex-smokers.

These findings are in agreement with well-established notion that obesity is a risk factor for

metabolic disorders [396, 397]. Indeed, in the current study, the dieting group with obesity was not representative of the general population with obesity as individuals with T2DM and other cardiovascular diseases (hypertension, previous myocardial infarction) were excluded. Despite this, they still had worse metabolic parameters compared to ex-smokers.

Furthermore, the dieting group with obesity, compared to ex-smokers, had greater prevalence of obesity in first degree relatives. On the other hand, in ex-smokers, there was higher prevalence of positive family history of smoking, alcohol problems and a trend for drug problems compared to dieting group with obesity. This is consistent with a genetic predisposition to obesity, smoking and other addictions such as alcohol and drug use disorders. This has been well recognised from genetic studies, including twin studies with heritability estimates for BMI of 0.57-0.75 [398], and nicotine dependence of up to 0.50 [399] Through genome-wide association studies (GWAS), different loci have been identified associated with obesity and smoking [400, 401], providing insights into the genetic aetiology of these conditions.

Eating behavioural traits

The dieting group with obesity had greater dietary restraint, emotional eating, uncontrolled eating, food addiction traits, and binge eating symptoms compared to ex-smokers. Furthermore, they tended to have a greater appetitive drive to consume palatable food and susceptibility to eating when hungry. However, there was no significant difference between the groups in DEBQ-external eating scores, indicating a similar tendency to overeating due to hedonic qualities of food. The higher prevalence of unhealthy eating behaviours in obesity, such as emotional eating [402], high dietary restraint [403], eating disinhibition [404], food addiction [405], and binge eating [406], contributes to overeating and weight regain after dieting.

Correlations of BMI with unhealthy eating behaviours

In ex-smokers, BMI *positively* correlated with dietary restraint, disinhibited eating and susceptibility to eat in response to hunger cues, indicating the higher the BMI, the unhealthier the eating behaviours. Disinhibition reflects tendency towards overeating, especially in obesogenic environment [407], whereas hunger-related eating, as measure in the TFEQ-hunger, describes the susceptibility to hunger feelings and the extent of subsequent eating [408].This finding concurs with studies showing higher susceptibility to eat when hungry, greater disinhibition and restraint is associated with higher BMI within a normal population [404, 408-

410]. Moreover, disinhibition was positively correlated to weight gain [411]. Restraint was also associated with weight changes but was sex-dependent. In females, high restraint promoted weight gain whereas the opposite was seen in males [412].

Interestingly, in dieting group with obesity, there was a trend for a *negative* correlation between BMI and TFEQ restraint scores, suggesting the higher the BMI, the less the dietary restraint. This is in agreement with some studies showing that within obesity, restraint correlated negatively with BMI [403, 404]. Dietary restraint may be initiated as a response to weight gain thereby explaining the positive correlation with BMI in lean participants[403]. However, in obesity, where overeating is ubiquitous, it is argued that dietary restraint is beneficial because it can help control food intake and combat overeating. Therefore, a negative correlation between restraint and BMI is seen in obesity [403].

Notably, there was no correlation found between BMI and other eating behavioural constructs in the dieting group with obesity. This could be due to the relatively small numbers in this study. Another possible explanation is that the dieting group with obesity were actively dieting with recent weight loss. This may have disrupted the relationship between BMI and eating behaviour traits, either because weight loss has altered eating behaviour possibly through influence of gut hormones [413-415], or because it was pre-diet BMI rather than current BMI which was related to eating behaviour. Unfortunately, pre-diet BMI was unavailable.

Correlations of severity of previous nicotine dependence with unhealthy eating behaviours

In ex-smokers, severity of nicotine dependence as measure by FTND correlated positively with BMI. This indicates the more severe the nicotine dependence, the higher the BMI. In a prospective study of people intending to quit smoking, an increased cigarette consumption was positively associated with BMI at baseline but not weight gain [416]. On the other hand, other studies have demonstrated a correlation between greater nicotine dependence and increased post-cessation weight gain [417, 418]. Collectively, these studies could explain the findings in the current study; a pre-existing positive correlation between severity of nicotine dependence and BMI in smokers was strengthened by post-cessation weight gain.

In the ex-smokers, FTND correlated positively with TFEQ-hunger scores. This suggests the more severe the nicotine dependence, the unhealthier the eating behaviour. Supporting this, Chao et

al also reported a positive correlation between nicotine dependence and cravings for unhealthy foods in current smokers. The finding in the current study is novel. This may be reflective of pre-existing unhealthy eating behaviour in heavier smokers prior to quitting or it may signify a change in the eating behaviour following nicotine abstinence.

However, no associations were seen with the duration of nicotine abstinence with BMI or unhealthy eating behaviour. In a prospective study, duration of nicotine abstinence was associated with significant smoking cessation weight gain [416]. Unfortunately, a pre-smoking cessation BMI was not available and hence calculating the change in weight was not possible in this current study. There remains a knowledge gap in this area, in particular what unhealthy eating behaviours smokers exhibit and if there are changes after smoking cessation. To address this, larger prospective studies in eating behaviour in smokers and after cessation will be needed to confirm the findings in the current study and may establish causality.

Other confounding personality traits

Ex-smokers, compared to dieting group with obesity, had a higher UPPS-sensation seeking score. UPPS-P Impulsivity scale measures five facets of impulsive behaviour, one of which is sensation seeking, and higher scores denote greater tendency to seek out novel experiences as part of an impulsive trait. Importantly, there were no significant differences between the groups in other impulsivity measures from other UPPS-P subscales or Barratt impulsivity scale (BIS-11), perceived stress, trait anxiety or sensitivity to reward and punishment. Overall, both groups had similar confounding traits.

It is important to explore any differences in confounding these traits between groups as they could alter food intake and neural responses to food cues. For instance, higher trait impulsivity, measured with Impulsivity scale of the Temperament and Character inventory, was associated with enhanced activation during anticipatory food reward in the ACC and amygdala in healthy volunteers [419]. In females, higher trait anxiety was positively associated with greater food intake and non-suppression of postprandial BOLD signal to HE food pictures vs. LE food in the amygdala, insula, dorsal striatum, NAcc and medial OFC [420]. Similarly, in healthy volunteers, trait reward sensitivity as measured by the Behavioural Activation Scale (BAS), predicted BOLD signal to HE foods vs. LE food in NAcc, amygdala, VTA, OFC and ventral pallidum [421].

Strengths and limitations

This comparison between the dieting group with obesity and ex-smokers covered a comprehensive list of variables which would be necessary for the interpretation of the study outcome measures. It was essential to demonstrate that the dieting group with obesity had higher BMI compared to ex-smokers so that interpretation of the outcome measures could take into account the difference in BMI and smoking status. In addition, a thorough comparison of other characteristics and personality traits was performed so as to account for any confounding factors relating to the outcome measures of the study.

In this study, having a group of ex-smokers also provided better insight into the relationship between unhealthy eating behaviours and previous nicotine dependence. Many studies tended to focus on current smokers but studies in ex-smokers are lacking. Studying ex-smokers could provide better understanding for the changes seen following nicotine abstinence and pave the way for development of strategies in prevention of smoking cessation weight gain and smoking relapse.

However, as alluded to, the limitations of this study are the small numbers of participants and its cross-sectional study design. This meant that the comparisons and correlations could have been underpowered. It was also impossible to then examine changes in weight and eating behaviour from dieting in group with obesity and smoking cessation in ex-smokers.

Another limitation of the study is the lack of a group of lean healthy controls who have never smoked. When comparing the dieting group with obesity to ex-smokers, any differences in variables could be attributed to either the difference in BMI or the previous nicotine dependence. An additional group of lean healthy participants would be a more suitable control for the dieting group with obesity and ex-smokers. However, doing so would have financial implications to the study and may slow down recruitment of the other participant groups thus underpowering the study.

CHAPTER 4:

EFFECT OF EXENATIDE ON EATING BEHAVIOUR IN DIETING ADULTS WITH OBESITY AND EX-SMOKERS

4.0 ABSTRACT

Introduction: GLP-1 analogues, such as Exenatide, has been successfully developed for the treatment of T2DM and more recently obesity, due to its beneficial effects on glucose homeostasis and food intake. GLP-1 receptor signalling can modulate central reward networks towards food and nicotine in preclinical and human studies which may partly contribute to its efficacy in weight loss.

Aims: In this study, the effects of acute administration of the GLP-1 analogue, Exenatide, was explored in dieting adults with obesity, or in abstinent nicotine-dependence (in a double blind randomised placebo controlled cross-over design), on food cue responsivity using fMRI in reward-processing regions, food intake, food reward and appetite.

Results: In dieting group with obesity, Exenatide increased BOLD signal to high-energy (HE) food pictures in the superior frontal gyrus (SFG) and frontal pole, implicated in decision-making and inhibitory control. In contrast, in the ex-smokers, Exenatide decreased BOLD signal to HE food pictures in SFG, paracingulate gyrus, caudate, putamen and thalamus, suggesting a reduction in anticipatory food reward with a concomitant decrease in executive control. Furthermore, ex-smokers had a higher average BOLD signal to food pictures in fROI than the dieting group with obesity at saline visit, indicating alterations in the mesocorticolimbic pathway following smoking cessation. Furthermore, Exenatide reduced HE food picture appeal, food intake in *ad libitum* meal, appetite score, and tended to decrease motivation for a chocolate reward in both participant groups. In dieting group with obesity, Exenatide also reduced food craving scores.

Conclusion: The novel findings in the ex-smokers supports the hypothesis that Exenatide attenuates anticipatory food reward and improves eating behaviour. Interestingly in dieting group with obesity, the increase in BOLD signal to HE food pictures in prefrontal regions similarly resulted in an improved eating behaviour. This study findings are in accord with the possibility that Exenatide and other GLP-1 analogues could be used as a treatment option for prevention of smoking cessation weight gain.

4.1 INTRODUCTION

Obesity, with its associated comorbidities, poses a significant public health problem and socioeconomic burden. Unhealthy eating behaviour in an obesogenic environment contribute to the increasing prevalence of obesity. Treatment options for obesity range from improving lifestyle choices and pharmacotherapy to bariatric surgery with varying success of weight loss [254]. Unfortunately, with dieting alone, rates of successful long-term weight loss remain low and many people regain weight after dieting. It is speculated that metabolic, hormonal and neural changes following weight loss favour conservation of energy and increase food intake thereby predisposing to weight regain. There are limited pharmacological anti-obesity treatment options currently but the most successful are GLP-1 analogues, such as Exenatide, Liraglutide and Semaglutide, resulting in 2-8 kg weight loss on average between a period of 4 to 12 months [422-426]. Furthermore, after a dietary and behavioural weight loss program, GLP-1 analogues, compared to placebo, maintained and induced further weight loss in a greater proportion of adults with obesity [427].

GLP-1 analogues have been effectively utilised in the treatment of T2DM for improving glycaemic control and cardiovascular risk along with weight loss [428, 429]. They enhance glucose-dependent insulin secretion, reduce post-prandial glucagon secretion, delay gastric emptying and also reduce appetite and food intake [141]. In rodents, GLP-1 reduces food intake in part through activation of the hepatic vagal nerve, as its effect is suppressed with subdiaphragmatic vagal deafferentation [430]. However, the hypophagic effect of exogenous Liraglutide was still sustained in rats with vagal deafferentation [272], highlighting the presence of other mechanisms of action. Endogenous GLP-1, with a half-life of several minutes, is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) and so its anorexic effect may be largely mediated through the vagal nerve. On the other hand, GLP-1 analogues, such as Liraglutide, have longer half-lives and could reach the brain by crossing the blood brain barrier and act on central GLP-1 receptors [431].

In pre-clinical studies, GLP-1 is synthesised in the brain by pre-proglucagon (PPG) neurons in the NTS, which innervates the hypothalamus, laterodorsal tegmental nucleus, VTA and NAcc [269, 275]. Indeed, activation of GLP-1R in these regions suppressed food intake [274, 275]. For instance, Exenatide acts directly on hypothalamic ARC neurons and activates anorexigenic alpha-MSH production but suppresses orexigenic NPY/AgRP expression, including via γ-aminobutyric acid (GABA) signalling [272, 431], resulting in a reduction of food intake.

Furthermore, many studies have also explored the role of mesolimbic GLP-1R activation on food intake. In pre-clinical studies, endogenous GLP-1, as well as activation of GLP-1 NTS neurons, reduces excitatory dopaminergic signal within the VTA [276, 277] . As a result, this preferentially reduces intake of chow, palatable food and high-fat food [267, 269]. Specifically, Exenatide decrease rewarding value of food, as evidence by a reduction in conditioned place preference and progressive ratio operant task [267, 278]. In addition, GLP-1 receptor signalling in hippocampus and amygdala mediates food reward behaviour [90, 278]. Thus, demonstrating a role of GLP-1 signalling in regulating higher-order cognitive aspects of feeding behaviour.

Moreover, human neuroimaging studies have shown that acutely GLP-1 analogues (Exenatide) attenuate anticipatory reward and attention-related responses to high-energy food cues (decreasing BOLD signal) in areas such as the insula, putamen, OFC and amygdala in adults who are lean, and with obesity with and without T2DM (in studies where plasma glucose concentrations are clamped), effects that are blocked by co-administration of the GLP-1R antagonist Exendin9-39 [141, 158, 226, 432]. Furthermore, the rise in GLP-1 following a meal or oral glucose tolerance test, is correlated with the reduction in BOLD signal to food cues and can be prevented by co-administration of the GLP-1R antagonist Exendin9-39 in adults with obesity [279]. On the other hand, GLP-1 and its analogues increases BOLD signal after consumption of palatable food in similar brain reward areas, such as the insula, putamen and amygdala. Taken together, these results suggest that GLP-1 analogues may help to prevent overeating not only by reducing appetite but also by reducing food anticipatory and increasing food consummatory reward.

Interestingly, chronic effects of GLP-1 analogues on brain responses to food cues differed depending on the duration of treatment. After 10 days of treatment with Liraglutide in adults with T2DM, BOLD signal to food cues decreased in insula when fasted, and in putamen when satiated, compared to long-acting glargine insulin therapy. Over time however, this effect diminished and no significant differences in brain BOLD signal to food cues was noted after 12 weeks of treatment with either insulin or Liraglutide [280]. This was replicated in another study in which no changes in BOLD signal to food cues were noted after 5 weeks of treatment with liraglutide when compared to placebo [433]. Indeed, when corrected for BMI or weight, there was greater BOLD signal in the right OFC to food cues on Liraglutide, indicating possible counter-

regulatory mechanisms that prevent further weight loss, which might be related to other homeostatic metabolic changes e.g. fall in plasma leptin or glucose.

This emerging evidence of the attenuation of food cue reactivity in mesolimbic reward networks by GLP-1 analogues introduces the possibility of GLP-1 analogues as a novel treatment option for weight gain following smoking cessation. The impact of smoking on health is well-documented, resulting in huge direct cost to the NHS and indirectly to society in lost income. It is the leading preventable cause of morbidity and mortality. Despite public health interventions and smoking cessation therapies, around 80% of the people who quit smoking will relapse within the first year. Importantly, weight gain after smoking cessation is one of the commonly cited reason for hindering successful quit attempts.

The link between smoking and body weight has been a subject of research for many years. Weight gain after smoking cessation could be attributed to an increase in appetite and a reduction in energy expenditure although reports on the latter have been inconsistent [197]. There is an average 4-5 kg increase in body weight after 12 months of abstinence, and 13% of people who quit smoking gained more than 10 kg in weight [194, 195]. The increase in weight poses an increased risk for developing T2DM initially but the overall health benefit of smoking cessation still outweighed this risk [196].

Nicotine exerts its rewarding and addictive properties through the mesocorticolimbic dopaminergic system via nicotinic acetylcholine receptors. Using fMRI, there is a distinct overlap of brain regions which are activated when anticipating food and drug rewards, indicating that both share the same reward processing pathways [213]. Indeed, nicotine alters food cue reactivity with current smokers having attenuated BOLD signal to favourite food cues in caudate, putamen, insula, thalamus and cerebellum compared to non-smokers [219]. This could be partially due to a decrease in dopamine concentration in the striatum following chronic use of nicotine [434]. Such alterations can disrupt homeostatic energy balance and predispose to unhealthy eating behaviour.

Furthermore, GLP-1 analogues have shown promising results in pre-clinical studies attenuating the rewarding behaviour and intake of drugs [435], including nicotine, alcohol and amphetamine, purportedly by reducing dopamine release in NAcc [286-288], and specifically in the case of

nicotine, stimulating habenular avoidance circuits [239]. Thus, GLP-1 analogues are being evaluated as a potential treatment option for smoking cessation in clinical studies, addressing both the weight gain and nicotine addiction [436]. The research in this chapter provides a proof-of-concept experimental medicine approach for such a clinical potential to assess the effects of acute administration of a GLP-1 analogue on food cue reactivity, appetite and food intake in dieting adults with obesity or ex-smokers.

4.2 HYPOTHESES

In dieting adults with obesity and in ex-smokers:

1. Exenatide will attenuate the appeal of and cue reactivity in mesolimbic brain regions to HE food pictures.
2. Exenatide will reduce appetite and food intake.
3. Exenatide will reduce food reward behaviour, including motivation to receive a food reward and approach bias.
4. Exenatide will decrease glucose concentrations and insulin.
5. BMI and eating behavioural traits can influence the impact of Exenatide on food cue reactivity, and in ex-smokers the severity of nicotine dependence or duration of abstinence.

4.3 AIMS

To investigate the effects of acute infusion of the GLP-1 analogue, Exenatide, on eating behaviour in dieting adults with obesity who are dieting, or in abstinent nicotine-dependence, in a double-blind randomised placebo-controlled cross-over design, on:

1. BOLD response in reward system during a HE food picture evaluation fMRI task.
2. appetite, food appeal, taste, and intake.
3. eating behaviour, namely motivation to eat using a progressive ratio task (PRT) and attentional bias to food using an approach-avoidance task (AAT).
4. plasma metabolites and hormones, including glucose and insulin.

   And to:
5. explore influence of BMI and eating behaviours associated with altered overeating (dietary restraint, uncontrolled eating, emotional eating, susceptibility to overeating from hunger cues and binge eating symptoms) on the HE food cue reactivity response to Exenatide, and in ex-smokers with severity of nicotine dependence (Fagerstrom Test for Nicotine Dependence) and duration of cigarette abstinence.

4.4 RESULTS

Summary statements of the findings are given in bold at the start of each section. In this section, data was available for n=24 dieting participants with obesity and n=23 ex-smokers for analysis of behavioural tasks. One participant had to be excluded from fMRI analysis due to frontal pole signal drop-out, leaving a final n=23 in each group for fMRI analysis.

4.4.1 Food Evaluation fMRI task

Summary: In dieting adults with obesity, Exenatide increased BOLD signal to high-energy (HE) food pictures in the SFG and frontal pole (Figure 4.2). In contrast, in the ex-smoker group, Exenatide decreased BOLD signal to HE food pictures in SFG, paracingulate gyrus, caudate, putamen and thalamus (Figure 4.3). At the saline visit, ex-smoker group had a higher average BOLD signal to food pictures in fROI than the dieting group with obesity. Furthermore, Exenatide decreased HE food picture appeal rating independent of group (Figure 4.1).

4.4.1.1 Food appeal rating

To examine the effect of Exenatide on food hedonics, a repeated measures ANOVA was performed including both groups, with visit (saline, Exenatide) as within subject factor, and group (obese, ex-smokers) as between subject factor. For HE food picture appeal rating (relative to neutral pictures), there was no significant interaction effect for visit*group [F(1,45)=0.58, P=0.45] in repeated measures ANOVA (Figure 4.1).

There was however an overall significant effect of visit [F(1,45)=8.12, P=0.007], with a decrease in HE food appeal during the Exenatide visit, independent of group [effect size mean ± SEM -0.28 ± 0.10 (95%CI -0.49, -0.83), P=0.007, Cohen's d=0.41]. This indicates that Exenatide reduces the appeal of HE food pictures in dieting group with obesity and ex-smokers with a small-moderate effect size.

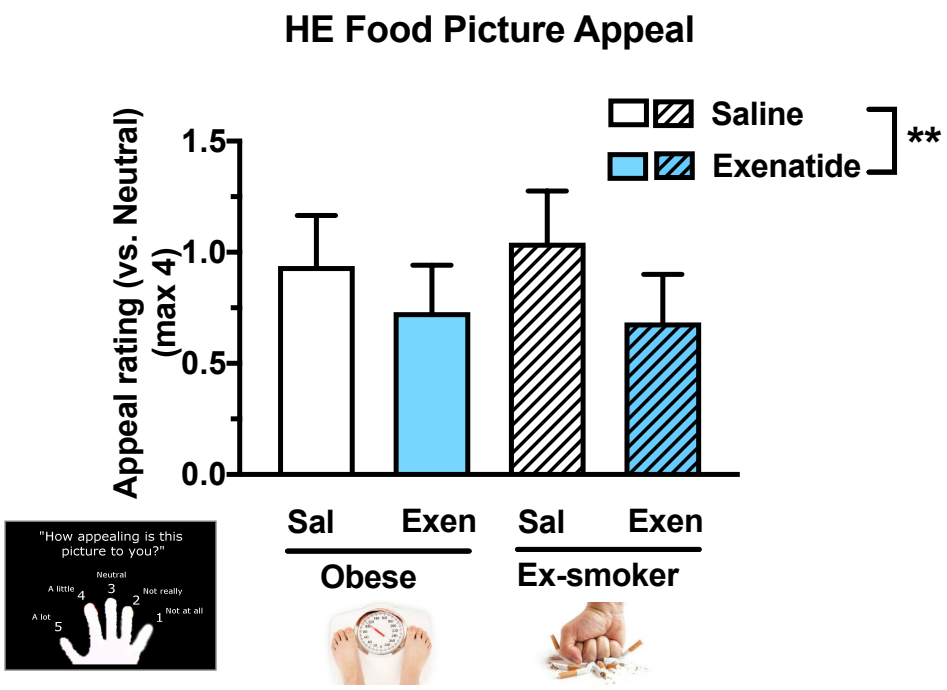


Figure 4.1. Exenatide reduced appeal rating of high-energy (HE) foods independent of group in picture evaluation fMRI task.

Comparison of appeal rating of HE food (vs. neutral) pictures between saline (Sal) and Exenatide (Exen) visits. Data presented as mean ± SEM, n=23-24. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor, and visit (saline, Exenatide) as within subject factors: **P<0.01 ***P<0.001.

4.4.1.2 Food cue reactivity: whole brain analysis

4.4.1.2a Dieting group with obesity

In the dieting group with obesity, Exenatide increased BOLD signal to HE food pictures in a frontal cluster, including the left superior frontal gyrus (SFG) and left frontal pole (Figure 4.2, Table 4.1). This cluster lies in the prefrontal cortex (PFC), an area that is involved with executive control. However, there was no associated reduction in BOLD signal in reward system areas, such as OFC, striatum or amygdala.

By contrast, this effect of Exenatide was only seen in the dieting group with obesity and not in ex-smokers. There was no difference in BOLD signal to food cues in this frontal cluster in the group of ex-smokers with Exenatide compared to saline (Figure 4.4). This was evident from a significant visit*group interaction [F(1,44)=7.71, P=0.008] and a higher BOLD signal to food cues in dieting group with obesity [effect size mean ± SEM 0.09 ± 0.04, (95% CI 0.02, 0.16), P=0.011], that was not seen in ex-smoker group [effect size mean ± SEM -0.04 ± 0.04, (95% CI -0.12, 0.03), P=0.21].

A

Contrast	Cluster	Number of voxels	Z-statistic	x	y	z	Side	Brain region
High-energy food pictures								
Exenatide > Saline	1	1013	3.77	-10	42	40	L	Frontal pole
			3.66	-14	30	62	L	SFG
			3.55	-8	22	56	L	SFG
			3.49	0	54	36	L	SFG
			3.47	-10	44	46	L	Frontal pole
			3.40	-6	52	28	L	SFG
Saline > Exenatide		nil significant						

B

Contrast	Cluster	Number of voxels	Z statistic	x	y	z	Side	Brain region
High-energy food pictures								
Exenatide > Saline		nil significant						
Saline > Exenatide	1	691	3.57	6	22	48	R	Paracingulate gyrus
			3.40	24	20	58	R	SFG
			3.32	20	26	52	R	SFG
			3.21	10	26	70	R	SFG
			3.19	14	24	48	R	SFG
			3.14	-6	18	54	L	SFG
	2	2196	4.05	-14	-12	18	L	Thalamus
			3.97	-26	-26	8	L	Putamen
			3.92	-14	-12	22	L	Caudate
			3.86	-28	10	2	L	Putamen
			3.69	-14	10	4	L	Caudate
			3.44	-14	12	18	L	Caudate

Table 4.1. Whole brain analysis for effect of Exenatide on HE food evaluation fMRI task in dieting group with obesity and ex-smokers.

Stereotactic coordinates (x, y, z in standard MNI space) for peak voxel of group activation for (A) obese (n=23) and (B) ex-smokers (n=23), at HE food vs. neutral pictures at Exenatide compared to saline visits. Cluster-wise threshold Z>2.3, family wise error (FWE) P<0.05. Abbreviations: SFG, superior frontal gyrus.

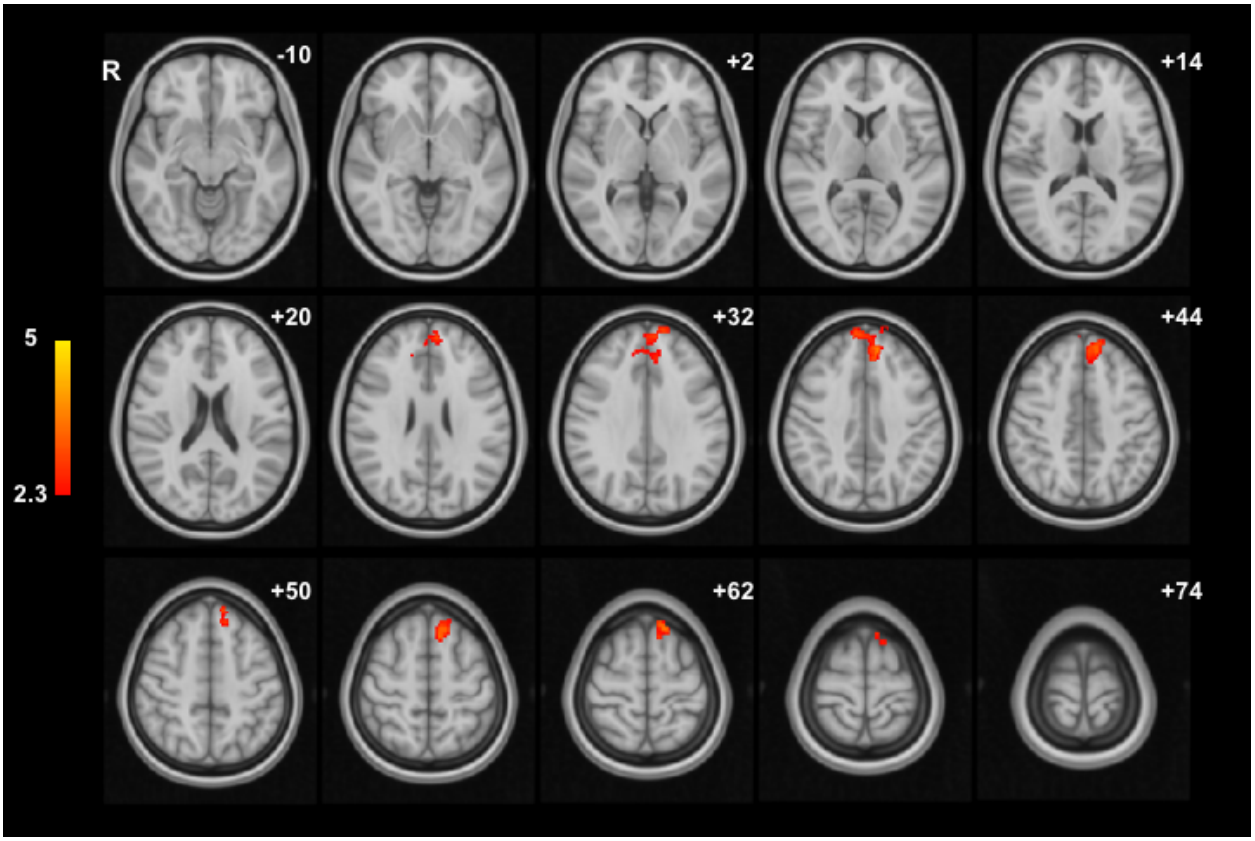


Figure 4.2. In dieting group with obesity, Exenatide increased BOLD signal to HE food pictures in prefrontal cortex.

Group activation map for HE food > neutral picture for Exenatide > saline visit in adults with obesity (n=23). Colour bar indicates Z score. Cluster-wise threshold Z>2.3, family-wise Error (FWE) P<0.05. z co-ordinates given in Montreal Neurological Institute (MNI) space. Abbreviations: R, right. See Table 4.1 for MNI co-ordinates.

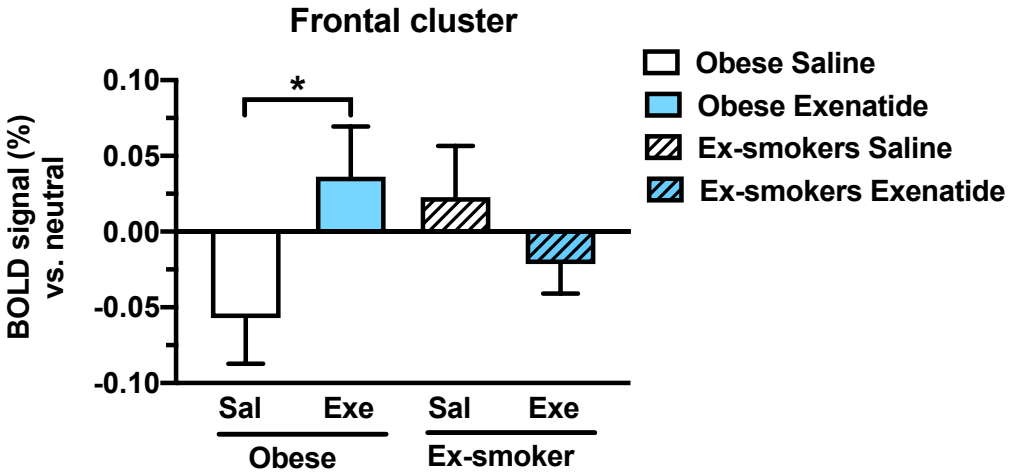


Figure 4.3. Increased frontal pole BOLD signal to HE food pictures by Exenatide in dieting group with obesity but not ex-smokers.

Comparison of BOLD signal to HE food pictures (vs. neutral) in frontal cluster (see Figure 4.2) between saline and Exenatide visits for group with obesity and ex-smokers. Data presented as mean ± SEM, n=23. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor and visit (saline, Exenatide) as within subject factors, with post-hoc Fisher LSD test: *P<0.05, **P<0.01. See Table 4.1 for MNI co-ordinates of cluster.

To test whether activity in this frontal cluster was indeed related to cognitive-control ability and other traits, a correlation between the median BOLD signal to HE food pictures at saline visit and BMI, individual scores of DEBQ (restraint, emotional, external), TFEQ (restraint, hunger, disinhibition) and BES was undertaken (Table 4.2). In the dieting group with obesity, there was no significant correlational relationship found between the BOLD signal to food pictures at saline visit and the above-mentioned variables.

Furthermore, to examine if Exenatide-induced BOLD signal changes to HE food pictures in this frontal cluster was influenced by BMI, change in pre-MRI glucose or eating behavioural traits, a correlation between change in BOLD signal to HE food pictures at Exenatide (relative to saline) visit and the aforementioned variables was performed (Table 4.2; Figure 4.3).

In the dieting group with obesity, BMI, Exenatide-induced change in pre-MRI glucose and the eating behavioural questionnaires did not show any significant associations with Exenatide-induced changes in BOLD signal to HE foods.

OBESE	BMI	Δ pre-MRI glucose	DEBQ-emotional	DEBQ-external	DEBQ-restaint	TFEQ-restraint	TFEQ-hunger	TFEQ-disinhibition	BES
BOLD at saline									
Correlation Coefficient	0.17	-	0.04	0.15	0.24	0.41	0.05	0.13	0.27
P value	0.42	-	0.88	0.49	0.26	0.059	0.84	0.56	0.21
Exenatide-induced changes in BOLD									
Correlation Coefficient	-0.30	0.09	-0.15	-0.09	0.32	0.42	-0.16	-0.13	-0.20
P value	0.17	0.70	0.49	0.70	0.13	0.053	0.48	0.57	0.35

Table 4.2. Correlations between BOLD signal to HE foods at saline and effects of Exenatide and BMI, eating behaviour and change in plasma glucose in dieting group with obesity.
Spearman correlation coefficient in dieting group with obesity (n=23) of BOLD signal to HE food pictures in frontal cluster (See Figure 4.2) at saline and changes of that at Exenatide (relative to saline) visit with BMI, change in pre-MRI glucose levels, DEBQ, TFEQ and BES. In obesity, TFEQ-restraint scores tended to correlate positively with the Exenatide-induced increases in BOLD signal to HE food pictures in frontal cluster and that at saline visit. Abbreviations: BES, Binge Eating Scale; BOLD, blood oxygen level dependent DEBQ, Dutch Eating Behavioural Questionnaire; MRI, magnetic resonance imaging; TFEQ, Three Factor Eating Questionnaire.

4.4.1.2b Ex-smokers

In keeping with the hypothesis, in the ex-smoker group, Exenatide reduced BOLD signal to HE food pictures in brain areas in frontal and striatal regions (Figure 4.4, Table 4.1). These included right paracingulate gyrus, bilateral SFG, caudate, putamen and thalamus. Both paracingulate gyrus and SFG are part of the PFC and implicated in executive decision-making and control. On the other hand, the caudate and putamen form the dorsal striatum that plays a role in anticipation of food rewards, conditioned learning and motivation in rewarding behaviours. Moreover, the thalamus is responsible for relaying sensory and motor signals between different regions of the cerebral cortex. Taken together, these findings may suggest a reduced anticipation from evaluating the food pictures in ex-smokers, and at the same time a diminished need for top-down inhibitory control.

Of note, this effect of Exenatide was only seen in ex-smoker group and not in dieting group with obesity. There was no difference in BOLD signal to HE food pictures in the corresponding frontal and striatal clusters in obesity at Exenatide compared to saline visits (Figure 4.5). For the frontal cluster, this was evident from a significant visit*group interaction [$F(1,44)=7.00$, $P=0.011$] and a lower BOLD signal to HE food pictures in ex-smoker group [effect size mean ± SEM -0.08 ± 0.03, (95% CI -0.13, -0.02), $P=0.010$], that was not seen in dieting group with obesity [effect size mean ± SEM 0.03 ± 0.03, (95% CI -0.03, 0.09), $P=0.30$]. As for the striatal cluster, there was a significant visit*group interaction [$F(1,44)=10.30$, $P=0.002$] and a lower BOLD signal to HE food pictures in ex-smokers [effect size mean ± SEM -0.09 ± 0.03, (95% CI -0.16, -0.02), $P=0.009$], that was not seen in obesity [effect size mean ± SEM 0.06 ± 0.03, (95% CI -0.007, 0.13), $P=0.078$].

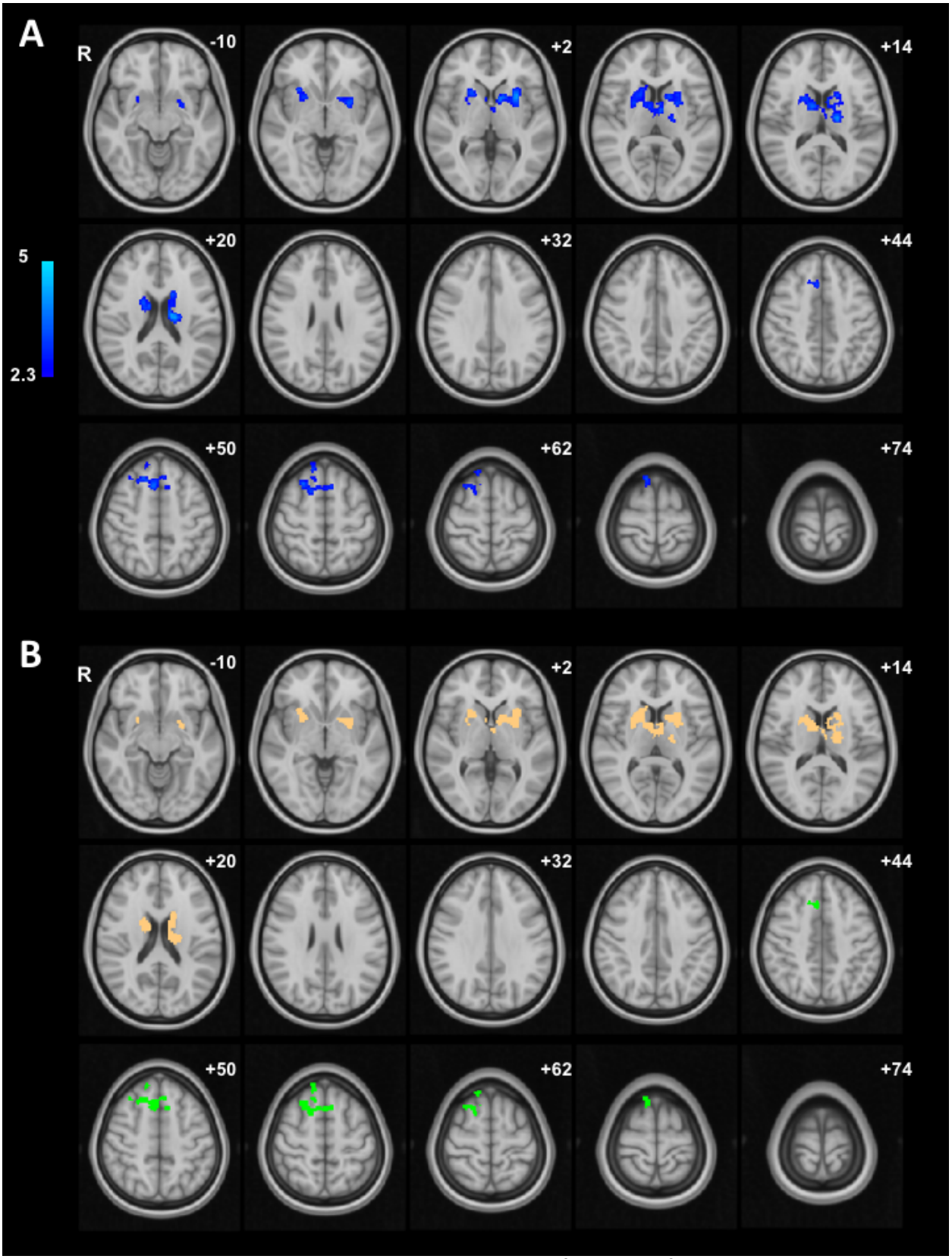


Figure 4.4. In ex-smokers, Exenatide reduced BOLD signal to HE foods in prefrontal cortex and dorsal striatum.

Group activation map for HE food > neutral pictures for saline > Exenatide visit in ex-smokers (n=23) in (A) whole brain analysis, colour bar indicates Z scores; (B) separate cluster in pre-frontal cortex (paracingulate gyrus, SFG, cluster; green) and dorsal striatum (caudate, putamen; beige). Cluster-wise threshold Z>2.3, family-wise error (FWE) P<0.05. z co-ordinates given in Montreal Neurological Institute (MNI) space. Abbreviations: R, right. See Table 4.1 for MNI co-ordinates.

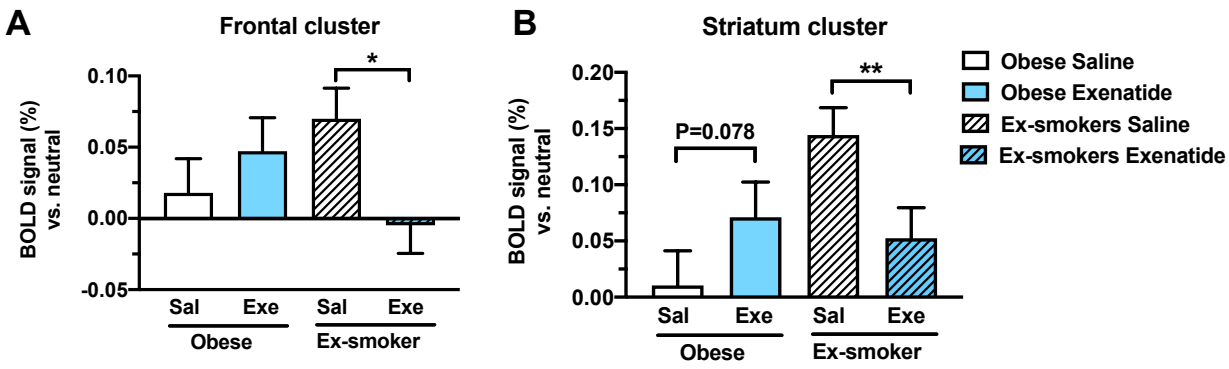


Figure 4.5. Decreased BOLD signal to HE food in frontal and dorsal striatum by Exenatide in ex-smokers, but not dieting group with obesity.

Comparison of BOLD signal to HE food pictures in (A) frontal and (B) dorsal striatum clusters (See Figure 4.5B) between saline and Exenatide visits in dieting group with obesity and ex-smokers. Data presented as mean ± SEM, n=23. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor and visit (saline, Exenatide) as within subject factors, with post-hoc Fisher LSD. See Table 4.1 for MNI co-ordinates of cluster.

As in the case with the dieting group with obesity, to further examine whether BOLD signal to HE food pictures in this frontal cluster for the ex-smoker group was related to cognitive-control ability and other traits, a correlation between the median BOLD signal to HE food pictures at saline visit and BMI, FTND, duration of cigarette abstinence, individual scores of DEBQ (restraint, emotional, external), TFEQ (restraint, hunger, disinhibition) and BES was undertaken (Table 4.3). There were no significant correlations of any variables to the BOLD signal to HE food pictures at saline visit.

Furthermore, to examine if Exenatide-induced BOLD signal changes to HE food pictures in this frontal cluster for ex-smoker group was influenced by BMI, change in pre-MRI glucose, FTND, duration of cigarette abstinence or eating behavioural traits, a correlation between change in BOLD signal to HE food pictures at Exenatide (relative to saline) visit and the aforementioned variables was performed (Table 4.3). There were no significant correlations of any variables to the Exenatide-induced decreases in BOLD signal to HE food pictures in this frontal cluster.

EX-SMOKERS: FRONTAL	BMI	Δ pre-MRI glucose	FTND	Abstinence	DEBQ-emotional	DEBQ-external	DEBQ-restaint	TFEQ-restraint	TFEQ-hunger	TFEQ-disinhibition	BES
BOLD at saline											
Correlation Coefficient	-0.15	-	-0.23	-0.01	0.28	-0.12	0.008	0.06	-0.27	-0.08	-0.27
P value	0.50	-	0.30	0.95	0.20	0.60	0.97	0.78	0.22	0.72	0.23
Exenatide-induced changes in BOLD											
Correlation Coefficient	0.08	-0.02	0.07	-0.04	-0.32	0.004	-0.32	-0.32	0.12	-0.08	0.10
P value	0.73	0.93	0.75	0.85	0.14	0.98	0.14	0.13	0.58	0.71	0.66
EX-SMOKERS: STRIATAL											
BOLD at saline											
Correlation Coefficient	-0.64	-	-0.14	-0.05	0.27	0.09	-0.41	-0.33	-0.07	-0.10	-0.03
P value	**0.001**	-	0.51	0.83	0.21	0.70	0.052	0.12	0.74	0.65	0.91
Exenatide-induced changes in BOLD											
Correlation Coefficient	0.36	-0.31	0.33	0.02	-0.39	-0.07	-0.009	-0.05	0.21	-0.004	0.18
P value	0.087	0.17	0.13	0.93	0.067	0.74	0.97	0.81	0.34	0.99	0.43

Table 4.3 In ex-smokers, BMI correlated negatively with the BOLD signal to HE food pictures in striatal cluster at saline visit.

Spearman correlation coefficient in ex-smoker group (n=23) of BOLD signal to HE food pictures in frontal and striatal clusters (See Figure 4.5B) at saline and changes of that at Exenatide (relative to saline) visit with BMI, change in pre-MRI glucose levels, FTND, duration of cigarette abstinence, DEBQ, TFEQ and BES. Abbreviations: BES, Binge Eating Scale; BOLD, blood oxygen level dependent DEBQ, Dutch Eating Behavioural Questionnaire; FTND, Fagerstrom Test for Nicotine Dependence; MRI, magnetic resonance imaging; TFEQ, Three Factor Eating Questionnaire.

Striatal cluster

A similar correlation analysis was done to further examine if any traits, including those involving eating behaviours, would affect BOLD signal to HE food pictures in the striatal cluster (See Figure 4.4B) of the ex-smoker group and influence the effect of Exenatide on it (Table 4.3).

In the ex-smoker group, BMI negatively correlated with BOLD signal to HE food pictures in dorsal striatal cluster [$r_s(21)= -0.64$, $P=0.0010$] and tended to positively associate with Exenatide-induced changes in BOLD signal to HE food pictures in the cluster [$r_s(21)= 0.36$, $P=0.087$] (Figure 4.6A-B). This suggests that, contrary to current literature, the higher the BMI, the lower the anticipatory food reward in dorsal striatal cluster. Similarly, BMI mediated the effects of Exenatide on BOLD signal, such that the higher the BMI, the lower the Exenatide-induced decrease in BOLD signal to HE food pictures in the striatal cluster.

DEBQ-restraint scores also tended to negatively correlate with BOLD signal to HE food pictures in striatal cluster at saline visit in ex-smokers [$r_s(21)= -0.41$, $P=0.052$] (Figure 4.6C). This may imply a possible link between the cognitive control in dietary restraint in related networks and the resulting decrease in anticipatory food reward in ex-smokers. Furthermore, DEBQ-emotional scores tended to negatively correlate with Exenatide-induced changes in BOLD signal to HE food pictures [$r_s(21)= -0.39$, $P=0.067$] (Figure 4.6D). This suggests the higher the emotional eating scores, the more Exenatide decreased the anticipatory food reward.

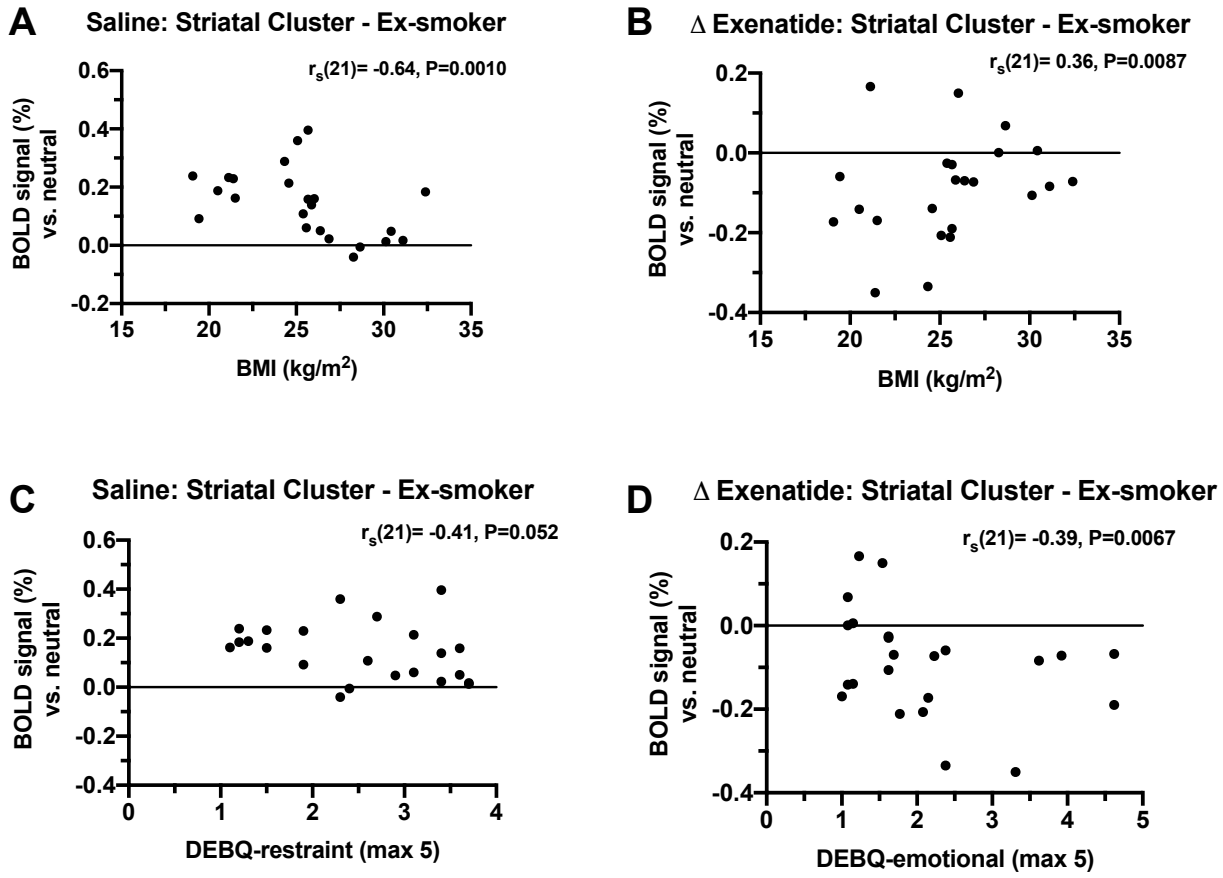


Figure 4.6. In ex-smokers, BMI correlated negatively with BOLD signal to HE food pictures at saline visit. In ex-smoker group (n=23), correlation between BMI with the BOLD signal to HE food pictures in striatal cluster (See Figure 4.5B) at (A) saline visit and (B) the change in BOLD signal at Exenatide (relative to saline) visit. Further correlation between (C) DEBQ-restraint and BOLD signal to HE food pictures at saline and (D) Exenatide-induced change in BOLD signal to HE food pictures and DEBQ-emotional scores are also shown. Analysis computed using Spearman correlation.

4.4.1.3 Food cue reactivity: functional ROI analysis

To assess the effects of Exenatide on BOLD signal during evaluation of HE food pictures vs. neutral in the *a priori* functional ROIs (nucleus accumbens, caudate, putamen, orbitofrontal cortex, amygdala, hippocampus, anterior insula, ventral anterior cingulate cortex, dorsolateral prefrontal cortex, Figure 2.5, Table 2.2), a repeated measures ANOVA was performed including both groups, with fROI and visit (saline, Exenatide) as within subject factors, and group (obese, ex-smokers) as between subject factor.

For BOLD signal to HE food pictures, there was no significant interaction effect for: (i) visit*group*ROI [F(4.23,185.89)=0.99, P=0.42 with Greenhouse-Geisser correction], (ii) visit*ROI [F(4.23,185.89)=0.80, P=0.54 with Greenhouse-Geisser correction], nor an overall effect of (iii) visit [F(1,44)=0.32, P=0.57].

There was however a significant interaction effect for: visit*group [F(1,44)=4.65, P=0.037]. This was driven by a significantly higher BOLD signal (averaged across all fROI), to HE food pictures in the ex-smokers at the saline visit compared to dieting group with obesity [effect size mean ± SEM 0.076 ± 0.033, (95% CI 0.010, 0.141), P=0.025, Cohen's d=0.70), that was not seen for the Exenatide visit (Figure 4.7). This indicates that ex-smokers had a higher BOLD signal to food pictures averaged across all fROI than the dieting group with obesity, that was lost with Exenatide administration.

In dieting group with obesity, Exenatide tended to increase the BOLD signal averaged across all fROI to food pictures compared to saline but this did not reach significance [effect size mean ± SEM 0.050 ± 0.026, (95%CI -0.002, 0.101), P=0.061, Cohen's d=0.40] (Figure 4.7). This was not seen in the ex-smokers [effect size mean ± SEM -0.029 ± 0.026, (95% CI -0.081, 0.023, P=0.27).

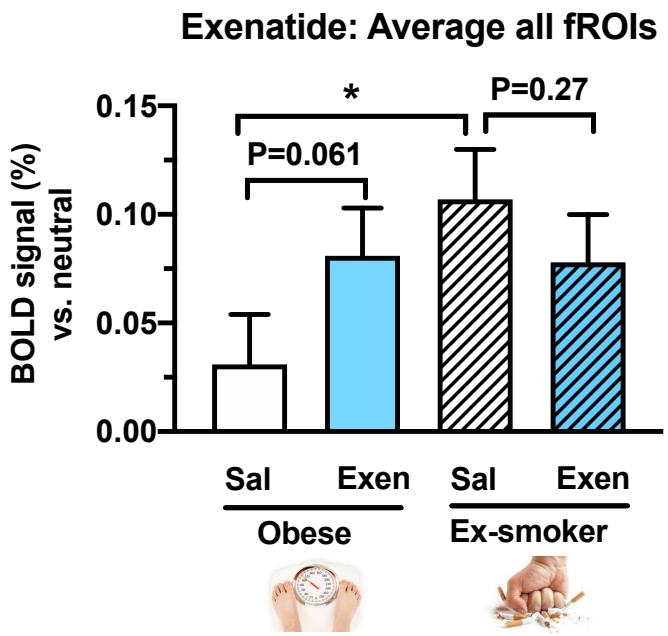


Figure 4.7. Ex-smokers have higher BOLD signal to HE food across all fROI than in dieting group with obesity at saline but not Exenatide visits.

Comparison of BOLD signal during evaluation of food (vs. neutral) pictures) averaged across all functional regions of interest (fROI: nucleus accumbens, caudate, putamen, orbitofrontal cortex, amygdala, hippocampus, anterior insula, ventral anterior cingulate cortex, dorsolateral prefrontal cortex) between saline and Exenatide visits in dieting group with obesity and ex-smokers. Data presented as mean ± SEM, n=23. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor and visit (saline, Exenatide) as within subject factors, post-hoc Fisher LSD test: *P<0.05.

Although there was no significant interaction effect for visit*group*ROI, exploratory analysis in the dieting group with obesity revealed a general pattern of increase in BOLD signal to HE food pictures with Exenatide in all the fROI, but this was only significant in the dlPFC, which is implicated in executive control (Figure 4.8A). In contrast, in ex-smoker group, there was a general pattern of reduction in BOLD signal to HE food pictures with Exenatide across most fROI although none individually reached significance (Figure 4.8B).

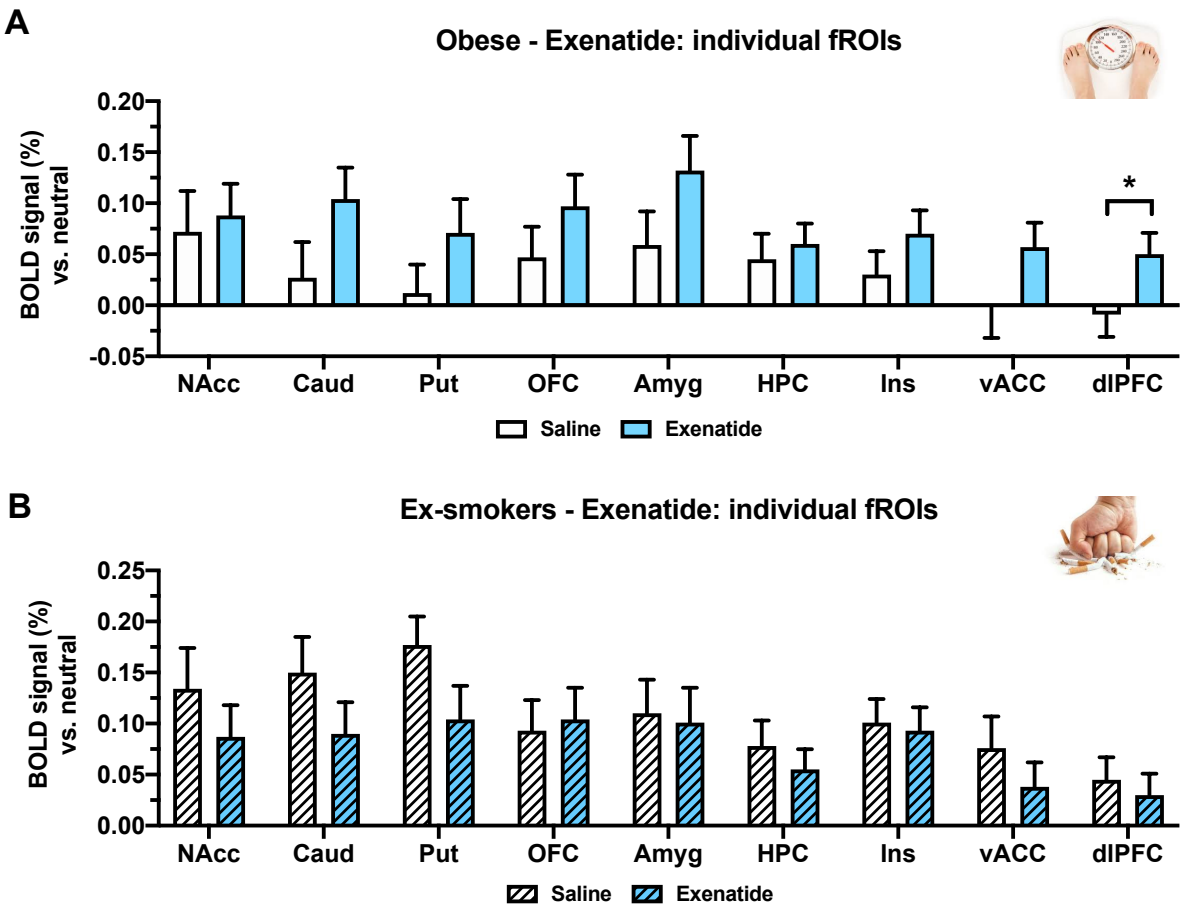


Figure 4.8. Effect of Exenatide on BOLD signal to food pictures in individual fROI in dieting group with obesity and ex-smokers.

Comparison of BOLD signal during evaluation of food pictures (vs. neutral pictures) in all functional regions of interest [fROI: nucleus accumbens (NAcc), caudate (Caud), putamen (Put), orbitofrontal cortex (OFC), amygdala (Amyg), hippocampus (HPC), anterior insula (Ins), ventral anterior cingulate cortex (vACC), dorsolateral prefrontal cortex (dlPFC)] between saline and Exenatide visits in (A) dieting group with obesity and (B) ex-smokers. Data presented as mean ± SEM, n=23. Statistics from repeated measures ANOVA, with group (obese, ex-smokers) as between subject factor, and visit (saline, Exenatide) and ROI as within subject factors, with post-hoc Fisher's LSD test *P<0.05.

Adjusting for Covariates

To assess whether the interaction effect for visit*group was altered when adjusting for co-variates, the following factors were included in separate repeated measures ANCOVA analyses:

(i) Visit order: The interaction effect for visit*group after adjusting for difference in visit order between visits (i.e. visit number of Exenatide minus saline visits) was F(1,43)=5.24, P=0.027. This was driven by the higher average BOLD signal to HE food pictures at saline in ex-smokers compared to dieting group with obesity [effect size mean ± SEM 0.08 ± 0.03, (95% CI 0.01, 0.15), P=0.021] and an increase in average BOLD signal to HE food pictures with Exenatide in the group with obesity [effect size mean ± SEM 0.05 ± 0.03, (95% CI 0.00, 0.10), P=0.049].

(ii) Plasma glucose: There was a trend for a significant interaction effect for visit*group after adjusting for change in plasma glucose (i.e. plasma glucose pre-fMRI scan at T=90mins at Exenatide minus saline visit, n=21-22) was F(1,40)=3.65, P=0.063. The average BOLD signal to HE food pictures in ex-smokers tended to be higher than that in dieting group with obesity [effect size mean ± SEM 0.06 ± 0.04, (95% CI -0.008, 0.13), P=0.082] and there was no longer a trend in effect of Exenatide on average BOLD signal to HE food pictures in dieting group with obesity [effect size mean ± SEM 0.04 ± 0.03, (95% CI -0.01, 0.10), P=0.12].

(iii) Nausea VAS: The interaction effect for visit*group after adjusting for change in nausea (i.e. nausea VAS rating pre-fMRI at T=90min at Exenatide minus saline visit) was F(1,43)=4.58, P=0.038. This was driven by the higher average BOLD signal to HE food pictures at saline in ex-smokers compared to group with obesity [effect size mean ± SEM 0.08 ± 0.03, (95% CI 0.01, 0.14), P=0.025]. The effect of Exenatide on average BOLD signal to HE food pictures in obesity remained a trend [effect size mean ± SEM 0.05 ± 0.03, (95% CI -0.003, 0.10), P=0.063].

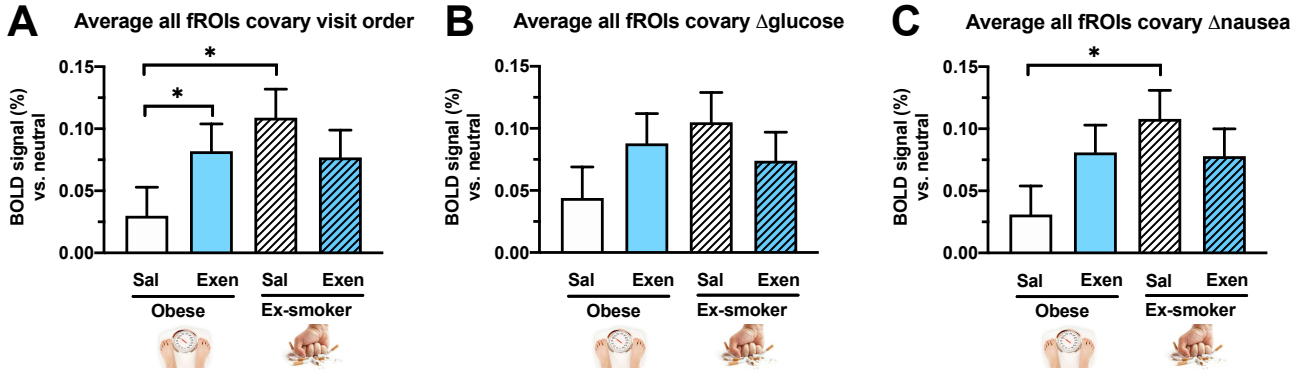


Figure 4.9. Average BOLD signal to HE food pictures across all fROI after adjusting for covariates
Comparison of average BOLD signal to HE food pictures across all fROI in dieting group with obesity (n=23) and ex-smokers (n=23) after adjusting for (A) visit order, (B) change in pre-MRI glucose (n=21-22) and (C) change in pre-MRI nausea VAS for Exenatide compared to saline visit. *P<0.05 **P<0.01.

4.4.2 Potential confounding factors for visit outcome variables

Summary: There were no significant differences in confounding factors that may account for within-subject differences in BOLD responses to food cues nor other behavioural outcomes (Table 4.4, Figure 4.10).

4.4.2.1 Confounds for food evaluation fMRI task

Several behavioural and state factors may confound comparison between visits. However, when assessing specific behavioural measures in the fMRI task, there were no significant interaction effects for visit*group*picture, visit*group, visit*picture, nor an overall effect of visit for:

(i) *rating accuracy* (defined as the percentage of recorded picture evaluation responses averaged across four picture types for a study visit);

(ii) *rating reaction time* (defined as the average of the duration between each picture appearing and recorded evaluation response in msec);

and no significant interaction effect for visit*group, nor an overall effect of visit for:

(iii) *relative motion per scan volume;*

(iv) *visit order* (defined as the visit number of Exenatide minus that of saline, performed to assess appropriate randomisation of study visits).

Furthermore*,* (v) *day of menstrual cycle* for women was assessed given influence of menstrual state on food cue reactivity [437-439]. In n=11 in dieting group with obesity and n=10 ex-smokers, there was a significant interaction effect for visit*group [$F(1,19)=4.66$, $P=0.044$]. This was driven by a later day of menstrual cycle at Exenatide visit for the women with obesity compared to ex-smokers [effect size mean ± SEM 14.0 ± 5.9, (95% CI 1.8, 26.3), $P=0.027$], that was not seen at the saline visit. Also, there was a trend for a later day of menstrual cycle for the women with obesity at the Exenatide compared to saline visit [effect size mean ± SEM 8.0 ± 4.0, (95% CI -0.4, 16.4), $P=0.062$], that was not seen for ex-smokers. Only women who had cycles of more than 3 months (n=1) or those who were post-menopausal (n=5) were excluded from this analysis as the accuracy of date of last menstrual period was deemed questionable.

4.4.2.2 Confounds for other outcomes

The following variables were measured at each study visit to assess differences in mood state: (i) *Profile of Mood States (POMS-2)* which included fatigue, tension, vigour, depression, anger and confusion, (ii-iii) *Positive affect and Negative Affect Schedule (PANAS)* as a measure of positive and negative mood, and (iv*) Spielberger State Anxiety Inventory (SSAI)* as a measure of state anxiety.

However, there were no significant visit*group interactions nor overall effect of visit for:
(i) *Profile of Mood States (POMS-2):*
(ii) *Positive affect (PANAS)*
(iii) *Negative affect (PANAS)*

(iv) For *Spielberger State Anxiety Inventory (SSAI)* there was no significant visit*group interaction nor overall effect of visit. However, there was a trend for an overall effect of visit, independent of group [$F(1,45)=3.74$, $P=0.059$, Cohen's $d=0.28$]. This was driven by a reduction of anxiety scores at Exenatide visit [effect size mean ± SEM -1.58 ± 0.82, (95% CI -3.22, 0.07), $P=0.059$]. Nonetheless, this was unlikely to be attributed to Exenatide as the questionnaires were performed just after the start of infusion and was unlikely to have caused an impact on the assessments between visits since the absolute change was clinically small relative to the maximum rating in this questionnaire (max 80).

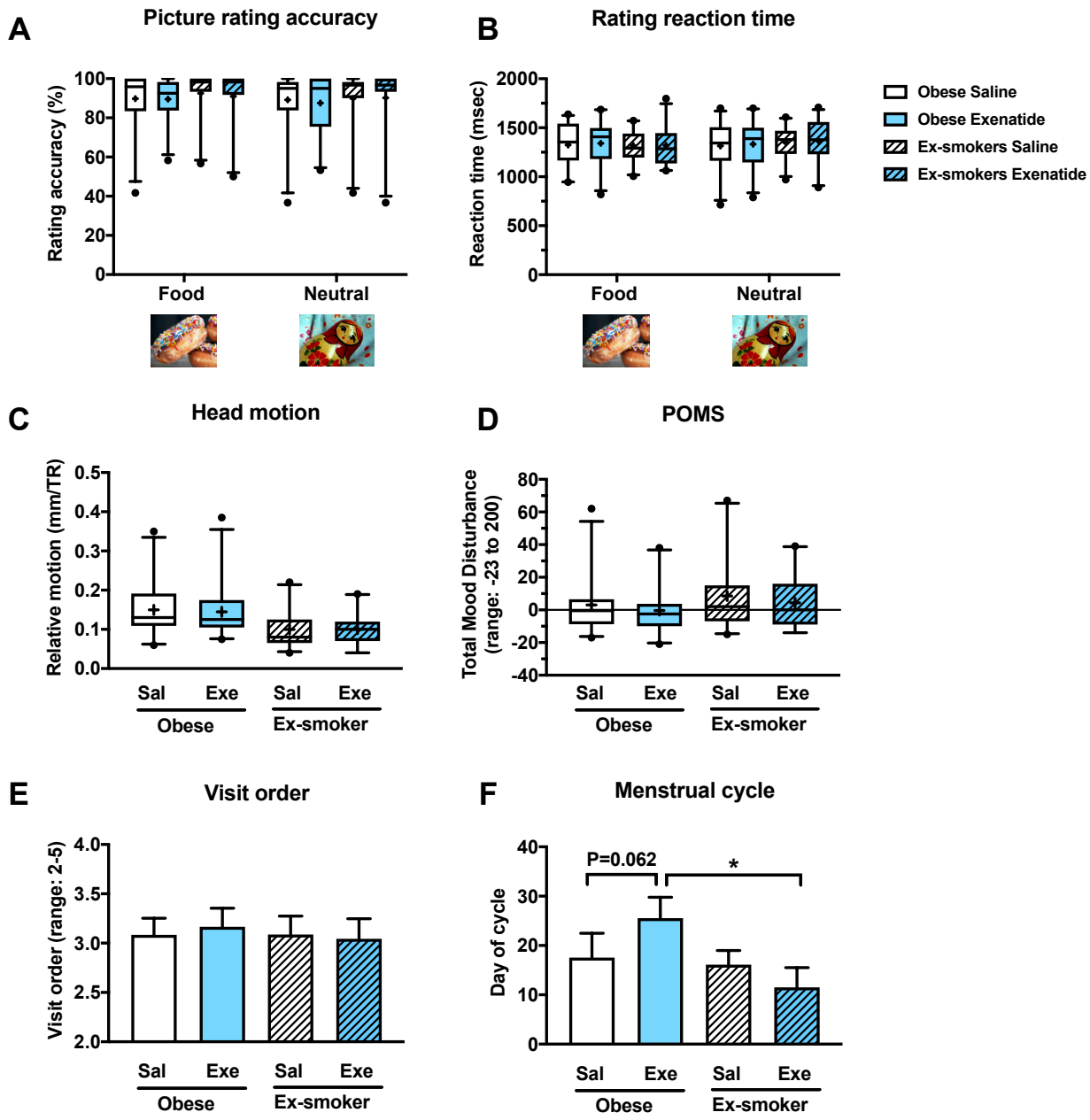


Figure 4.10. No significant within-subject differences in confounding factors of picture evaluation fMRI task. Comparison of (A) rating accuracy (%), (B) rating reaction time (msec), (C) head motion artefact (mm/TR) during the picture evaluation fMRI task, (D) Profile of Mood States-2 (POMS), (E) visit order and (F) day of menstrual cycle (n=10-11) between saline (white) and Exenatide (blue) visits for obese (clear) and ex-smoker (hatched) groups. (A-D) Data presented as box plots with median as line, mean as +, box 25th and 75th percentiles and bars 5th and 95th percentiles, n=23-24. (E-F) Data presented as bar graphs (mean) and SEM error bars. Abbreviations: Exe, Exenatide; POMS, Profile of mood states 2; Sal, saline; TR, time to repeat.

	Picture type	Obese		Ex-smoker		Interactions	F	P
		Saline	Exenatide	Saline	Exenatide			
Rating accuracy[a] (%)	Food	89.8 ± 14.0	89.5 ± 11.0	91.8 ± 12.9	90.9 ± 14.9	Visit	0.08	0.78
						Visit*picture	0.02	0.90
	Neutral	89.3 ± 15.4	87.5 ± 14.6	89.5 ± 14.9	90.2 ± 14.9	Visit*group	0.003	0.95
						Visit*group*picture	1.81	0.19
Reaction time (msec)	Food	1323 ± 215	1341 ± 230	1312 ± 147	1319 ± 189	Visit	0.53	0.47
						Visit*picture	0.05	0.83
	Neutral	1317 ± 240	1332 ± 238	1335 ± 163	1367 ± 210	Visit*group	0.18	0.68
						Visit*group*picture	0.12	0.73
Motion[a] (mm/TR)	All	0.147 ± 0.067	0.145 ± 0.069	0.104 ± 0.052	0.101 ± 0.040	Visit	0.001	0.98
						Visit*group	0.20	0.66
POMS (range: -23 to 200)		3.04 ± 4.00	-0.33 ± 3.10	8.52 ± 4.09	4.35 ± 3.17	Visit	2.13	0.15
						Visit*group	0.02	0.88
SSAI (max 80)		27.33 ± 1.33	24.96 ± 1.19	29.00 ± 1.36	28.22 ± 1.21	Visit	3.74	0.059
						Visit*group	0.95	0.34
PA (max 50)		34.92 ± 1.31	34.96 ± 1.30	32.74 ± 1.34	33.87 ± 1.33	Visit	0.37	0.55
						Visit*group	0.32	0.58
NA (max 50)		13.00 ± 0.95	13.13 ± 0.98	15.04 ± 0.97	14.44 ± 1.00	Visit	0.10	0.76
						Visit*group	0.22	0.64

Table 4.4. Potential confounding factors for HE food picture evaluation task.

Analyses looked for differences in rating accuracy and reaction time between groups using repeated measures ANOVA with visit (saline, Exenatide) and picture type (Food, Neutral) as within subject factors, and group (obese, ex-smoker) as between subject factor. Results from repeated measures ANOVA for state mood questionnaires with visit (Exenatide, saline) as within subject factor and group (obese, ex-smoker) as between subject factor Data presented as mean ± SEM, n=23-24. [a] Data used in repeated measures ANOVA was normalised to log10 values. Abbreviations: NA, Negative Affect; PA, Positive Affect; POMS, Profile of Mood States; SSAI, Spielberger State Anxiety Inventory

4.4.3 Lunch Taste Visual Analogue Scale Ratings

Data was available for n=24 dieting participants with obesity and n=23 ex-smokers for analysis of initial visual analogue scale (VAS) taste ratings, and overall consumption (see next section), of the individual dishes at the *ad libitum* test lunch meal after scanning. All participants had the tomato soup on their study visits, apart from one in the ex-smoker group who had the chicken soup. In these analyses, a single repeated measures ANOVA was performed across both groups and all 4 dishes, with visit (saline, Exenatide), dish sweetness (savoury, sweet), dish fat content (low fat, high fat) as within subject factors, and group (obese, ex-smoker) as between subject factor, primarily to look at interactions and overall effect of visit.

Summary: At the Exenatide visit the liking and pleasantness ratings for yogurt was lower in ex-smokers than in dieting group with obesity, but not the saline visit, consistent with Exenatide lowering these ratings in ex-smokers (Figure 4.11AB, Table 4.5). Furthermore, Exenatide increased the rating of ideal sweetness for yogurt, independent of group (Figure 4.11F, Table 4.5).

4.4.3.1 Liking

Summary: Exenatide reduced liking of low-fat yoghurt in ex-smokers more than in dieting group with obesity (Figure 4.11A, Table 4.5).

For rating of liking, there was a significant interaction effect for visit*sweet*fat*group [F(1**,**45)=4.68, P=0.036]. This was driven predominately by the ex-smokers liking yogurt less than dieting group with obesity at the Exenatide visit [effect size mean ± SEM -17.98 ± 6.71, (95%CI -31.50, -4.46), P=0.010, Cohen's d=0.18], but not at the saline visit [effect size mean ± SEM -4.84 ± 5.31, (95%CI -15.53, 5.85), P=0.37, Cohen's d=0.27].

4.4.3.2 Pleasantness

Summary: Exenatide reduced pleasantness of low-fat yoghurt in ex-smokers more than in dieting group with obesity (Figure 4.11B, Table 4.5).

Similarly, for rating of pleasantness, there was a significant interaction effect for visit*group*sweet*fat [F(1**,**45)=6.66, P=0.013]. This was driven predominately by the ex-smokers rating yogurt less pleasant than in dieting group with obesity at the Exenatide visit [effect size

mean ± SEM -16.23 ± 6.76, (95%CI -29.85, -2.61), P=0.021, Cohen's d=0.45], but not the saline visit effect size mean ± SEM -6.13 ± 5.54, (95%CI -17.29, 5.02), P=0.27, Cohen's d=0.71].

4.4.3.3 Creaminess intensity and ideal creaminess

For rating of creaminess intensity or ideal creaminess, there were no significant interaction effects for: (i) visit*sweet*fat*group, (ii) visit*sweet*fat, (iii) visit*fat*group, (iv) visit*fat, (v) visit*sweet*group, (vi) visit*sweet, (vii) visit*group, nor an overall effect of (viii) visit (Figure 4.11C-D, Table 4.5).

4.4.3.4 Sweetness intensity

For rating of sweetness intensity of the desserts (yogurt and ice cream), there were no significant interaction effects for: (i) visit*fat*group, (ii) visit*fat, (iii) visit*group, nor an overall effect of (iv) visit (Figure 4.11E, Table 4.5).

4.4.3.5 Ideal sweetness

Summary: Exenatide increased rating of ideal sweetness for yogurt, independent of group (Figure 4.11F, Table 4.5).

There were no significant interaction effects for: (i) visit*fat*group, nor (ii) visit*group, but there was a significant interaction effect for (iii) visit*fat, independent of group [F(1,45)=5.40, P=0.025]. This was driven mainly by a significant increase in rating of ideal sweetness rating for yogurt at Exenatide compared to saline visit, independent of group [effect size mean ± SEM 4.04 ± 1.99, (95%CI 0.03, 8.04), P=0.048, Cohen's d=0.30], that was not seen for ice-cream [effect size mean ± SEM -0.70 ± 1.59, (95%CI -3.91, 2.51), Cohen's d=0.06].

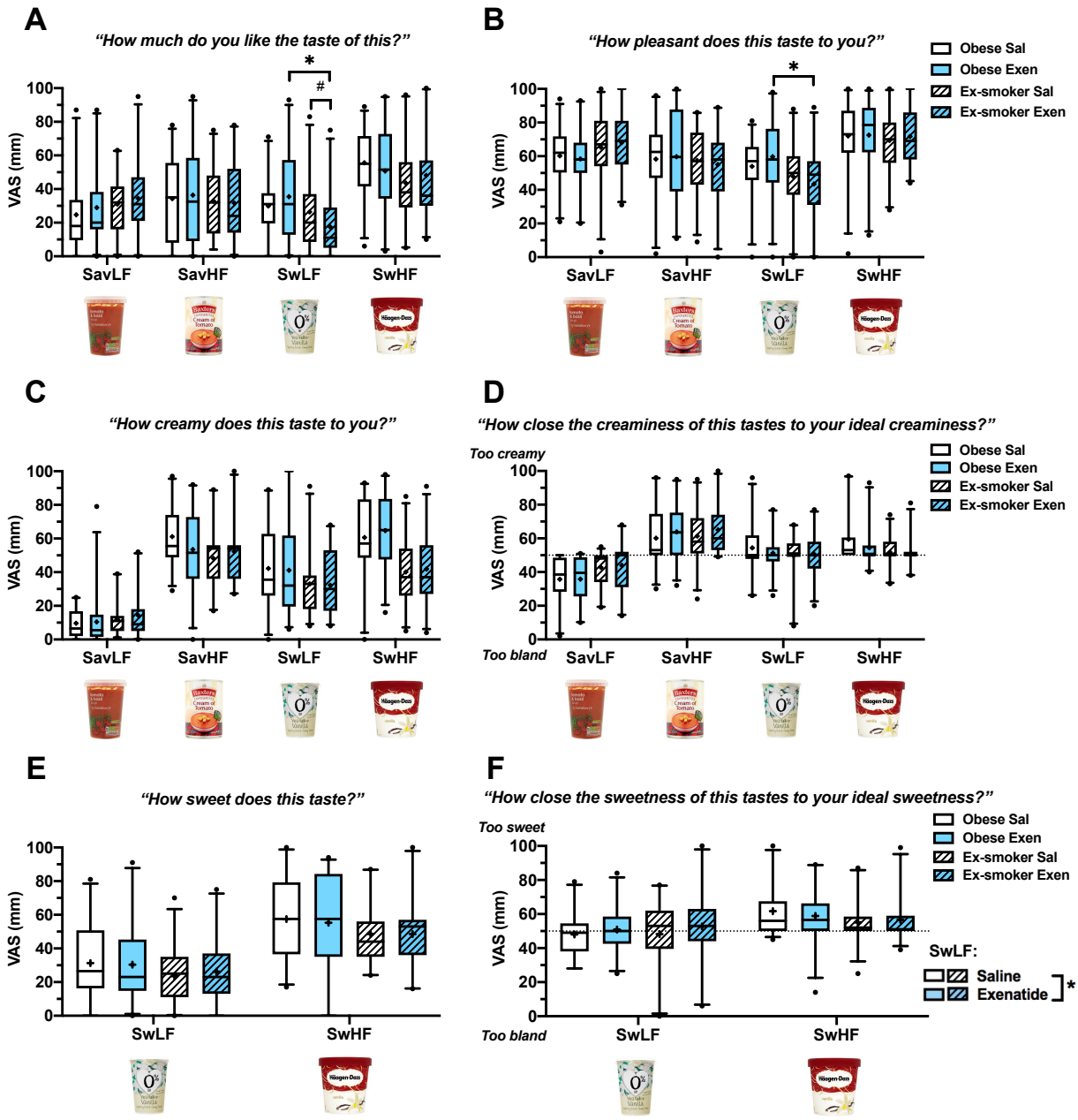


Figure 4.11. Effects of Exenatide on taste ratings of lunch dishes in dieting group with obesity and ex-smokers. Comparison of visual analogue scale ratings of (A) liking, (B) pleasantness, (C) creaminess intensity, (D) ideal creaminess, (E) sweetness intensity, (F) ideal sweetness, tasted at the start of the *ad libitum* test lunch meal, for (A-D) individual dishes (savoury LF broth, savoury HF cream soup, sweet LF yogurt, sweet HF ice-cream), or (E,F) just desserts (yogurt, ice-cream), between visits (Exenatide, Saline) and group (obese, ex-smoker). Data presented as boxplots showing median (line), mean (cross), interquartile range (box) and 5th – 95th percentile (bars), n=23-24. Statistics from repeated measures ANOVA for taste measures, with group (obese, ex-smoker) as between subject factor, and dish fat content (low fat, high fat), dish sweetness (savoury, sweet) and visit (Saline, Exenatide) as within subject factors. , with post hoc Fisher LSD test: *P<0.05 #P=0.082

Interactions: Liking	***df***	***F***	***P***	***Interactions: Pleasantness***	***df***	***F***	***P***
Visit	(1,45)	0.28	0.60	Visit	(1,45)	0.11	0.75
Visit*group	(1,45)	0.33	0.57	Visit*group	(1,45)	0.30	0.59
Visit*sweet	(1,45)	1.21	0.28	Visit*sweet	(1,45)	0.28	0.60
Visit*sweet*group	(1,45)	0.032	0.86	Visit*sweet*group	(1,45)	1.14	0.29
Visit*fat	(1,45)	0.49	0.49	Visit*fat	(1,45)	0.004	0.95
Visit*fat*group	(1,45)	3.67	0.062	Visit*fat*group	(1,45)	0.13	0.72
Visit*sweet*fat	(1,45)	1.07	0.31	Visit*sweet*fat	(1,45)	0.20	0.66
Visit*sweet*fat*group	(1,45)	4.68	**0.036***	Visit*sweet*fat*group	(1,45)	6.66	**0.013***
Interactions: Creaminess				***Interactions: Ideal creaminess***			
Visit	(1,45)	0.06	0.81	Visit	(1,45)	0.009	0.92
Visit*group	(1,45)	0.63	0.43	Visit*group	(1,45)	1.85	0.18
Visit*sweet	(1,45)	0.18	0.67	Visit*sweet	(1,45)	3.66	0.062
Visit*sweet*group	(1,45)	2.15	0.15	Visit*sweet*group	(1,45)	0.63	0.43
Visit*fat	(1,45)	0.01	0.91	Visit*fat	(1,45)	0.12	0.73
Visit*fat*group	(1,45)	0.61	0.44	Visit*fat*group	(1,45)	0.005	0.94
Visit*sweet*fat	(1,45)	1.64	0.21	Visit*sweet*fat	(1,45)	1.68	0.20
Visit*sweet*fat*group	(1,45)	1.30	0.26	Visit*sweet*fat*group	(1,45)	0.06	0.81
Interactions: Sweetness				***Interactions: Ideal sweetness***			
Visit	(1,45)	0.004	0.95	Visit	(1,45)	1.26	0.27
Visit*group	(1,45)	0.43	0.52	Visit*group	(1,45)	1.67	0.20
Visit*fat	(1,45)	0.17	0.68	Visit*fat	(1,45)	5.40	**0.025***
Visit*fat*group	(1,45)	0.02	0.88	Visit*fat*group	(1,45)	0.06	0.80

Table 4.5. Repeated measures ANOVA for effect of Exenatide on taste ratings of lunch dishes.
Results from repeated measures ANOVA for taste measures (liking, pleasantness, creaminess, ideal creaminess) for soups and desserts, and sweet and ideal sweetness for the desserts only, with group (obese, ex-smoker) as between subject factor, and dish fat content (low fat, high fat), dish sweetness (savoury, sweet) and visit (saline, Exenatide) as within subject factors. Significant results are in bold, *P<0.05, n=23-24.

4.4.4. Ad libitum lunch meal energy intake

Summary: Exenatide reduced total energy intake, and energy intake from the savoury soups, independent of group (Figure 4.12, Table 4.6).

At the *ad libitum* test meal, Exenatide reduced *total* energy intake (both for absolute amount or as % of estimated 24 hour resting energy expenditure, calculated using Cunningham equation), independent of group [effect size mean ± SEM (absolute) -71.95 ± 30.26, (95% CI -132.90, -11.00), P=0.022, Cohen's d=0.34; (%REE) -4.10 ± 1.76, (95% CI -7.64, -0.56), P=0.024, Cohen's d=0.33] (Figure 4.12 AB, Table 4.6).

When examining individual dishes, there were no significant interaction effects on either absolute or REE energy intake for: (i) visit*sweet*fat*group, (ii) visit*sweet*fat, (iii) visit*fat*group, (iv) visit*fat, (v) visit*sweet*group, nor (vi) visit*group.

However, there was a significant interaction effect for (vii) visit*sweet, independent of group. This was driven by a reduction in energy intake from both soups (savoury) at the Exenatide visit [effect size mean ± SEM (absolute) -31.98 ± 9.55, (95% CI -51,22, -12,75), P=0.002; (%REE) -1.87 ± 0.57, (95% CI -3.02, -0.71), P=0.002].

Interactions: Energy intake by dishes (kcal)	***df***	***F***	***P***	***Interactions: Energy intake by dishes (%REE)***	***df***	***F***	***P***
Visit	(1,45)	5.65	**0.022***	Visit	(1,45)	5.43	**0.024***
Visit*group	(1,45)	0.29	0.59	Visit*group	(1,45)	0.32	0.58
Visit*sweet	(1,45)	4.90	**0.032***	Visit*sweet	(1,45)	5.70	**0.021***
Visit*sweet*group	(1,45)	0.67	0.42	Visit*sweet*group	(1,45)	0.84	0.37
Visit*fat	(1,45)	2.63	0.11	Visit*fat	(1,45)	2.91	0.095
Visit*fat*group	(1,45)	1.41	0.24	Visit*fat*group	(1,45)	1.58	0.22
Visit*sweet*fat	(1,45)	0.84	0.37	Visit*sweet*fat	(1,45)	1.15	0.29
Visit*sweet*fat*group	(1,45)	0.01	0.91	Visit*sweet*fat*group	(1,45)	0.03	0.97

Table 4.6. Repeated measures ANOVA for effect of Exenatide on energy intake of *ad libitum* test meal.
Results from repeated measures ANOVA for energy intake [absolute, or as a percentage of estimated 24 hour resting energy expenditure (REE)] for the individual dishes, with group (obese, ex-smoker) as between subject factor, and visit (Exenatide, saline), dish sweetness (savoury, sweet) and dish fat content (LF, HF) as within subject factors. N=23-24 per group, significant results are in bold: *P<0.05.

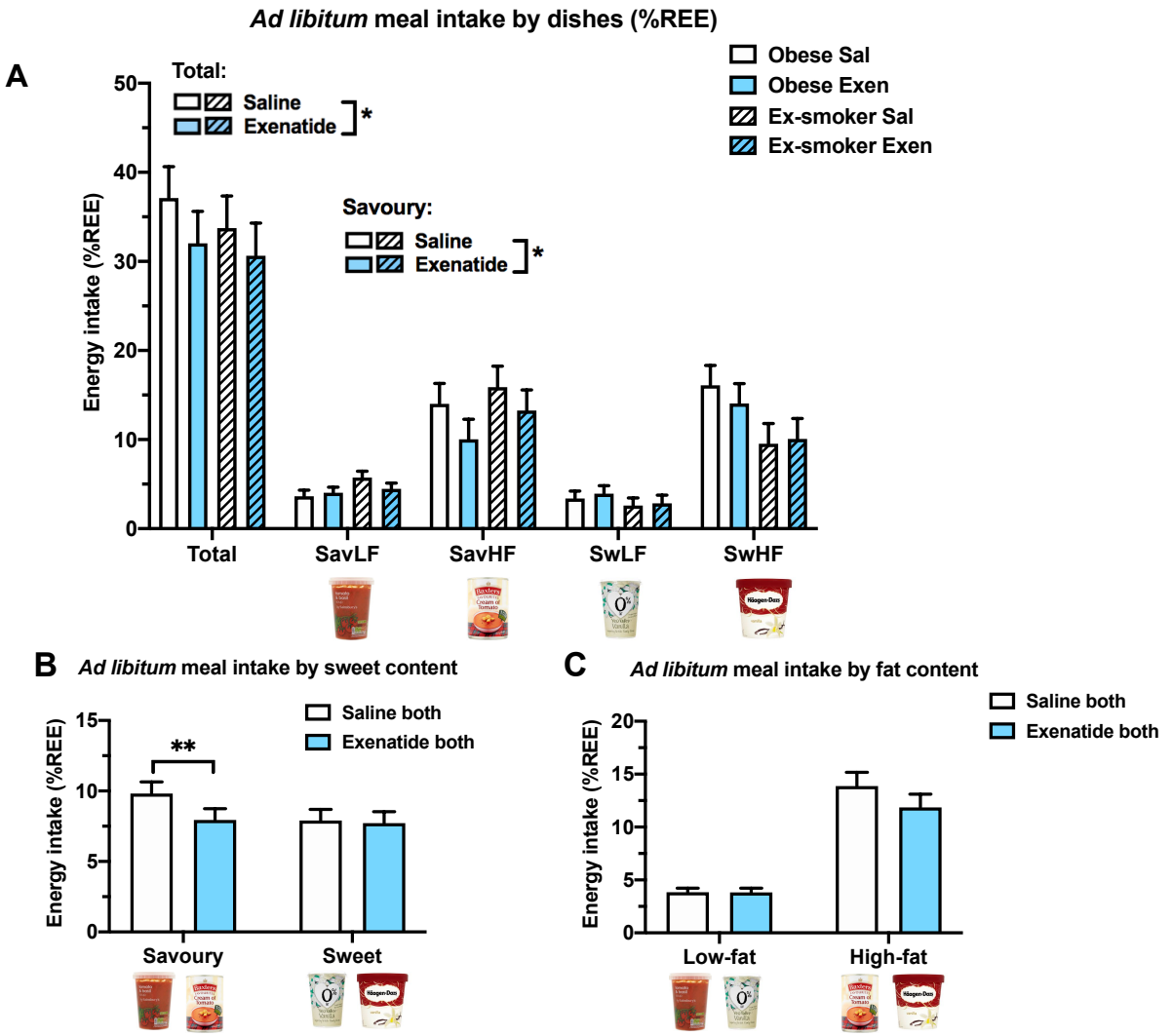


Figure 4.12. Effects of Exenatide on energy intake at *ad libitum* test meal in dieting group with obesity and ex-smokers.

Comparison of energy intake for: (A) total food and individual dishes [(SavLF) savoury low-fat broth; (SavHF) savoury high-fat cream soup; (SwLF) sweet low-fat yogurt; (SwHF) sweet high-fat ice-cream] as percentage of estimated 24 hour resting energy expenditure (REE), and (B,C) energy intake as %REE by dish (B) sweetness and (C) fat content, between Exenatide and saline visits in (A) in obese and ex-smokers separately, or (B-C) independent of group. Data presented as mean ± SEM, n=23-24 per group. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor, and visit (Exenatide, saline), dish sweetness (savoury, sweet) and dish fat content (LF, HF) as within subject factors: *P<0.05 **P<0.01.

Adjusting for Covariates: total intake in kcal as %REE

To assess whether the overall effect for visit was altered when adjusting for co-variates, the following factors were included in separate repeated measures ANCOVA analyses:

(i) *Visit order*: The overall effect for visit after adjusting for difference in visit order between visits (i.e. visit number of Exenatide minus saline visits) was F(1,44)=5.97, P=0.019]. This was driven by a reduction in intake (%REE) at Exenatide visit compared to saline, independent of group [effect size mean ± SEM -4.10 ± 1.70, (95%CI -7.52, -0.67), P=0.020].

(ii) *Plasma glucose*: There was no longer a significant overall effect of visit after adjusting for change in plasma glucose (i.e. plasma glucose pre-meal at T=315mins at Exenatide minus saline visit, n=20-21) was [F(1,38)=0.07, P=0.79].

(iii) *Nausea VAS*: There was a trend for significant overall effect of visit after adjusting for change in nausea (i.e. nausea VAS rating pre-meal at T=315min at Exenatide minus saline visit) [F(1,44)=4.04, P=0.051]. This was driven by a reduction in total intake (%REE) at the Exenatide compared to saline visit independent of group [effect size ± SEM -4.10 ± 1.77, (95%CI -7.66, -0.53), P=0.025].

4.4.5 Progressive Ratio Task (PRT)

Summary: There was a trend for Exenatide to significantly reduce motivation to receive a chocolate sweet, independent of group. This remained a trend after adjusting for nausea (Figure 4.13).

Data was available for PRT in n=24 dieting group with obesity and n=23 ex-smokers. However, 1 participant with obesity had to be excluded as they disliked M&Ms and declined to do the task. Another 6 participants with obesity and 1 ex-smoker had to be excluded as they did not understand the task instructions or had a more than 2 chocolate discrepancy between number of chocolates eaten and the number of completed ratios on software records at either Exenatide or saline visit. This resulted in a final n=17 with obesity and n=22 ex-smokers respectively. Data was normalised by $\log_{10}$ transformation for statistical analysis given the exponential nature of the progressive ratio design.

Outcome measures of PRT

For the breakpoint (last completed click to earn an M&M), there was no significant interaction effects for: (i) visit*group, nor overall effect of (ii) visit. However, there were trends for an overall effect of visit for breakpoint [F(1,37)=2.92, P=0.096], independent of group, suggesting a decrease in motivation to receive a sweet taste between the saline and Exenatide visits, independent of group. Exenatide tended to reduce breakpoint by 81.10% (95% CI 63.25, 104.00). The effect size of Exenatide on breakpoint was calculated as a percentage change using this equation $100/10^{-x}$ where x is the effect size of $\log_{10}$ transformed data taken from the repeated measures ANOVA.

Adjusting for Covariates

To assess whether the overall effect of visit was altered when adjusting for co-variates, the following factors were included in separate repeated measures ANCOVA analyses:

(i) *Visit order*: After adjusting for visit order (See section 4.4.2.1 for definition), there was a trend for an overall effect of visit [F(1,36)=3.37, P=0.075]. This indicates that Exenatide tended to reduce breakpoint independent of group [effect size 80.91% (95% CI 63.09, 103.52%)].

(ii) *Plasma glucose*: After adjusting for change in glucose concentrations (i.e. plasma glucose level pre-PRT T=270min at Exenatide minus that at saline visit, n=12-20), there was no significant overall effect of visit [F(1,29)=0.02, P=0.89]. However, this was partially attributed to the much smaller sample size when in fact the effect size of Exenatide reduction on breakpoint was similar [effect size 86.70%, (95%CI 63.98, 117.50)].

(iii) *Nausea*: After adjusting for change in nausea VAS rating (i.e. nausea VAS pre-PRT at T=270min at Exenatide minus that at saline visit), there was a trend for significant overall effect of visit [F(1,36)=3.37, P=0.075]. This indicates that Exenatide tended to reduce breakpoint independent of group [effect size 81.29%, (95% CI 63.37, 104.00)].

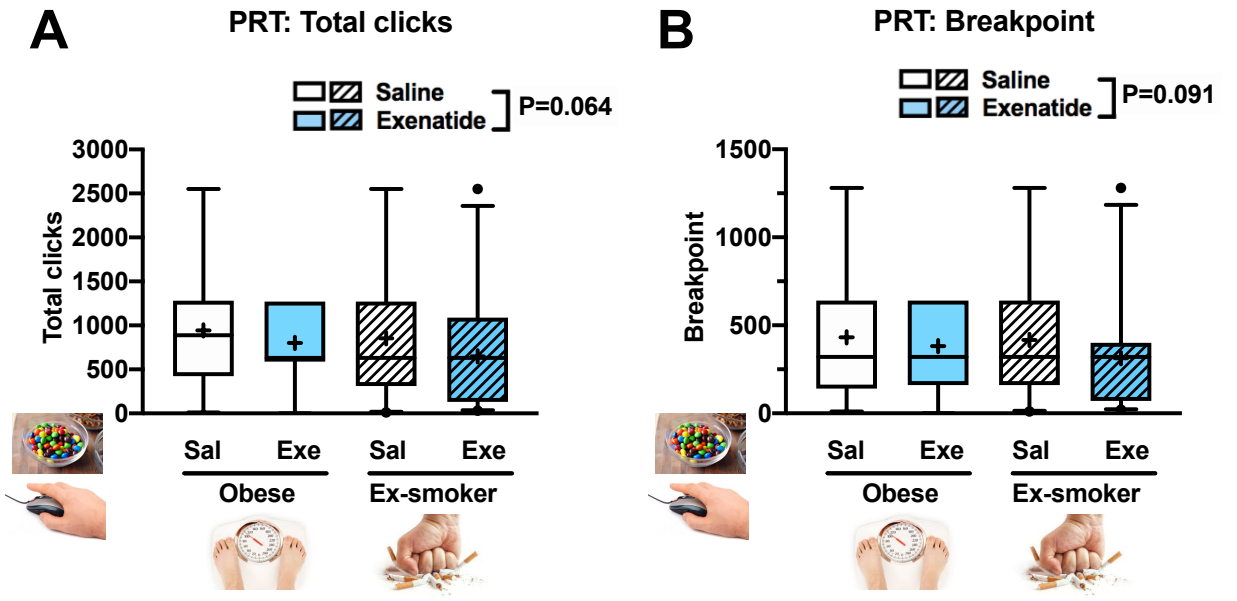


Figure 4.13 Exenatide tended to reduce total number of clicks and breakpoint in PRT in dieting group with obesity and ex-smokers.

Comparison of [A] total number of clicks and [B] breakpoint (last completed click) to receive an M&M chocolate between saline and Exenatide visits in the obese and Ex-smoker groups. Data presented as boxplots showing median (line), mean (cross), interquartile range (box) and 5^{th} – 95^{th} percentile (bars), n=17-22. Data normalised by log transformation for [A-B] outcome measures in task for statistical analysis. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor and Visit (Exenatide, saline) as within subject factors, with post hoc Fisher LSD test.

4.4.6 Approach Avoidance Task (AAT)

Summary: Exenatide did not have an effect on approach bias to food pictures in the AAT (Figure 4.14).

Data was available for AAT in n=24 dieting group with obesity and n=23 ex-smokers. However, only participants achieving an average accuracy of 75% and over for each visit were included, giving a final n=21 and n=19 in dieting group with obesity and ex-smokers respectively.

Outcome measures of AAT

For the relative food bias (median pull reaction time of food pictures subtracted from median push reaction time of food pictures compared to that of neutral pictures), there were no significant interaction effects for: (i)visit*group [F(1,38)=0.16, P=0.70] nor overall effect of (ii)visit [F(1,38)=0.05, P=0.82], indicating there was a similar approach bias to food pictures in both saline and Exenatide visits. This remained non-significant after adjusting for difference in visit order (saline visit number subtracted from Exenatide visit number). Data was normalised using log transformation for statistical analysis.

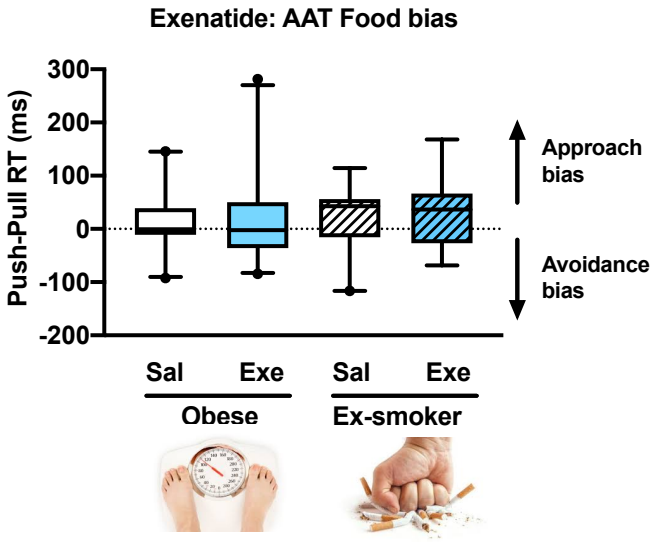


Figure 4.14. No effect on Exenatide on HE food approach bias in dieting group with obesity or ex-smokers. Comparison of relative food bias (vs. neutral) [i.e. median pull reaction time subtracted from median push reaction time to food pictures vs. that of neutral pictures] during the task between saline and Exenatide visits in the obese and ex-smoker groups. Data presented as boxplots showing median (line), mean (cross), interquartile range (box) and 5^{th} – 95^{th} percentile (bars), n=19-21. Data normalised by log transformation for outcome measures in task for statistical analysis. Statistics from repeated measures ANOVA, with Group (obese, ex-smoker) as between subject factor and Visit (Exenatide, saline) as within subject factors.

4.4.7 Visual analogue scale (VAS) ratings of appetite and confounding factors

Summary: Exenatide reduced appetite rating independent of group (Figure 4.15). Exenatide also reduced food craving rating in the dieting group with obesity, and not ex-smokers (Figure 4.16). Exenatide increased nausea ratings independent of group although overall ratings remained low (Figure 4.17). Exenatide increased anxiety rating at T=45 in ex-smokers and not dieting group with obesity (Figure 4.18). Exenatide did not affect sleepiness and stress VAS ratings (Figure 4.19-4.20).

Data was available for n=24 participants in dieting group with obesity and n=23 participants in ex-smoker group.

4.4.7.1. Composite appetite VAS rating (Figure 4.15, Table 4.7)

For appetite VAS rating throughout the whole study visit, there were no significant interaction effects for: (i) visit*time*group, (ii) visit*time, nor (iii) visit*group. However, there was an overall effect for (iv) visit, [F(1,45)=4.92, P=0.032]. This was driven by a reduction of composite appetite rating at the Exenatide visit (effect size mean ± SEM -3.91 ± 1.76, (95%CI -7.46,-0.36), P=0.032), across the visit and independent of group.

For area under the curve (AUC) of appetite VAS rating from T=-10mins to T=-315mins, there was no significant interaction effects for visit*group. However, there was an overall effect for visit, [F(1,45)=5.88, P=0.019]. This was driven by the reduction of appetite rating from the start of the Exenatide infusion till the beginning of the *ad libitum* meal (effect size mean ± SEM -1683.82 ± 694.66, (95%CI -3082.92, -284.71), P=0.019).

For the ΔAUC of appetite VAS rating from T=-10mins to T=-315mins, there were no significant interaction effects for visit*group, nor overall effect of visit. However, there was a trend to a significant overall reduction of ΔAUC for appetite rating during the Exenatide infusion, independent of group [F(1,45)=4.00, P=0.052, Cohen's d; effect size mean ± SEM -1361.95 ± 681.00, (95%CI -2733.56, 9.66)].

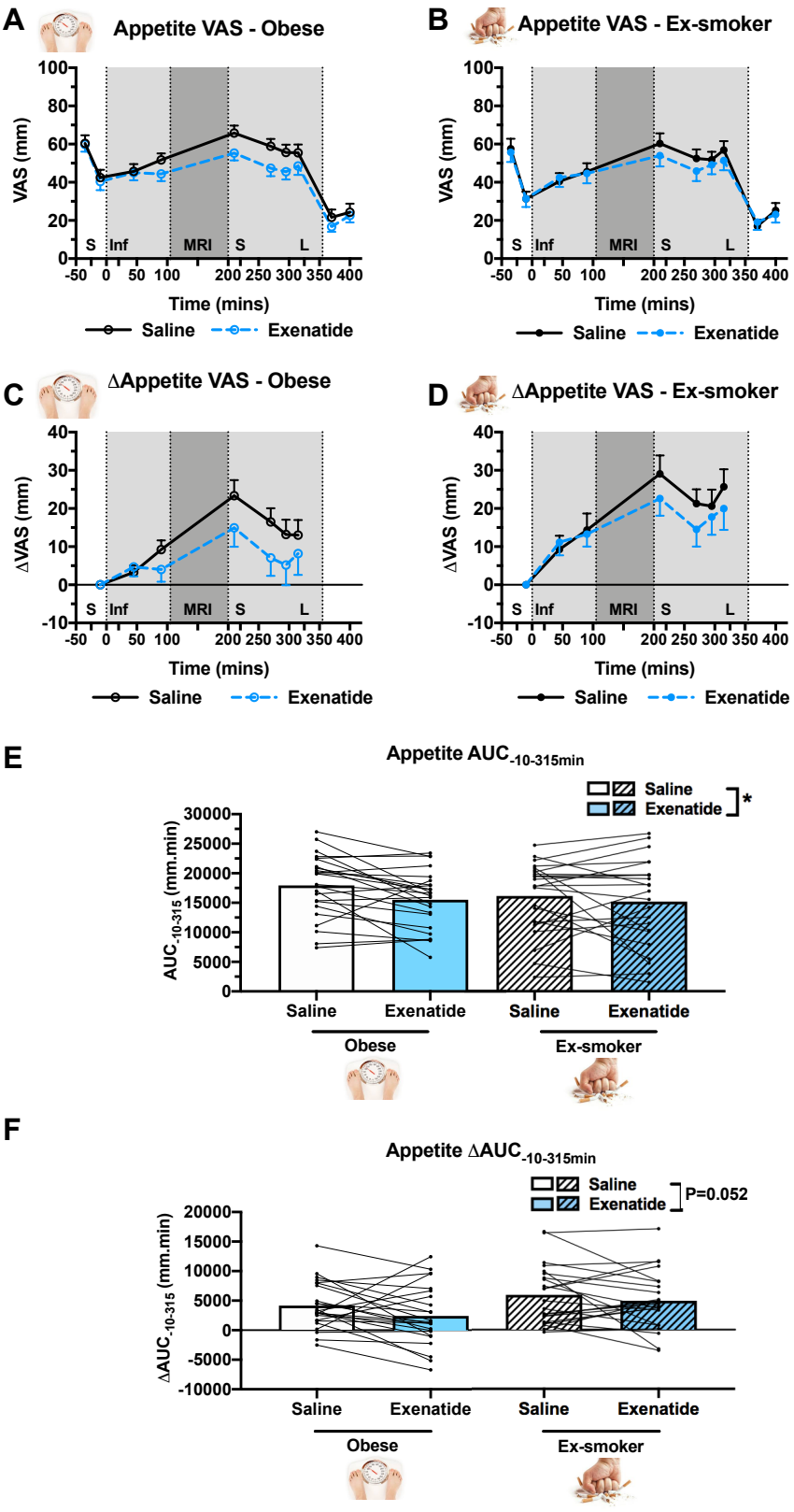


Figure 4.15 Exenatide decreased appetite VAS AUC (T=-10 to +315min) independent of group.
Comparison of composite appetite [A-B] visual analogue scale (VAS) ratings (max 100mm), [C-D]delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and Exenatide visits in the [A,C,E,F] obese and [B,D,E,F] ex-smoker groups. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=23-24. [A-D] VAS ratings plotted against time in group with [A,C] obesity and [B,D] ex-smoker during infusion of saline (black line) and Exenatide (blue dotted line). Light grey shaded area denotes time of infusion (T=0 to +355min), darker grey shaded area denotes period of fMRI scan (T=+105 to +205min). S indicates time of snacks and L indicates time of *ad libitum* test meal. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 4.7.

4.4.7.2. Composite food craving VAS rating (Figure 4.16, Table 4.7)

For food craving VAS rating throughout the whole study visit, there was a significant interaction effect for visit*group [F(1,45)=6.27, P=0.016]. This was driven by a reduction in food craving VAS at the Exenatide visit for the dieting group with obesity, which was not seen in ex-smokers (effect size mean ± SEM, -1.07 ± 0.38, (95%CI -1.83, -0.31), P=0.007) (Figure 4.16E-F). There were no significant interaction effects for: (i) visit*time*group, (ii) visit*time, nor overall effect for (iii) visit.

For AUC of food craving VAS rating from T=-10mins to T=-315mins, there was a significant interaction effect for visit*group [F(1,45)=4.46, P=0.040)]. This was driven by the reduction AUC of appetite rating from the start of the Exenatide infusion till the beginning of the *ad libitum* meal in the dieting group with obesity, which was not seen in ex-smokers (effect size mean ± SEM -373.96 ± 155.65, (95%CI -687.46, -60.46), P=0.020).

For the ΔAUC of food craving VAS rating from T=-10mins to T=-315mins, there were no significant interaction effects for visit*group, nor overall effect of visit.

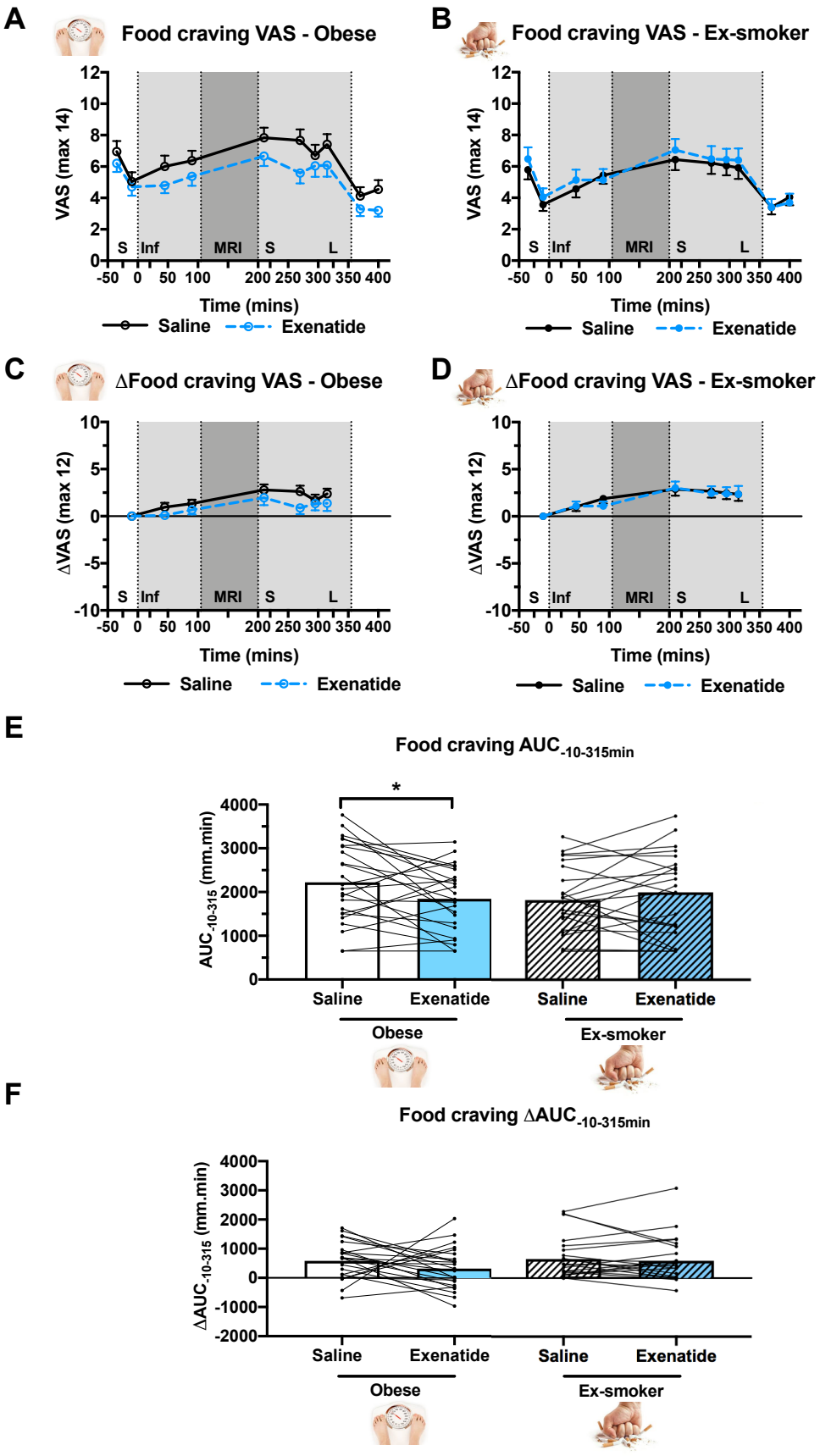


Figure 4.16. In dieting group with obesity, Exenatide decreased food craving visual analogue scale AUC(T=-10 to +315min). Comparison of composite food craving [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and Exenatide visits in the [A,B,E,F] obese and [C,D,E,F] ex-smokers. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=23-24. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 4.7.

Interactions: Appetite VAS	*df*	*F*	*P*	*Interactions: Food craving VAS*	*df*	*F*	*P*
Visit	(1,45)	4.92	**0.032***	Visit	(1,45)	2.13	0.15
Visit*group	(1,45)	0.95	0.34	Visit*group	(1,45)	6.27	**0.016***
Visit*time (GG)	(4.79, 215.45)	1.78	0.12	Visit*time (GG)	(4.78, 215.09)	0.73	0.59
Visit*time*group (GG)	(4.79, 215.45)	0.35	0.87	Visit*time*group (GG)	(4.78, 215.09)	0.52	0.75
Interactions: Appetite AUC -10 - +315				***Interactions: Food craving AUC -10 - +315***			
Visit	(1,45)	5.88	**0.019***	Visit	(1,45)	1.56	0.22
Visit*group	(1,45)	1.14	0.29	Visit*group	(1,45)	4.46	**0.040***
Interactions: Appetite ΔAUC -10 - +315				***Interactions: Food craving ΔAUC -10 - +315***			
Visit	(1,45)	4.00	0.052	Visit	(1,45)	2.02	0.16
Visit*group	(1,45)	0.32	0.58	Visit*group	(1,45)	0.81	0.37

Table 4.7. Repeated measures ANOVA results for effect of Exenatide on appetite and food craving VAS.
Results from repeated measures ANOVA for appetite and food craving VAS ratings with group (obese, Ex-smoker) as between subject factor and visit (Exenatide, saline), time (timepoints 1-10) as within subject factors. Results from repeated measures ANOVA for AUC (T=-10mins to T=+315mins) and ΔAUC (T=-10mins to T=+315mins) for appetite and food craving with group (obese, Ex-smoker) as between subject factor and visit (Exenatide, saline) as within subject factors. Significant results are in bold. Abbreviations: GG Greenhouse-Geisser correction.

Interactions: Nausea VAS	*df*	*F*	*P*	*Interactions: Anxious VAS*	*df*	*F*	*P*
Visit	(1,45)	7.15	**0.010***	Visit	(1,45)	1.81	0.19
Visit*group	(1,45)	1.71	0.20	Visit*group	(1,45)	4.27	**0.045***
Visit*time (GG)	(5.66, 254.82)	0.43	0.85	Visit*time (GG)	(4.35, 195.76)	1.24	0.29
Visit*time*group (GG)	(5.66, 254.82)	0.96	0.45	Visit*time*group (GG)	(4.35, 195.76)	2.90	**0.020***
[a] Interactions: Nausea AUC -10 - +315				*[a] Interactions: Anxious AUC -10 - +315*			
Visit	(1,45)	5.93	**0.019***	Visit	(1,45)	0.24	0.63
Visit*group	(1,45)	0.06	0.81	Visit*group	(1,45)	1.05	0.31
[a] Interactions: Nausea ΔAUC -10 - +315				*[a] Interactions: Anxious ΔAUC -10 - +315*			
Visit	(1,45)	0.99	0.33	Visit	(1,45)	1.24	0.27
Visit*group	(1,45)	0.84	0.37	Visit*group	(1,45)	1.59	0.21
Interactions: Stress VAS	***df***	***F***	***P***	***Interactions: Sleepy VAS***	***df***	***F***	***P***
Visit	(1,45)	0.29	0.59	Visit	(1,45)	1.91	0.17
Visit*group	(1,45)	1.88	0.18	Visit*group	(1,45)	0.02	0.88
Visit*time (GG)	(5.18, 233.13)	1.09	0.37	Visit*time (GG)	(4.91, 220.88)	1.05	0.39
Visit*time*group (GG)	(5.18, 233.13)	1.10	0.36	Visit*time*group (GG)	(4.91, 220.88)	1.08	0.37
[a] Interactions: Stress AUC -10 - +315				*[a] Interactions: Sleepy AUC -10 - +315*			
Visit	(1,45)	0.23	0.64	Visit	(1,45)	0.41	0.52
Visit*group	(1,45)	1.34	0.25	Visit*group	(1,45)	0.12	0.73
[a] Interactions: Stress ΔAUC -10 - +315				*[a] Interactions: Sleepy ΔAUC -10 - +315*			
Visit	(1,45)	1.08	0.30	Visit	(1,45)	0.13	0.72
Visit*group	(1,45)	1.41	0.24	Visit*group	(1,45)	1.32	0.26

Table 4.8. Repeated measures ANOVA for effect of Exenatide on nausea, anxiety, stress and sleepiness VAS ratings.
Results from repeated measures ANOVA for nausea, anxiety, stress and sleepiness VAS ratings with group (obese, ex-smoker) as between subject factor and visit (Exenatide, saline), time (timepoints 1-10) as within subject factors. Results from repeated measures ANOVA for AUC (T=-10mins to T=+315mins) and ΔAUC (T=-10mins to T=+315mins) for nausea, anxiety, stress and sleepiness with group (obese, ex-smoker) as between subject factor and visit (Exenatide, saline) as within subject factors. Significant results are in bold. [a] All data used in repeated measures ANOVA was normalised to log10 values. Abbreviations: GG Greenhouse-Geisser correction.

4.4.7.3. Nausea VAS rating (Figure 4.17, Table 4.8)

For nausea VAS rating throughout the whole study visit, there were no significant interaction effects for: (i) visit*time*group, (ii) visit*time nor (iii) visit*group. However, there was a significant overall effect of visit, independent of group [F(1,45)=7.15, P=0.010]. This was driven by an increase in nausea ratings at the Exenatide visit (effect size mean ± SEM 2.55 ± 0.96, (95%CI 0.63, 4.48), P=0.010).

For AUC of nausea VAS rating from T=-10mins to T=-315mins, there was no significant interaction effect for visit*group but there was a significant overall effect of visit, independent of group [F(1,45)=5.93, P=0.019]. This was driven by an increase in AUC of nausea rating at the Exenatide visit (effect size mean ± SEM 0.42 ± 0.17, (95%CI 0.07, 0.76), P=0.019].

For the ΔAUC of nausea VAS rating from T=-10mins to T=-315mins, there were no significant interaction effects for visit*group nor overall effect of visit.

4.4.7.4. Anxiety VAS rating (Figure 4.18, Table 4.8)

For anxiety VAS rating throughout the whole study visit, there was a significant interaction effect for visit*time*group [F(1,45)=2.90, P=0.020]. This was driven by a significant increase in anxiety rating during Exenatide infusion in Ex-smoker group at T=45min [effect size mean ± SEM 11.00 ± 4.02, (95%CI 2.91, 19.09), P=0.009], and a trend for significant increase at T=270min [effect size mean ± SEM 5.35 ± 2.95, (95%CI -0.60, 11.29), P=0.077]. Whilst there was a significant increase in anxiety rating at the start of Exenatide visit in dieting group with obesity and a trend in ex-smokers, this preceded the start of the infusion and hence not caused by Exenatide.

For AUC of anxiety VAS rating from T=-10mins to T=-315mins, there were no significant interaction effect for visit*group nor overall effect of visit.

For the ΔAUC of anxiety VAS rating from T=-10mins to T=-315mins, there were no significant interaction effects for visit*group nor overall effect of visit.

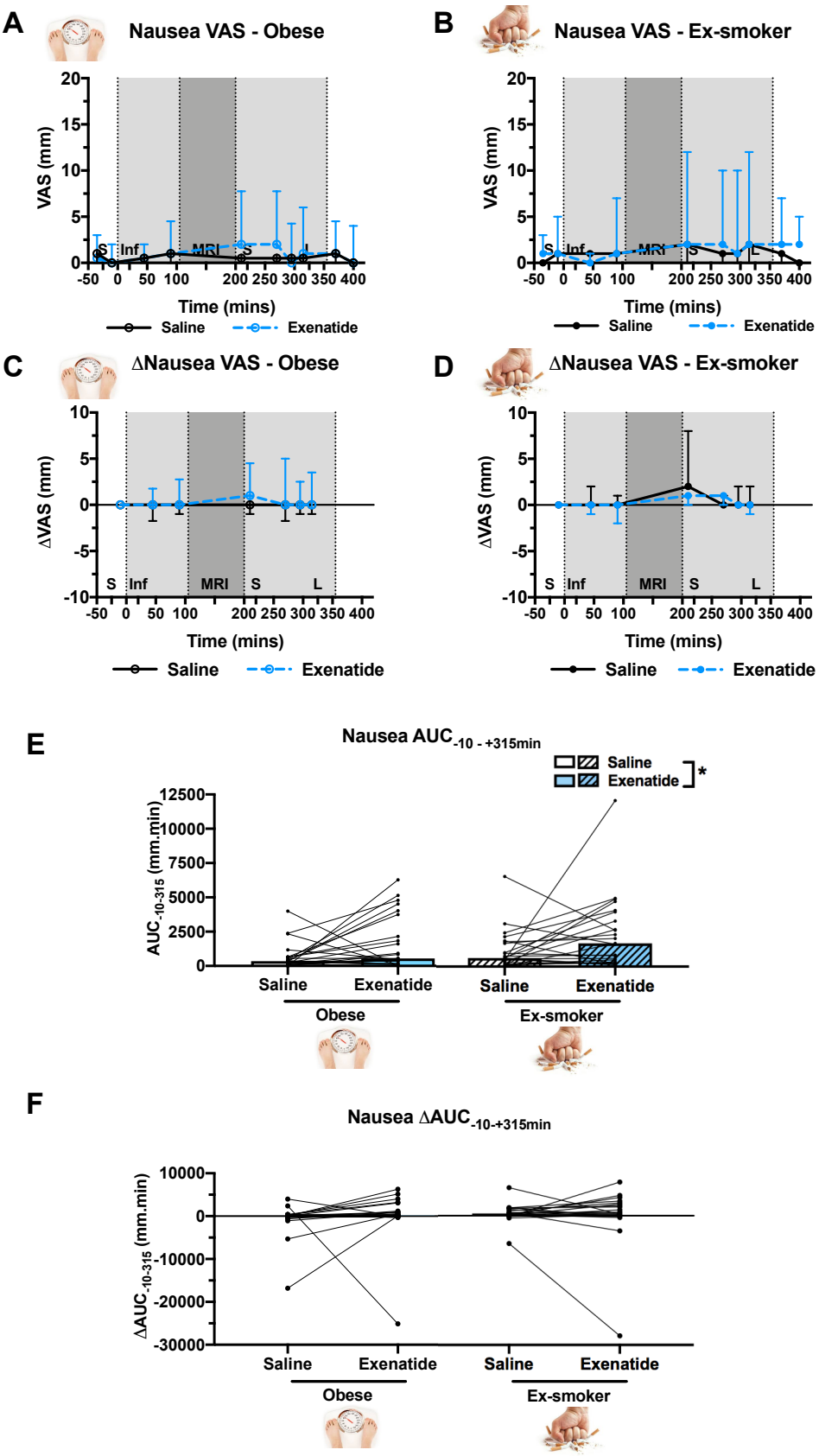


Figure 4.17 Exenatide increased nausea visual analogue scale AUC (T=-10 to +315min) independent of group. Comparison of nausea [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and Exenatide visits in the [A,C,E,F] obese and [B,D,E,F] ex-smoker groups. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=23-24. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 4.8.

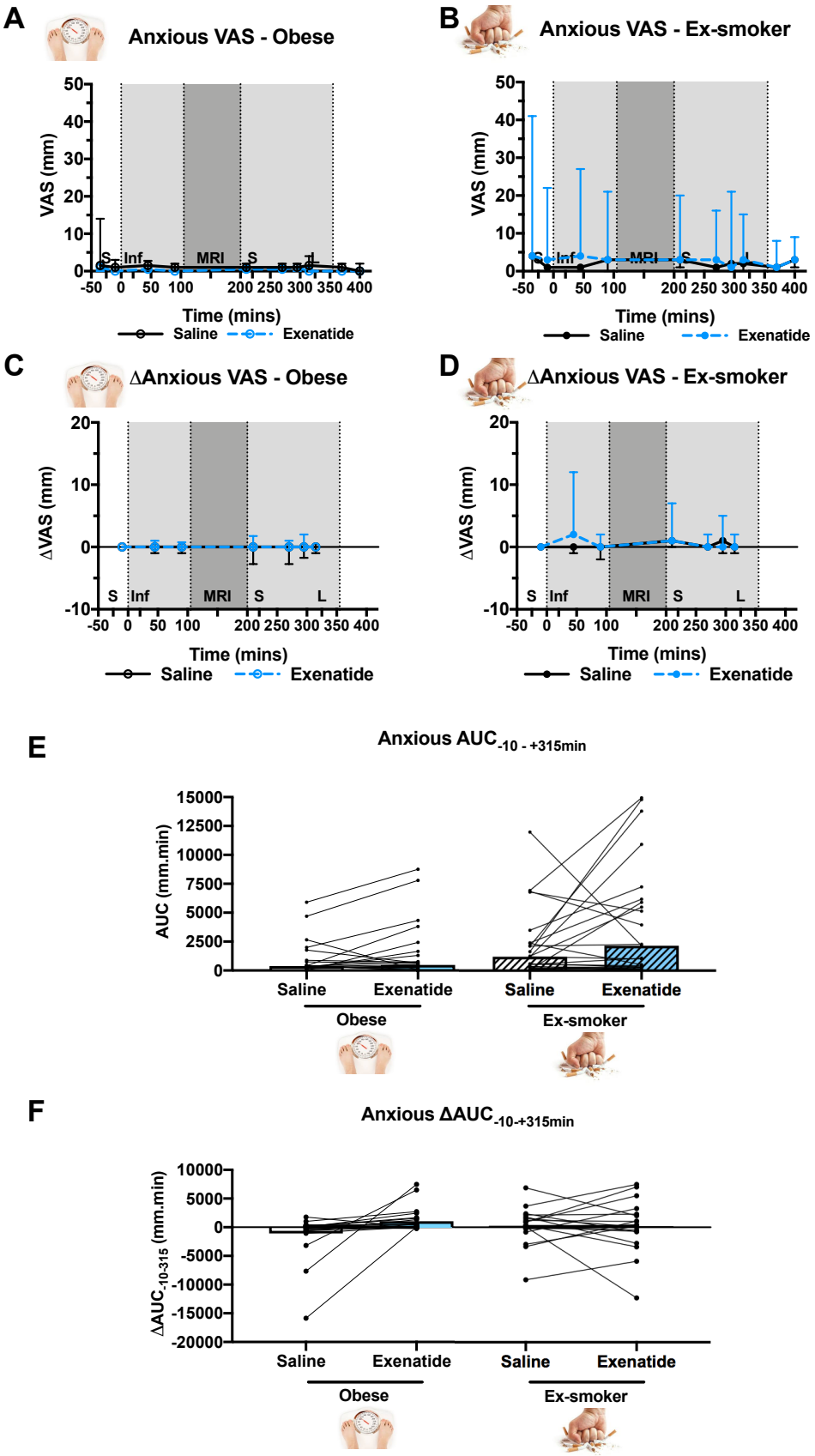


Figure 4.18 In the ex-smokers, there was increased anxiety VAS at T=-10min and T=+45min.
Comparison of anxiety [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and Exenatide visits in the [A,C,E,F] obese and [B,D,E,F] ex-smoker groups. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=23-24. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 4.8.

4.4.7.5. Sleepy VAS rating (Figure 4.19, Table 4.8)

For sleepy VAS rating throughout the whole study visit, there were no significant interaction effects for: (i) visit*time*group, (ii) visit*time, (iii) visit*group, nor overall effect of (iv) visit.

For AUC of sleepy VAS rating from T=-10mins to T=-315mins, there were no significant interaction effect for visit*group nor overall effect of visit.

For the ΔAUC of sleepy VAS rating from T=-10mins to T=-315mins, there were no significant interaction effects for visit*group nor overall effect of visit.

4.4.7.6. Stress VAS rating (Figure 4.20, Table 4.8)

For stress VAS rating throughout the whole study visit, there were no significant interaction effects for: (i) visit*time*group, (ii) visit*time, (iii) visit*group, nor overall effect of (iv) visit.

For AUC of stress VAS rating from T=-10mins to T=-315mins, there were no significant interaction effect for visit*group nor overall effect of visit.

For the ΔAUC of stress VAS rating from T=-10mins to T=-315mins, there were no significant interaction effects for visit*group nor overall effect of visit.

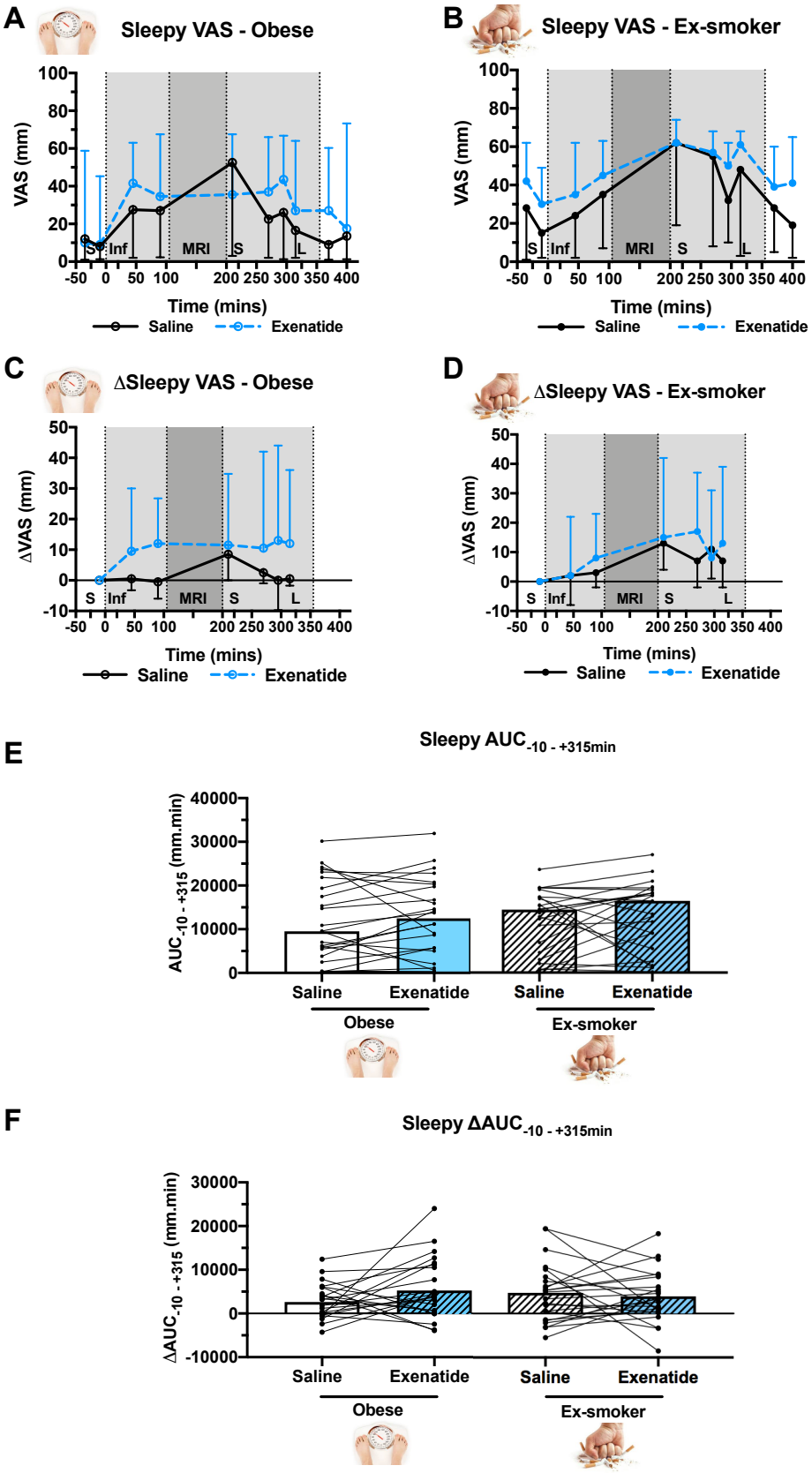


Figure 4.19. There was no difference in sleepiness during Exenatide and saline visits across both groups. Comparison of sleepy [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and Exenatide visits in the [A,C,E,F] obese and [B,D,E,F] ex-smoker groups. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=23-24. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 4.8.

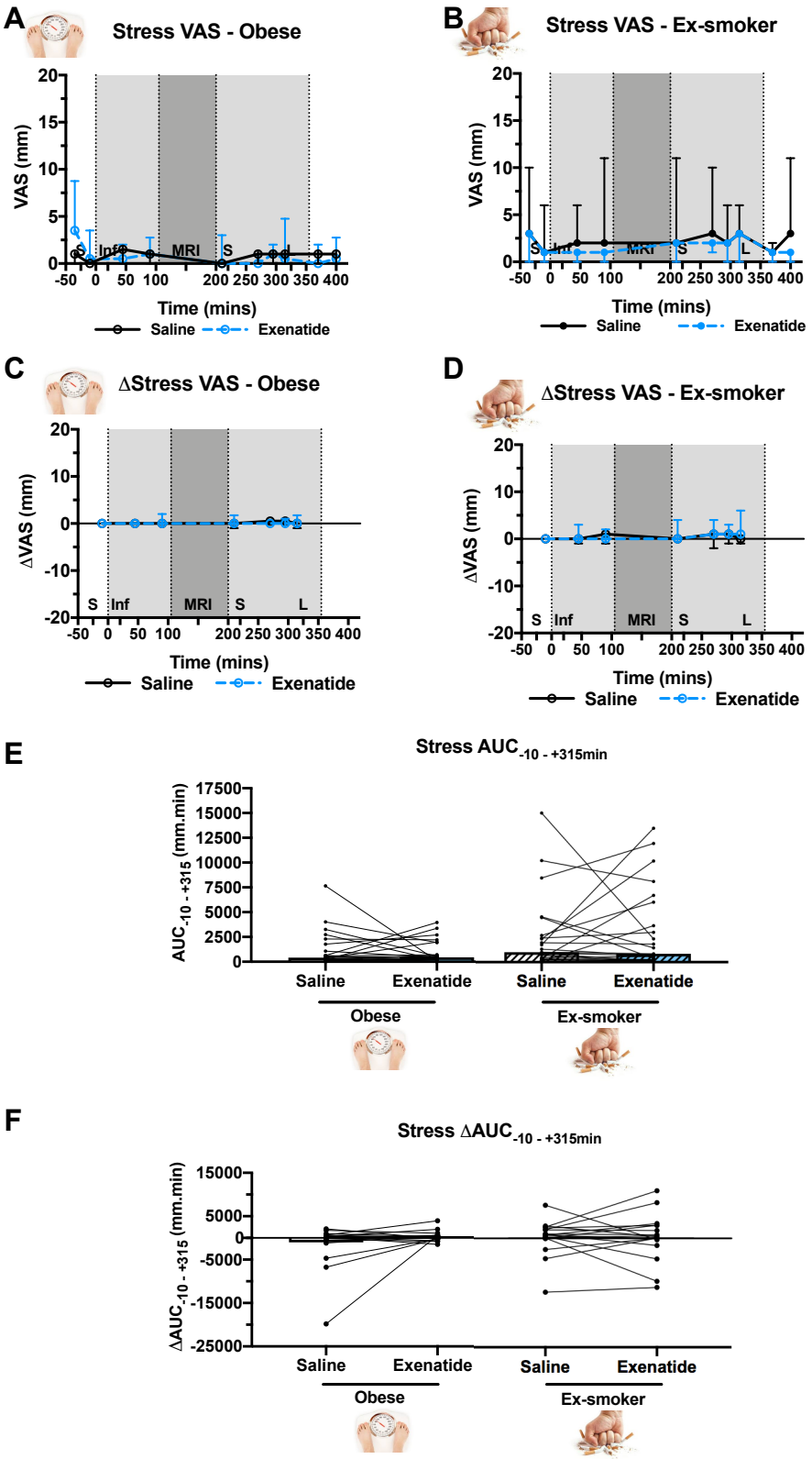


Figure 4.20. There was no difference in stress VAS during Exenatide and saline visits across both groups.
Comparison of stress [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and Exenatide visits in the [A,C,E,F] obese and [B,D,E,F] ex-smoker groups. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=23-24. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 4.8.

4.4.8. Plasma Glucose, Hormones and Lipid Profile

Summary: Exenatide decreased plasma glucose, insulin and growth hormone concentrations but increased cortisol and prolactin concentrations. Results for gut hormones (GLP-1, PYY, AG, DAG) and Exenatide are awaited.

Results were combined into a single linear mixed model ANOVA analysis. This resulted in blood data across six timepoints being available for n=20-22 in dieting group with obesity and n= 20-23 in ex-smoker group. Furthermore, blood AUC data was available for n=16-19 in dieting group with obesity and n=20-21 in ex-smoker group.

4.4.6.1 Plasma Glucose

Summary: Exenatide reduced plasma glucose in both dieting group with obesity and ex-smokers (Figure 4.21; Table 4.9).

For plasma glucose, there was no significant visit*time*group interaction, but there was a significant interaction effect for: (i) visit*group [F(1, 469.03)=5.82, P=0.016] and (ii) visit*time [F(5, 450.25)=31.50, P<0.001]. This was driven by a reduction in glucose levels at the Exenatide visit independent of timepoints in both dieting group with obesity and ex-smokers [effect size mean ± SEM obese: -0.96 ± 0.06, (95%CI -1.08, -0.84), P<0.001, Cohen's d=0.92; ex-smokers: -0.76 ± 0.06, (95%CI -0.87, -0.64), P<0.001, Cohen's d=1.17]. This indicates that Exenatide reduced plasma glucose in both dieting group with obesity and ex-smokers with a large effect size independent of timepoints. In addition, there was also a reduction in glucose levels at the Exenatide visit independent of groups at all timepoints [T=45, 90, 210, 270, 315 (P<0.001 – P=0.009)] apart from the baseline (T=-35min).

For change in glucose from baseline (Δglucose from T=-35), there was no significant visit*time*group interaction, but there was a significant interaction effect for visit*time [F(4, 362.79)=18.38, P<0.001]. This was driven by a reduction at the Exenatide visit in delta glucose from baseline at all timepoints independent of group (P<0.001-P=0.008).

For area under the curve between T=-35 and T=315 ($AUC_{-35\text{-}315min}$) for plasma glucose, there was a trend for significant effect of visit*group interaction [F(1,37.86)=3.48, P=0.070] and a significant overall effect of visit [F(1,37.86)=277.64, P<0.001]. This was driven by a reduction in $AUC_{-35\text{-}315min}$

glucose independent of group [effect size mean ± SEM -287.01± 17.23, (95%CI -321.88, -252.14), P<0.001, Cohen's d=2.58]. This indicates that Exenatide reduced AUC $_{-35\text{-}315min}$ glucose independent of group with a large effect size.

For the change in area under the curve between T=-35 and T=315 (ΔAUC $_{-35\text{-}315min}$) from baseline for plasma glucose, there was no significant effect of visit*group interaction but there was a significant overall effect of visit [F(1, 36.79)=204.40, P<0.001]. This was driven by a reduction in ΔAUC $_{-35\text{-}315min}$ glucose at the Exenatide visit independent of group [effect size mean ± SEM – 281.35 ± 19.68, (95%CI -321.23, -241.47), P<0.001, Cohen's d=2.47]. This indicates that Exenatide reduced ΔAUC $_{-35\text{-}315min}$ glucose independent of group with a large effect size.

GLUCOSE	*Visit*		*Group*		*Group*visit*		*Visit*time*		*Group*visit*time*	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
glucose	(1,469.03)=410.60	**<0.001**	(1,44.97)=2.48	0.12	(1,469.03)=5.82	**0.016**	(5,450.25)=31.50	**<0.001**	(5,450.25)=0.90	0.48
Δglucose	(1,379.69)=476.17	**<0.001**	(1,43.2)=2.41	0.13	(1,379.69)=3.04	0.082	(4,362.79)=18.38	**<0.001**	(4,362.79)=1.15	0.33
glucose AUC	(1,37.86)=277.64	**<0.001**	(1,44.49)=2.32	0.14	(1,37.86)=3.48	0.070				
glucose ΔAUC	(1,36.79)=204.40	**<0.001**	(1,42.16)=3.49	0.069	(1,36.79)=1.88	0.18				
INSULIN										
insulin	(1,471.22)=17.84	**<0.001**	(1,46.10)=10.17	**0.003**	(1,471.22)=0.006	0.94	(5,456.27)=6.20	**<0.001**	(5,456.27)=1.34	0.25
Δinsulin	(1,390.23)=21.41	**<0.001**	(1,47.38)=0.20	0.66	(1,390.23)=3.52	0.061	(4,371,57)=5.43	**<0.001**	(4,371,57)=1.37	0.24
insulin AUC	(1,38.06)=16.32	**<0.001**	(1,45.69)=13.37	**0.001**	(1,38.06)=0.56	0.46				
insulin ΔAUC	(1,43.19)=8.78	**0.005**	(1,45.92)=0.001	0.97	(1,43.19)=2.68	0.11				
GROWTH HORMONE										
growth hormone	(1,471.70)=0.34	0.56	(1,44.17)=4.47	**0.040**	(1,471.70)=1.41	0.24	(5,436.71)=3.55	**0.004**	(5,436.71)=0.49	0.78
Δgrowth hormone	(1,370.01)=0.07	0.79	(1,46.36)=0.18	0.68	(1,370.01)=0.12	0.73	(4,353.89)=4.00	**0.003**	(4,353.89)=0.53	0.71
growth hormone AUC	(1,35.97)=1.07	0.31	(1,39.71)=6.99	**0.012**	(1,35.97)=1.41	0.24				
growth hormone ΔAUC	(1,35.45)=0.70	0.41	(1,42.29)=0.52	0.47	(1,35.45)=0.23	0.64				
CORTISOL										
cortisol	(1,461.23)=20.21	**<0.001**	(1,44.82)=2.94	0.093	(1,461.23)=0.03	0.86	(5,445.94)=0.54	0.74	(5,445.94)=0.42	0.84
Δcortisol	(1,371.18)=4.42	**0.036**	(1,42.93)=1.54	0.22	(1,371.18)=0.45	0.50	(4,359.22)=0.47	0.76	(4,359.22)=0.41	0.81
cortisol AUC	(1,39.24)=18.35	**<0.001**	(1,44.38)=2.49	0.12	(1,39.24)=1.23	0.27				
cortisol ΔAUC	(1,40.07)=3.22	0.080	(1,43.57)=0.90	0.35	(1,40.07)=0.23	0.64				
PROLACTIN										
prolactin	(1,456.67)=1.67	0.20	(1,43.95)=0.15	0.70	(1,456.67)=7.20	**0.008**	(5,450.93)=1.78	0.12	(5,450.93)=0.32	0.90
Δprolactin	(1,371.84)=21.99	**<0.001**	(1,44.58)=2.11	0.15	(1,371.84)=2.49	0.12	(4,365.69)=1.23	0.30	(4,365.69)=0.26	0.91
prolactin AUC	(1,34.08)=0.09	0.76	(1,39.12)=0.03	0.86	(1,34.08)=1.43	0.24				
prolactin ΔAUC	(1,36.49)=4.32	**0.045**	(1,41.31)=2.66	0.11	(1,36.49)=0.23	0.64				

Table 4.9. Mixed model ANOVA results for effect of Exenatide on plasma glucose and hormones.

Results from fixed effects mixed model ANOVA for plasma glucose and hormones with group as between subject factor (obese, ex-smoker) and visit (saline, Exenatide) and time (T=-35, +45, +90, +210, +270, +315) as within subject factors. Significant results are in bold.

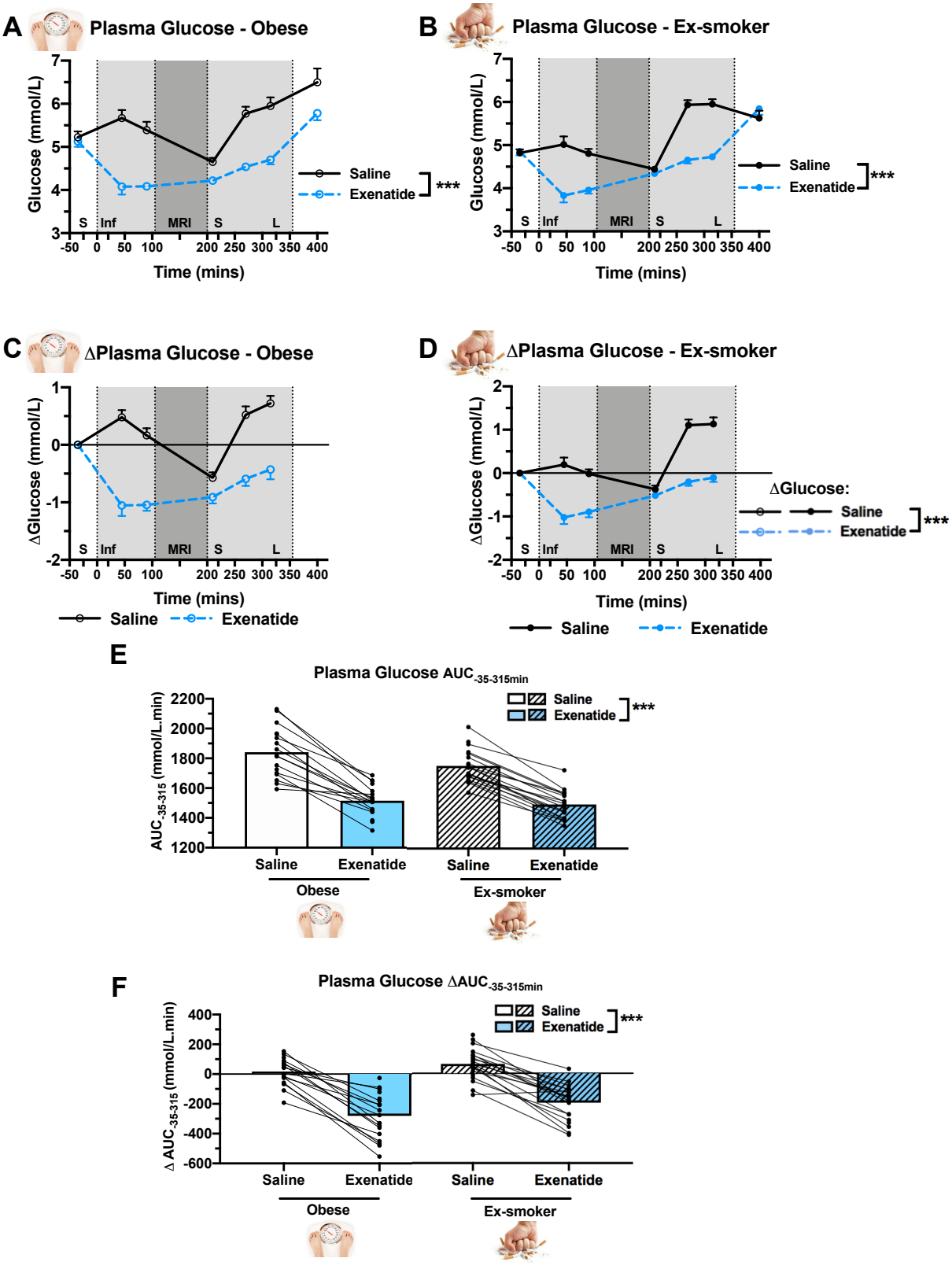


Figure 4.21. Exenatide reduced plasma glucose independent of group.
[A,B] Glucose concentrations (mmol/L) across seven timepoints, [C,D] change in glucose from baseline, [E] AUC glucose (T=-35 to 315min) and [F] Δ AUC glucose (T=-35 to 315min) for [A,C] obese or [B,D] ex-smoker or [E,F] both groups at the saline and Exenatide visits. Data presented as [A-D] mean ± SEM, n=18-22 or (E,F) before-after with bar graph (mean), n=21-23. Fixed effects mixed model ANOVA with post-hoc LSD test. Exenatide vs. saline: P<0.001***. For ANOVA results see Table 4.9.

4.4.6.2 Serum Insulin

Summary: Exenatide reduced serum insulin concentrations independent of group (Figure 4.22; Table 4.9).

For serum insulin, there was no significant visit*time*group interaction, but there was a significant interaction effect for visit*time [F(5, 456.27)=6.20, P<0.001]. This was driven by a decrease in insulin levels at T=45min and T=270min independent of group [effect size mean ±SEM T=45: -5.67 ± 1.73, (95%CI -9.07, -2.28), P=0.001, Cohen's d=0.61; T=270: -10.24 ± 1.73, (95%CI -13.64, -6.84), P<0.001, Cohen's d=1.09]. This indicates that Exenatide reduced insulin significantly at T=45min and T=270min independent of group with a large effect size.

For change in insulin from baseline (∆insulin from T=-35), there was no significant visit*time*group interaction, but there was a significant interaction effect for visit*time [F(4, 371.57)=5.43, P<0.001]. Similarly, this was driven by a reduction in ∆insulin at T=45min and T=270min independent of group [effect size mean ±SEM T=45: -6.61 ± 1.99, (95%CI -10.51, -2.70),P=0.001, Cohen's d=0.69; T=270: -10.58 ± 1.95, (95%CI -14.41, -6.74), P<0.001, Cohen's d=1.11]. This indicates that Exenatide reduced ∆insulin concentrations with moderate-large effect size at T=45min and T=270min (pre-AAT).

For area under the curve between T=-35 and T=315 ($AUC_{-35\text{-}315min}$) for insulin, there was no significant effect of visit*group interaction but there was a significant overall effect of visit [F(1, 38.06)=16.32, P<0.001]. This was driven by a reduction in $AUC_{-35\text{-}315min}$ insulin at the Exenatide visit independent of group [effect size mean ± SEM -1010.54 ± 250.19, (95%CI -1516.99, -504.09), P<0.001, Cohen's d=0.55]. This indicates that Exenatide decreased $AUC_{-35\text{-}315min}$ insulin independent of group with a moderate effect size.

For the change in area under the curve between T=-35 and T=315 ($\Delta AUC_{-35\text{-}315min}$) from baseline for insulin, there was no significant effect of visit*group interaction but there was a significant overall effect of visit [F(1, 43.19)=8.78, P=0.005]. This was driven by a reduction in $\Delta AUC_{-35\text{-}315min}$ insulin at the Exenatide visit independent of group [effect size mean ± SEM -1205.51 ± 406.83, (95%CI -2025.87, -385.15), P=0.005, Cohen's d=0.60]. This indicates that Exenatide reduced $\Delta AUC_{-35\text{-}315min}$ insulin independent of group with a moderate effect size.

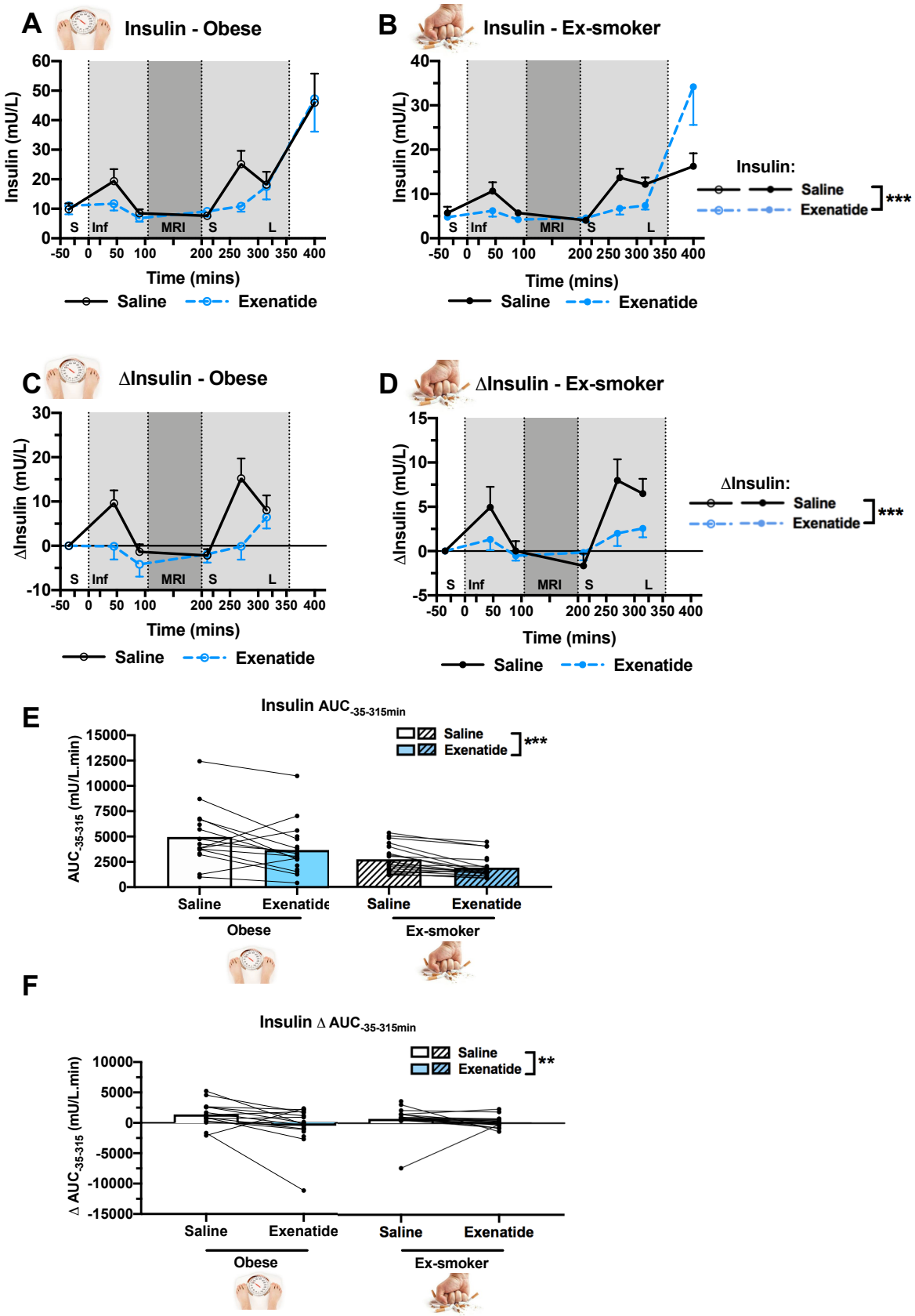


Figure 4.22. Exenatide reduced serum insulin concentrations independent of group.
(A, B) Serum insulin concentrations (mU/L) across seven timepoints, (C,D) change in insulin from baseline, (E) AUC insulin (T=-35 to 315min) and (F) Δ AUC insulin (T=-35 to 315min) for (A,C) obese or (B,D) ex-smoker or (E,F) both groups at the saline and Exenatide visits. Data presented as (A-D) mean ± SEM, n=21-23 or (E,F) before-after with bar graph (mean), n=18-21. Fixed effects mixed model ANOVA with post-hoc LSD test. Exenatide vs. saline: P<0.01** P<0.001***. For ANOVA results see Table 4.9.

4.4.6.3 Growth hormone

Summary: Exenatide suppressed GH levels after the fMRI scan (T=210min) across the dieting group with obesity and ex-smokers (Figure 4.23; Table 4.9).

For growth hormone (GH) concentrations, there was no significant visit*time*group interaction, but there was a significant interaction effect for visit*time [F(5, 436.71)=3.55, P=0.004]. This was driven by a significant decrease in GH levels at T=210min independent of group [effect size mean ± SEM T=210: -1.30 ± 0.34, (95%CI -1.98, -0.63), P<0.001, Cohen's d 0.80]. This indicates Exenatide suppressed GH levels after the fMRI scan across the dieting group with obesity and ex-smokers with a large effect size.

For change in GH from baseline (ΔGH from T=-35), there was no significant visit*time*group interaction, but there was a significant interaction effect for visit*time [F(4, 353.89)=4.00, P=0.003]. This was driven by a reduction in ΔGH at T=210 and a trend for an increase in ΔGH at T=90 independent of group [effect size mean ±SEM T=210: -1.24 ± 0.36, (95%CI -1.95, -0.53), P=0.001, Cohen's d=0.61; T=90: 0.68 ± 0.36, (95%CI -0.04, 1.39), P=0.063]. This indicates Exenatide suppressed ΔGH levels after the fMRI scan across the dieting group with obesity and ex-smokers with a moderate effect size.

For area under the curve between T=-35 and T=315 ($AUC_{-35\text{-}315min}$) for GH, there was no significant effect of visit*group interaction nor overall effect of visit.

For the change in area under the curve between T=-35 and T=315 ($\Delta AUC_{-35\text{-}315min}$) from baseline for GH, there was no significant effect of visit*group interaction nor overall effect of visit.

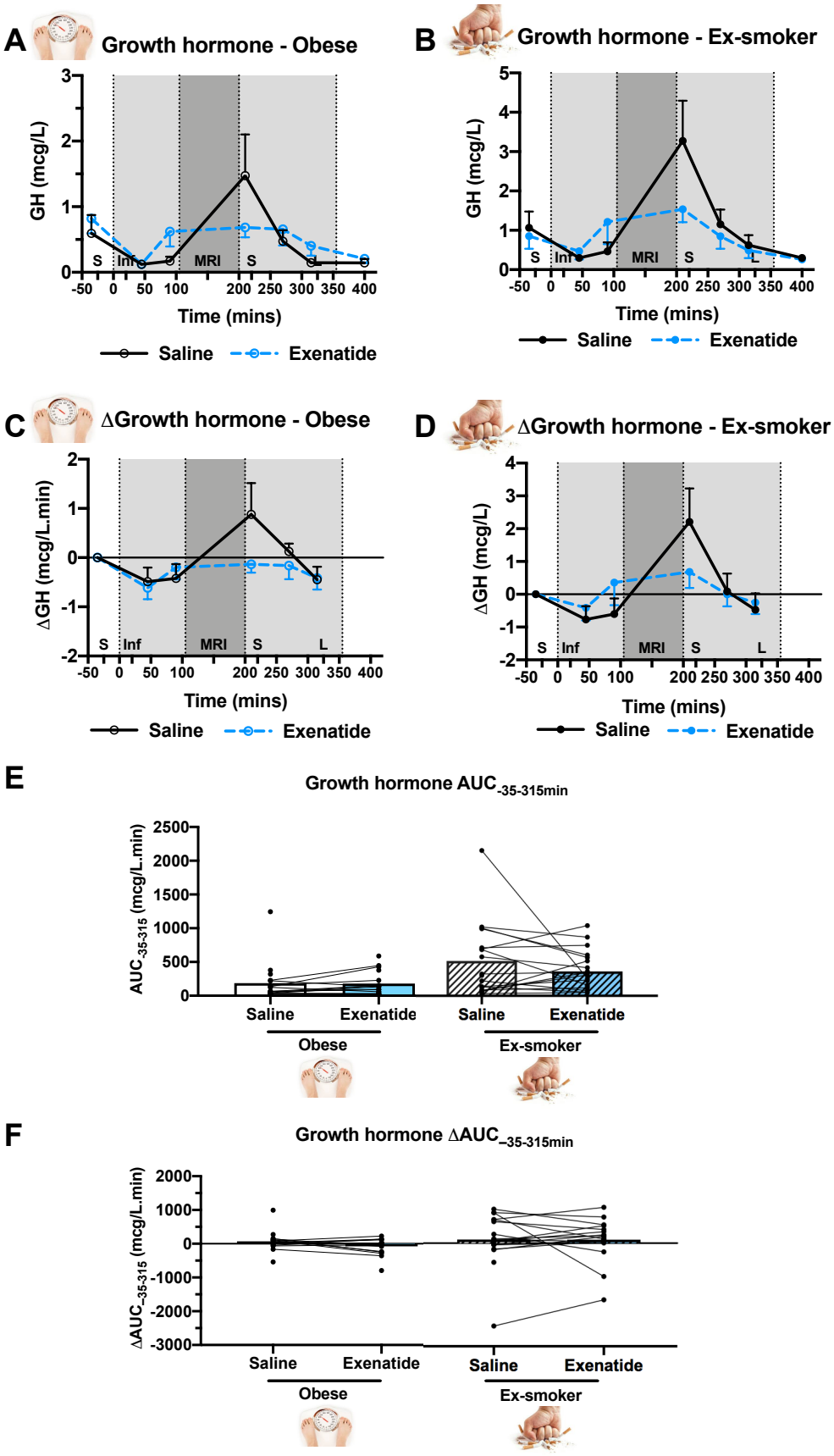


Figure 4.23. Exenatide suppressed growth hormone (GH) secretion at T=210 mins independent of groups.
(A, B) GH concentrations (mcg/L) across seven timepoints, (C,D) change in GH from baseline, (E) AUC GH (T=-35 to 315min) and (F) Δ AUC GH (T=-35 to 315min) for (A,C) obese or (B,D) ex-smoker or (E,F) both groups at the saline and Exenatide visits. Data presented as (A-D) mean ± SEM, n=21-23 or (E,F) before-after with bar graph (mean), n=19-21. Fixed effects mixed model ANOVA with post-hoc LSD test. Exenatide vs. saline: P<0.01** P<0.001***. For ANOVA results see Table 4.9.

4.4.6.4 Cortisol

Summary: Exenatide increased cortisol levels across the dieting group with obesity and ex-smokers (Figure 4.24; Table 4.9).

For cortisol concentrations, there was no significant interaction effect of (i) visit*time*group, (ii) visit*time, (iii) visit*group but there was a significant overall effect of visit [F(1,461.23)=20.21, P<0.001]. This was driven by a significant increase in cortisol levels at Exenatide visit compared to saline independent of group [effect size mean ± SEM 29.72 ± 6.61, (95%CI 16.73, 42.72), P<0.001, Cohen's d=0.22]. This indicates Exenatide increased cortisol levels across the dieting group with obesity and ex-smokers with a small effect size.

For change in cortisol from baseline (Δcortisol from T=-35), there was no significant interaction effect of (i) visit*time*group, (ii) visit*time, (iii) visit*group but there was a significant overall effect of visit [F(1,371.18)=4.42, P=0.036]. This was driven by an increase in Δcortisol at Exenatide visit compared to saline independent of group [effect size mean ±SEM 16.92 ± 8.05, (95%CI 1.09, 32.75), P=0.036, Cohen's d=0.11]. This indicates with Exenatide, there was a smaller reduction in cortisol levels from T=-35 compared to saline across the dieting group with obesity and ex-smokers with a small effect size.

For area under the curve between T=-35 and T=315 ($AUC_{-35\text{-}315min}$) for cortisol, there was no significant effect of visit*group interaction but there was a significant overall effect of visit [F(1, 39.24)=18.35, P<0.001]. This was driven by an increase in $AUC_{-35\text{-}315min}$ cortisol by Exenatide compared to saline independent of group [effect size mean ±SEM 14696 ± 3431, (95%CI 7758, 21635), P<0.001, Cohen's d=0.71]. This indicates Exenatide increased $AUC_{-35\text{-}315min}$ cortisol across the dieting group with obesity and ex-smokers with a moderate-large effect size.

For the change in area under the curve between T=-35 and T=315 ($\Delta AUC_{-35\text{-}315min}$) from baseline for cortisol, there was no significant effect of visit*group interaction nor overall effect of visit. However, there was a similar trend for a smaller decrease in $\Delta AUC_{-35\text{-}315min}$ cortisol at the Exenatide visit compared to saline independent of groups. This was driven by an increase in $\Delta AUC_{-35\text{-}315min}$ cortisol by Exenatide compared to saline [effect size mean ±SEM 8093 ± 4513, (95%CI -1027, 17213), P=0.080].

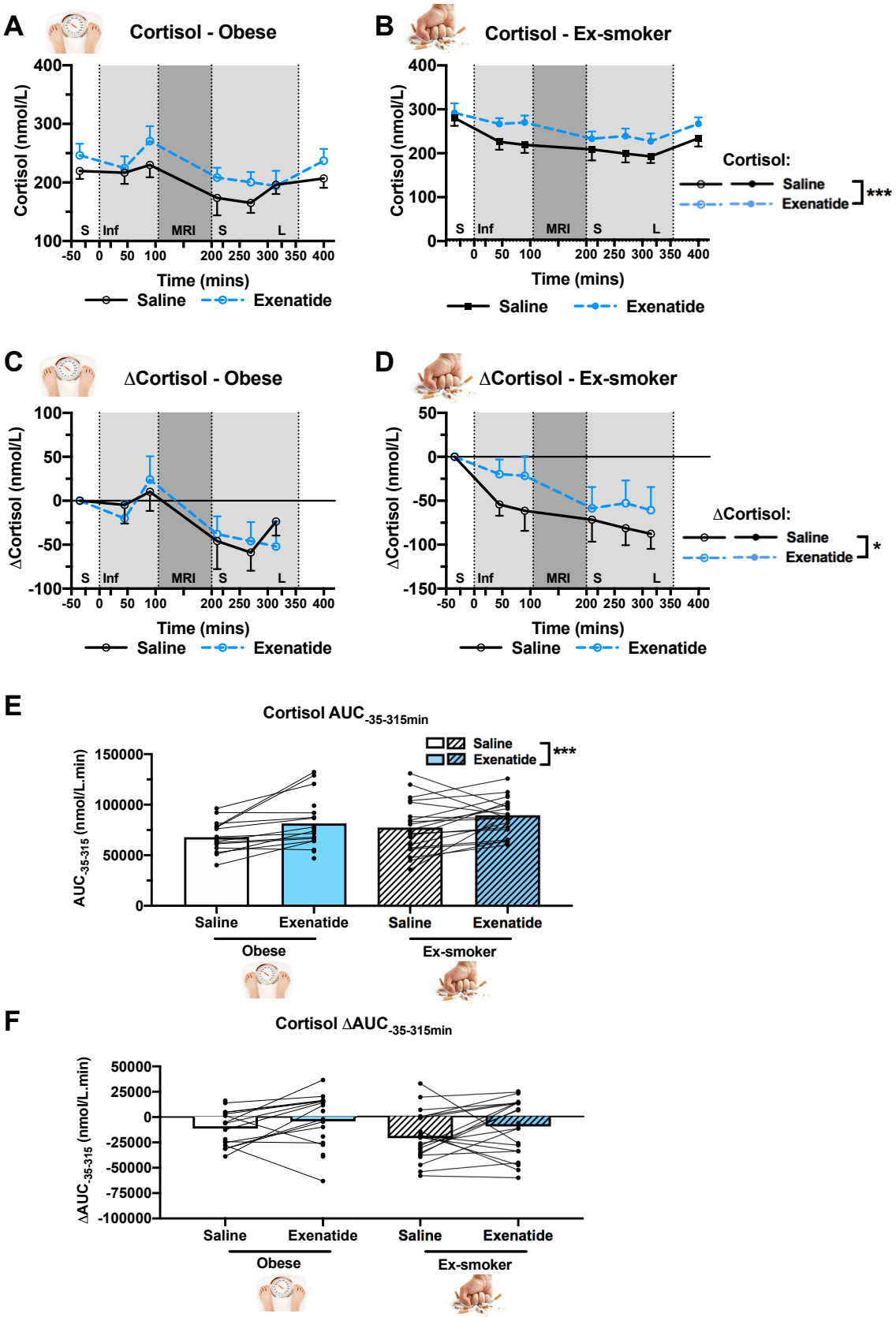


Figure 4.24. Exenatide increased cortisol levels independent of group.
(A, B) Cortisol concentrations (mmol/L) across seven timepoints, (C,D) change in cortisol from baseline, (E) AUC cortisol (T=-35 to 315min) and (F) Δ AUC cortisol (T=-35 to 315min) for (A,C) obese or (B,D) ex-smoker or (E,F) both groups at the saline and Exenatide visits. Data presented as (A-D) mean ± SEM, n=21-23 or (E,F) before-after with bar graph (mean), n=19-21. Fixed effects mixed model ANOVA with post-hoc LSD test. Exenatide vs. saline: P<0.01** P<0.001***. For ANOVA results see Table 4.9.

4.4.6.5 Prolactin

Summary: Exenatide caused a smaller reduction of prolactin levels from baseline (T=-35min) compared to saline independent of groups (Figure 4.25; Table 4.9).

For prolactin concentrations, there was no significant interaction effect of (i) visit*time*group, (ii) visit*time but there was a significant overall effect of visit*group [F(1,456.67)=7.20, P=0.008]. This was driven by a significant increase in prolactin levels at Exenatide visit compared to saline in dieting group with obesity [effect size mean ± SEM 22.00 ± 8.11, (95%CI 6.06, 37.93), P=0.007, Cohen's d=0.13]. This indicates Exenatide increased prolactin levels in the dieting group with obesity with a small effect size.

For change in prolactin from baseline (∆prolactin from T=-35), there was no significant interaction effect of (i) visit*time*group, (ii) visit*time, (iii) visit*group but there was a significant overall effect of visit [F(1,371.84)=21.99, P<0.001]. This was driven by an increase in ∆prolactin at Exenatide visit compared to saline independent of group [effect size mean ±SEM 26.06 ± 5.56, (95%CI 15.13, 36.99), P<0.001, Cohen's d=0.20]. This indicates with Exenatide, there was a smaller reduction in prolactin levels from T=-35 compared to saline across the dieting group with obesity and ex-smokers with a small effect size.

For area under the curve between T=-35 and T=315 ($AUC_{-35\text{-}315min}$) for prolactin, there was no significant effect of visit*group interaction nor overall effect of visit.

For the change in area under the curve between T=-35 and T=315 ($\Delta AUC_{-35\text{-}315min}$) from baseline for prolactin, there was no significant effect of visit*group interaction but an overall effect of visit [F(1,36.49)=4.32, P=0.045]. This was driven by an increase in $\Delta AUC_{-35\text{-}315min}$ prolactin by Exenatide compared to saline independent of group [effect size mean ± SEM 6963 ± 3352, (95%CI 168, 13758), P=0.045, Cohen's d=0.35]. This indicates Exenatide decreased prolactin levels less from T=-35min compared to saline with a small-moderate effect size.

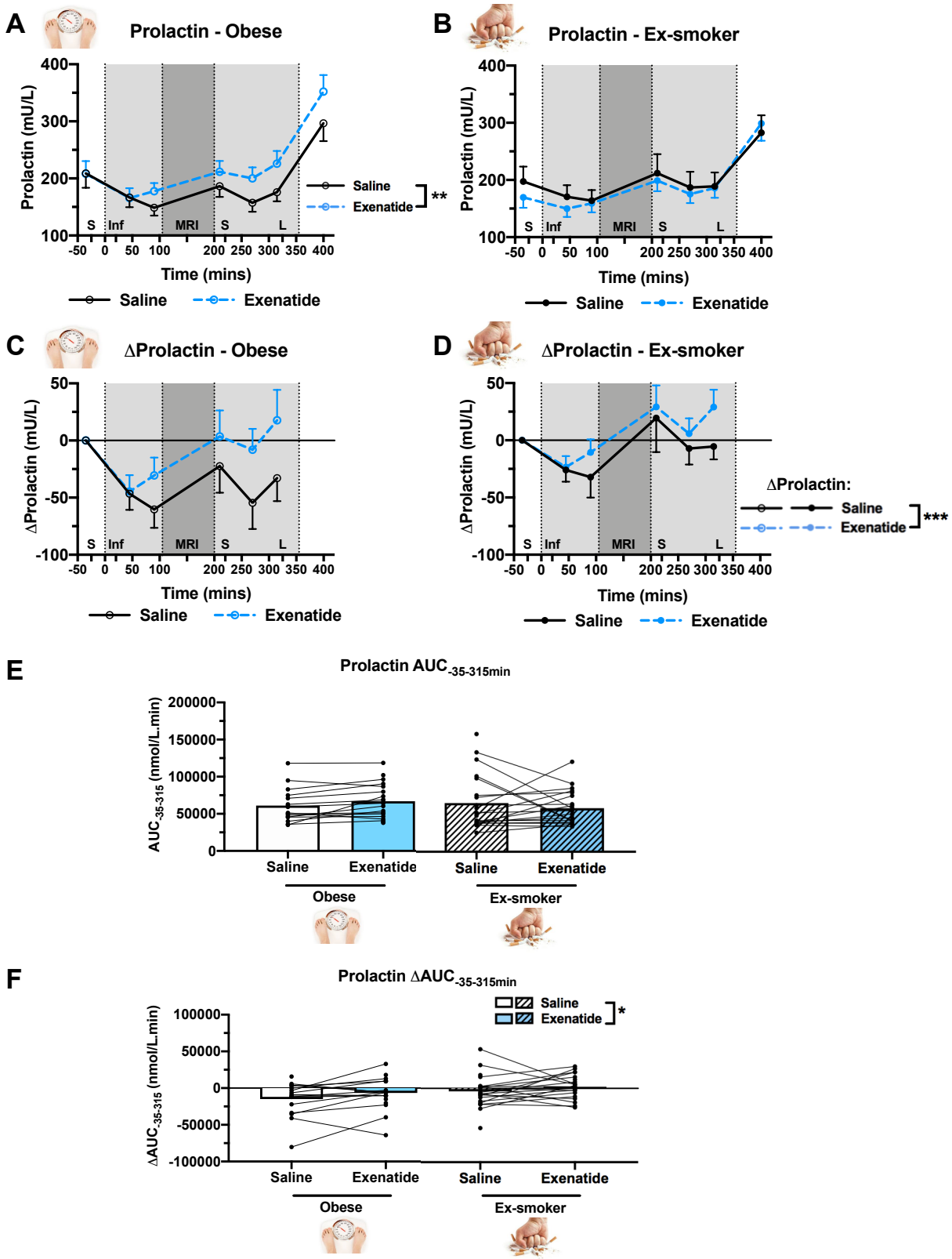


Figure 4.25. At Exenatide visit, there was a smaller reduction of prolactin levels from baseline (T=-35min) compared to saline independent of group.

(A, B) Prolactin concentrations (nmol/L) across seven timepoints, (C,D) change in prolactin from baseline, (E) AUC prolactin (T=-35 to 315min) and (F) Δ AUC prolactin (T=-35 to 315min) for (A,C) obese or (B,D) ex-smoker or (E,F) both groups at the saline and Exenatide visits. Data presented as (A-D) mean ± SEM, n=21-23 or (E,F) before-after with bar graph (mean), n=19-21. Fixed effects mixed model ANOVA with post-hoc LSD test. Exenatide vs. saline: P<0.01** P<0.001***. For ANOVA results see Table 4.9.

4.5 DISCUSSION

The main findings from analysis of BOLD signal outcomes from the fMRI high energy food picture evaluation task are summarised in Table 4.10. It demonstrates a difference in effect of the GLP-1 analogue, Exenatide, between dieting group with obesity and ex-smokers on the BOLD signal during HE food vs. neutral picture evaluation. In the dieting group with obesity, there was a general pattern of enhanced BOLD signal to HE food pictures, in particular in the PFC, with Exenatide vs. saline. On the contrary, in the ex-smokers, there was a reduction in BOLD signal to HE food pictures in the PFC, thalamus and dorsal striatum with Exenatide.

Despite the different patterns of Exenatide-induced changes in BOLD signal to HE food pictures, between groups, Exenatide had similar effects on the behavioural outcomes in both groups (Table 4.10). Exenatide reduced the relative appeal of food pictures and significantly reduced energy intake at the *ad libitum* meal independent of groups. Furthermore, in the PRT, Exenatide also tended to reduce motivation for a chocolate reward even after adjusting for nausea but had no effect on approach bias to food cues in AAT independent of groups. In the VAS, Exenatide reduced appetite and food craving ratings but increased nausea rating, albeit to a small effect size. As expected, Exenatide decreased plasma glucose, serum insulin, growth hormone and TG levels but increased cortisol and prolactin concentrations.

Measure	Obese	Ex-smoker
Saline		
fMRI: average fROI	Ex-smoker > obese	
Exenatide vs. saline		
fMRI: WBA	↑ SFG/FP	↓ DS & ↓ SFG/PCG
fMRI: average fROI	(↑)	→
Food appeal	↓	↓
Taste VAS		
Liking	→	(↓) SwLF
Pleasantness	→	→
Creaminess	→	→
Ideal creaminess	→	→
Sweetness	→	→
Ideal sweetness	↑ SwLF	↑ SwLF
Food intake		
Total	↓	↓
Savoury	↓	↓
Sweet	→	→
High-fat	(↓)	(↓)
Low-fat	→	→
PRT	(↓)	(↓)
AAT	→	→
VAS rating		
Appetite	↓	↓
Food craving	↓	→
Confounding VAS		
Nausea	↑	↑
Anxiety	→	↑ [a]
Stress	→	→
Sleepiness	→	→

Table 4.10. Summary table of effect of Exenatide on food-related fMRI and behavioural outcome measures

Comparison between dieting group with obesity and ex-smokers at saline visit alone or effects of Exenatide vs. saline on BOLD signal to HE foods vs. neutral from whole brain analysis and *a priori* functional region of interest analysis, and other behavioural outcome measures.

Trends are denoted in parentheses. [a] at T= 45min

Abbreviations: AAT, approach avoidance task; DS, dorsal striatum (caudate/putamen); Exen, Exenatide; FP, frontal pole; fROI, functional region of interest (including nucleus accumbens, caudate, putamen, orbitofrontal cortex, amygdala, hippocampus, anterior insula, ventral anterior cingulate cortex, dorsolateral prefrontal cortex); PCG, paracingulate gyrus; PRT, progressive ratio task; SFG, superior frontal gyrus; SwLF; sweet low-fat (yogurt); VAS, visual analogue scale; WBA, whole brain analysis.

Hypothesis: Exenatide will attenuate the appeal of and BOLD response in striatal regions to HE food pictures in both groups of participants.

Result: In dieting group with obesity, Exenatide increased BOLD signal to HE food pictures in the left SFG and frontal pole and tended to do so across all fROI.

In contrast to the hypothesis, in dieting group with obesity, Exenatide increased BOLD signal to HE food pictures in the left SFG and left frontal pole using whole brain analysis and tended to do so in the fROI analysis averaged across all fROI. As the interaction effect of visit*group*roi did not reach significance in the repeated measures ANOVA in fROI analysis, it was not appropriate to analyse any effects of Exenatide on individual fROI. However, exploratory post-hoc analysis showed the increase in the BOLD signal to HE foods to be significant only in the dlPFC, a functional subregion of the PFC implicated in executive control.

The prefrontal cortex is a large heterogenous region and is attributed a multifaceted role in decision making, emotional regulation, social cognition and memory formation [440-442]. Specifically, it has been postulated that SFG, an anatomical subregion, may play a role in self-control [171]. Individuals with successful weight loss maintenance and higher restraint, compared to those with obesity, had greater BOLD signal to food pictures in the SFG [171]. Interestingly, the cortical thickness of the left SFG is reduced in people with obesity compared to lean [443], and cortical thickness of the right SFG is inversely related to BMI in lean people which is mediated by inhibitory control [444], suggesting a dysregulation in this region implicated in executive control could predispose to overeating and obesity. In line with this, an increase in BOLD signal to food cues in dlPFC, as well as other PFC regions, was associated with weight loss in people with obesity on a diet [56] and predicted a lower subsequent energy intake [445, 446]. The increase in BOLD signal to HE food pictures in SFG by Exenatide seen in the current study could indicate a heightened inhibitory control when evaluating HE food pictures in the dieting group with obesity. This presumption is strengthened by the finding that in the dieting group with obesity, TFEQ dietary restraint scores tended to positively correlate with BOLD signal to HE food pictures in this frontal cluster and the Exenatide-induced increase of that. This suggests the higher the dietary restraint, the higher the BOLD signal to HE foods in the frontal cluster and therefore a role in inhibitory control.

Interestingly, in females with obesity and food addiction as measured by the Yale Food Addiction Score, there was an elevated BOLD signal to highly processed food cues in the superior frontal

gyrus and a diminished BOLD signal to minimally processed food cues as compared to those without food addiction [137]. It would therefore be interesting to see if a diagnosis of food addiction influences the effect of Exenatide on BOLD signal in the frontal cluster in the dieting group with obesity. Using an unpaired t-test comparing those with and without food addiction, there was no significant difference in the effect of Exenatide on BOLD signal to HE foods in the frontal cluster in dieting group with obesity (P=0.78). However only a third of participants (n=9) within the dieting group with obesity had food addiction as measured by YFAS meaning that the study may well have been under-powered for such an analysis.

Similarly, the frontal pole has a modulatory role in guiding goal-directed behaviour and co-activates with regions including dlPFC and anterior insula [447]. In people with obesity undergoing a diet, the change in BOLD signal to food pictures in regions that regulate executive control including frontal pole and dlPFC, were negatively correlated with the change in sweet food and fast-food cravings [414]. In other words, the increase in BOLD signal to food pictures in frontal pole and dlPFC reflected a reduction in food cravings following a diet, suggesting a role in controlling cravings for these prefrontal regions. In the current study, even though the increase in BOLD signal to HE foods in prefrontal cluster in dieting group with obesity with Exenatide did not corelate with the change in food appeal or food craving ratings ($r_s(21)$= -0.06, P=0.78 and $r_s(21)$= 0.34, P=0.11 respectively), this was more likely to be a reflection of the heterogeneity of the prefrontal cluster.

Unexpectedly in the fROI analyses there was also a trend for Exenatide to increase the average BOLD signal in non-frontal areas involved in reward processing in obesity, including NAcc, caudate, putamen, OFC, amygdala, hippocampus, anterior insula, vACC and dlPFC.

These findings are in contrast to other studies examining effects of GLP-1 analogues on food cue reactivity in obesity where typically a reduction of BOLD signal to food cues was demonstrated. In one study, Exenatide infusion decreased BOLD signal to food cues in adults with obesity without T2DM in the amygdala, insula (both HE and LE foods vs. neutral) and OFC (HE foods vs. neutral), and additionally in people with obesity and T2DM in the putamen (HE foods vs neutral)[141]. However, there were important differences in study protocol and participant characteristics between both studies in that their participants:

(i) were older and all females were post-menopausal;

(ii) had a somatostatin pancreatic-pituitary clamp which included a somatostatin infusion to suppress endogenous insulin, glucagon, GH and GLP-1 production concentrations, and infusions of exogenous glucagon, GH and insulin. Also, plasma glucose was clamped at 5.0 mmol/L whereas in this current study there were mean differences in plasma glucose of 4.43 ± 0.05 to 5.29 ± 0.05 mmol/L between Exenatide and saline visits respectively;

(iii) viewed HE and LE food pictures passively, whereas in the current study participants had to evaluate the picture's appeal, and included only HE foods;

(iv) had their fMRI in the fasted nutritional state while in current study participants were studied 2.5 hours after a small snack;

(v) were not dieting; and

(vi) their fMRI analysis was conducted with a voxel-wise family-wise error correction within just 4 selected ROI spheres using a small volume correction (SVC) for amygdala, insula, putamen and OFC. By contrast, the current study analysis used both whole brain and fROI approaches. The latter included a larger number of relevant regions in the reward processing and inhibitory control network, and averaged BOLD signal within the whole fROI rather than looking for individual voxels/clusters within the SVC ROI.

In another study, Liraglutide, a GLP-1 analogue, decreased BOLD signal to HE foods (vs LE foods) in the parietal cortex, insula and putamen in people with obesity and T2DM after 17 days [271]. Interestingly in a longer-term study, Liraglutide *increased* the BOLD signal in OFC in response to HE food cues in people with obesity after 5 weeks when adjusted for BMI [281]. This increase in BOLD signal in this reward-related region was suggested to be a counter-regulatory mechanism to halt further weight loss. However, the latter two studies were: (i) conducted over weeks of GLP-1 analogue treatment, that may also result in some adaptations to the GLP-1 signalling system, (ii) conducted in fasted state, and (iii) used the above-mentioned SVC analysis method therefore confounding any direct comparisons with the current study.

Result: In ex-smokers, Exenatide reduced BOLD signal in SFG, paracingulate gyrus, caudate, putamen and thalamus.

The novel findings in the ex-smokers supports the hypothesis that Exenatide attenuates HE food reward. Exenatide decreased BOLD signal to HE food pictures in parts of the dorsal striatum, caudate and putamen, and thalamus, which are regions related to reward-processing, in the whole brain analysis of the ex-smokers. This is of clinical significance as Exenatide, or other GLP-

1 analogues, could be used in prevention of smoking cessation weight gain by reducing anticipatory food reward.

This is in contrast to the dieting group with obesity, which did not show these effects in whole brain analysis, and indeed showed a trend for an *increase* in average BOLD signal to HE foods in fROI. There are several potential explanations for the difference in effect of Exenatide of HE food cue reactivity between the ex-smokers and dieting group with obesity:

(i) BMI itself, since the ex-smokers had a lower BMI than the dieting group with obesity, and only 17% of the former were obese. Indeed, there was a trend for a negative correlation of BMI with the Exenatide-induced reduction in BOLD signal to HE foods in the striatal cluster in the ex-smokers (Figure 4.7, Table 4.3), indicating that the higher the BMI, the less Exenatide reduced BOLD signal in the striatum. This might be related to a floor effect since the BOLD signal to HE foods in the striatal cluster in ex-smokers at the saline visit was negatively correlated with BMI. Notwithstanding the difference in protocols as discussed above, in a previous study of lean participants, acute infusion of GLP-1 analogue tended to reduce BOLD signal to HE and LE food cues vs. neutral in OFC [141] which is distinct from the current findings of decreased BOLD signal to HE foods in the dorsal striatum in ex-smokers.

(ii) Previous nicotine dependence, since nicotine dependence is mediated though the mesolimbic dopaminergic reward system [203, 448], and causes desensitisation of reward neurocircuitry [198] with blunting of BOLD signal in the striatum to favourite foods [219]. With nicotine abstinence, there could be a rebound of these changes to the common reward processing network for HE foods and nicotine. This may explain why ex-smokers, compared to dieting group with obesity, have a heightened BOLD response to HE foods across all fROI. As such, there is a difference in effect of Exenatide of HE food cue reactivity between the both groups (see further discussion in Section 6.1).

Additionally, Exenatide decreased BOLD signal to HE food pictures in bilateral SFG, right paracingulate gyrus in the whole brain analysis of ex-smokers, that was the opposite to that seen in dieting group with obesity. As discussed above, SFG is implicated in cognitive control. The paracingulate gyrus, also part of PFC, is thought to be involved in reward-processing, self-monitoring and executive decision-making [449-451]. The decrease in the anticipatory food reward response in the striatum with Exenatide in ex-smokers may result from less attention and

less inhibitory control towards HE food being required therefore contributing to a reduction in BOLD signal to HE food pictures in the frontal cluster.

A confounding factor in interpretation of the fMRI results in both groups is the fact that Exenatide lowered plasma glucose concentrations from an average of 5.00 ± 0.10 to 4.02 ± 0.05 mmol/L just before participants entered the MRI scanner. Circulating glucose can modulate brain reward processing and motivation for food cues. Using a hyperinsulinaemic-euglycaemic-hypoglycaemic pump, an enhanced BOLD signal to HE-/LE- food cues vs. neutral in reward-related areas including VTA, thalamus, striatum and insula during the hypoglycaemic phase (around 3.7 mmol/L) was seen in healthy volunteers, including those with obesity [175]. This could partially explain the tendency for Exenatide to increase average BOLD signal to HE foods in fROI in dieting group with obesity, but not in ex-smokers in the current study. Importantly, despite the lowering of glucose concentrations with Exenatide, in the ex-smokers, there was a *decrease* in BOLD signal to HE foods in parts of the dorsal striatum and thalamus in the whole brain analysis, contrary to the aforementioned study.

Similarly, Page et al also reported a decrease in BOLD signal to HE-/LE-food cues in the PFC and ACC during hypoglycaemia, compared to euglycaemia (around 4.9 mmol/L), in adults who were lean but not in those with obesity [175]. This concurs with the finding in the current study of Exenatide reducing BOLD signal to HE foods in ex-smokers in frontal cluster. However, there was no correlation between changes in pre-MRI plasma glucose and the Exenatide-induced changes in BOLD signal to HE food pictures in the frontal cluster in ex-smokers, suggesting glucose changes may not be a major contributory factor for the reduction in food cue responses in this frontal cluster.

Hypothesis: BMI and eating behavioural traits can influence the Exenatide response on food cue reactivity in both groups of participants.

Result: In dieting group with obesity, TFEQ-restraint scores tended to correlate positively with the Exenatide-induced increases in BOLD signal to HE food pictures in frontal cluster.

As discussed in earlier in the section, this indicates the higher the dietary restraint, the higher the increase in BOLD signal to HE foods by Exenatide in the frontal cluster in dieting group with obesity (Figure 4.4B). This supports the hypothesis that dietary restraint influences the Exenatide response on BOLD signal to HE foods in frontal cluster in dieting group with obesity.

In dieting group with obesity, there were no significant associations with other eating behavioural constructs, including emotional eating and disinhibition, and Exenatide response on HE food cue reactivity. This could be because the frontal cluster may not be involved in regulating these facets of eating behaviour therefore no influence on the Exenatide response in this frontal cluster was observed. For instance, it was in the amygdala and insula that emotional eating scores negatively correlated with Exenatide-induced reductions in BOLD signal to HE-/LE-foods vs. neutral in obesity and T2DM [129].

In spite of this, it is also interesting to note that there were no associations with BMI and Exenatide-induced increases in frontal cluster in dieting group with obesity. A possible explanation is that BMI may not be an appropriate measure in this analysis as there are a heterogeneity of causes for obesity. BMI is not a measure of a specific eating behaviour or a direct measure of an appetitive hormone. Moreover, there may have been recent change in BMI from dieting that may have disrupted any pre-existing correlations. Furthermore, the groups were relatively small, and this may be underpowered to find any correlations.

Results: In ex-smokers, BMI tended to positively, while emotional eating scores tended to negatively correlate with Exenatide-induced changes of BOLD signal to HE food pictures in striatal cluster.

As discussed in the earlier section, in the ex-smokers, the higher the BMI, the less Exenatide reduced BOLD signal to HE foods in the striatal cluster (Figure 4.7, Table 4.3). This might be related to a floor effect since the BOLD signal to HE foods in the striatal cluster in ex-smokers at the saline visit was negatively correlated with BMI. This is unexpected as obesity is usually associated with higher BOLD signal to HE foods in the mesolimbic reward regions [63], although this viewpoint has been challenged with the suggestion that food reward processing in the brain is dynamic and changes with cognitive state of individual, such as in dieting [452]. One possible reason for the negative association in ex-smokers between BMI and BOLD signal to HE foods in striatal cluster is dietary restraint. Dietary restraint is correlated positively with BMI in ex-smokers (Figure 3.3A-B) and also tended to negatively correlate with BOLD signal to HE foods in striatal cluster (Figure 4.7C).

In ex-smokers, the higher their emotional eating score, the greater the Exenatide-induced *reduction* of BOLD signal to HE food pictures in the striatal cluster. This suggests that in ex-smokers, emotional eaters may be more sensitive to the effects of Exenatide to reduce food cue reactivity in striatum. By contrast, in a previous fMRI study, emotional eating scores correlated positively with Exenatide-induced *increases* to BOLD signal to HE-/LE- foods vs. neutral in the amygdala in obesity, but no correlations were observed in lean [129]. However, direct comparison between the 2 studies are confounded by the differences in BMI of participants and brain regions.

There was no correlation found between other eating behaviours and Exenatide-induced changes in BOLD response to HE foods in ex-smokers. This may be due to the small numbers of participant and hence reduced power. Also, the ex-smokers score lower in eating behavioural questionnaires which may impede the possibility of finding a significant correlation.

To further examine the influences of these variables on the effects of Exenatide, future analyses should examine the effects of BMI, dietary restraint and emotional eating on HE food cue reactivity using the current data set using a whole brain approach to see which regions in the brain have a BOLD signal change with Exenatide that depends on the relevant covariate.

Hypothesis: Exenatide will reduce appetite and food intake in both groups of participants.

Result: Exenatide reduced total energy intake at ad libitum meal independent of group.

Consistent with the anorexigenic effect of Exenatide, Exenatide reduced total energy intake at the *ad libitum* meal and energy intake from the savoury food (soups). In addition, Exenatide tended to reduce energy intake specifically from high-fat foods (cream soup and ice-cream) independent of group. Furthermore, Exenatide decreased ratings of appetite independent of group, and reduced that of food craving in the dieting group with obesity. These findings are consistent with studies demonstrating suppression of food intake and appetite in humans by GLP-1 [260, 262]. GLP-1 analogues act on central GLP-1 receptors to inhibit food intake, primarily via the hypothalamic arcuate nucleus [272, 453] and via NAcc GLP-1 receptor activation [430, 454]. GLP-1 and its analogues also mediate its satiating effects via vagal afferents in preclinical studies[387]. In humans, clinical studies have also supported the finding that GLP-1 analogues inhibit food intake through brain networks [141, 157] and to some extent, via vagal afferents [455].

Interestingly, the findings that Exenatide tended to reduce energy intake from the high-fat foods (cream soup and ice-cream) is consistent with a study where Exenatide preferentially reduced consumption of high-fat diet in rats when given a choice between that and chow [269], raising the possibility of GLP-1 analogues reducing food palatability [456]. In line with this, 16 weeks of liraglutide treatment reduced the taste preference for sweet, salty, fatty and savoury compared to placebo in obesity [457].

Result: Exenatide increased rating of ideal sweetness for sweet low-fat food (yogurt), independent of group.

No effect of Exenatide was seen on palatability of the high-fat foods. Instead, Exenatide increased the rating of ideal sweetness for yogurt, independent of group. During tasting , participants were asked, "How close the sweetness of this taste to your ideal sweetness?" with the term "Just right" anchored at 50mm mark. A rating of ideal sweetness above 50mm is considered sweeter than ideal and vice versa. The increase in rating of ideal sweetness for yogurt with Exenatide was from 47.8 mm to 51.8 mm. Thus, an increase above the 'just right' 50mm mark with Exenatide might lead to participants finding the yoghurt too sweet. This effect would encourage reduced consumption of yoghurt. This finding supports the notion from preclinical studies that GLP-1 signalling enhances sweet taste sensitivity [458]. However, the clinical relevance of these findings may be limited as: (i) the effect sizes were small, (ii) there was no corresponding reduction in the consumption of yogurt at the test meal, and (iii) no change was seen in the sweetness ratings of the ice cream which provides a much greater contribution to total energy intake than the yoghurt.

Hypothesis: Exenatide will reduce food reward behaviour, including motivation to receive a food reward and approach bias, in both groups.

Result: Exenatide tended to reduce motivation to receive a chocolate sweet in the progressive ratio task, independent of group.

There is a consensus emerging, mainly from preclinical studies, that GLP-1 analogue decreases reward behaviours including reducing sucrose or food reward behaviour using a progressive ratio operant conditioning task [267, 459]. Consistent with this, in the current study Exenatide tended to reduce motivation to receive a chocolate independent of group, even after adjusting for nausea, in the progressive ratio task (PRT) independent of group. This finding is in support of the

hypothesis that GLP-1 analogue, Exenatide can reduce food reward behaviour in dieting group with obesity and ex-smokers. The lack of overall statistical significance is likely related to the study being under-powered as there is great inter-individual variability in this task, and therefore perhaps also in response to Exenatide. This does mean however that the interpretation of these results should be cautious, and needs confirming in larger studies.

Result: Exenatide did not have an effect on approach bias to food pictures in the approach-avoidance task.

Despite the above findings, Exenatide did not have any effect on approach bias towards HE food pictures in the AAT, in either of the two groups. In other literature, this approach-avoidance bias has been correlated with food cravings [460, 461]. Given that food craving ratings in the dieting group with obesity were reduced with Exenatide, it would have been expected that Exenatide would consequently decrease approach bias to HE food as well.

However, there may be reasons for our negative findings. Firstly, as with the PRT, the study was likely under-powered as there was great inter-individual variability in this AAT, and therefore perhaps also in response to Exenatide. Secondly, in this task, pulling or pushing the joystick was dependent on the colour of the image frame. Pulling the joystick enlarged the image simulating approach whereas pushing the joystick shrank the image thus simulating avoidance. These actions draw similarities to approaching or avoiding these foods in natural environment and are meant to measure implicit responses as a conscious evaluation of the picture content is avoided [386]. Nonetheless, it may be that by making the image content task-relevant in the AAT, such as instructing participants to pull or push depending on whether it is edible, a stronger approach bias can be measured as attention is drawn to processing the image content [461]. Such versions of AAT may have been better suited to elicit any effects of Exenatide.

Finally, it is possible that the brain regions where Exenatide has an effect, such as the SFG and dorsal striatum in the food picture evaluation fMRI task, may not be the same network that govern approach-avoidance tendencies. Indeed, the dlPFC, ACC, striatum, hippocampus and amygdala have been implicated in approach-avoidance decision-making [462-464]. Specifically, in individuals with obesity who have undergone AAT training to enhance approach to healthy food and avoidance of unhealthy food, there was decreased BOLD signal when avoiding unhealthy food in the angular gyrus and increased BOLD signal in the ACC [126]. GLP-1 analogues

may not impact function of these brain areas and therefore may not reduce the subconscious impulsive bias towards food as measured in AAT. In fact, AAT is more commonly utilised as part of a cognitive bias modification therapy to improve addictive behaviour, for instance in groups with alcohol-dependence to reduce alcohol consumption [465, 466].

Hypothesis: Exenatide will decrease glucose concentrations and insulin in both groups.

Result: Exenatide decreased plasma glucose, insulin and growth hormone but increased cortisol and prolactin.

GLP-1 analogues have been developed successfully for the treatment of T2DM based on its insulinotropic and glucose-lowering effects [377, 387]. In line with this, Exenatide reduced plasma glucose in both groups of participants. Although Exenatide is insulinotropic, the serum insulin levels were appropriately reduced across both groups of participants in response to the low plasma glucose levels in accordance with other studies [377].

In humans, GLP-1 and its analogues transiently stimulate the hypothalamus-pituitary-adrenal (HPA) axis, via GLP-1 receptors expressed in the hypothalamus [467], to increase cortisol [468]. This is confirmed in the current study which demonstrated an increase in serum cortisol with acute administration of Exenatide. This alludes to the role of GLP-1 in acute stress response amid concerns that long-term treatment with GLP-1 analogues may induce a chronic stress-resembling state with interrupted circadian cortisol secretion in humans [469, 470]. However, with prolonged GLP-1 analogue administration, there was no difference in hypothalamic-pituitary-adrenal axis activity in healthy volunteers compared to placebo [470]. Certainly, in the current study, there was no acute effect of Exenatide on stress VAS rating in either group.

At the saline visit, the increase in serum GH concentrations at one timepoint (post-MRI at T=210min) was likely a counter-regulatory response to the drop in plasma glucose levels following the fMRI scan. Conversely, this change in GH was not seen at the Exenatide visit as the plasma glucose concentrations were more constant (around 4 mmol/L).

The increase in prolactin levels could be a response to the lower plasma glucose concentrations at the Exenatide visit [471].

Conclusion

GLP-1 analogues are widely used in the treatment for T2DM and more recently in obesity. GLP-1 analogues are insulinotropic and causes weight loss, reasons for which are multifactorial. By studying the effects of acute administration of GLP-1 analogue, Exenatide, on the HE food cue reactivity and food-related behaviour in dieting adults with obesity and ex-smokers, it helps to gain insight into the mechanisms by which GLP-1 analogues alter eating behaviours. This can contribute towards developing strategies for maintenance of weight loss after dieting and prevention of weight gain following smoking cessation.

The finding in current study that Exenatide reduced anticipatory food cue reactivity in ex-smokers in brain regions including SFG, paracingulate gyrus, caudate, putamen and thalamus, is novel. Furthermore, the effect of Exenatide in enhancing the BOLD signal to food cues in dieting group with obesity in brain regions such as the SFG and frontal pole is in direct contrast with this finding. Interestingly, despite the mismatch in the effect of Exenatide on BOLD signal in both groups of participants, they both correspondingly reduced food appeal rating and total energy intake from the *ad libitum* meal. Exenatide also tended to reduce the motivation for sweet food reward in both groups. Each task, whether fMRI or behavioural, has its own strengths and weaknesses with inter- and intra-subject variability therefore interpretation of these results should be cautious, and needs confirming in larger studies. Notwithstanding, these results are in accord with the possibility that Exenatide and other GLP-1 analogues could be used as a treatment option for prevention of smoking cessation weight gain.

Currently, data analysis of the other cues in the fMRI picture evaluation tasks (including cigarette pictures) and related behavioural tasks is still ongoing. This may reveal additional beneficiary effects of Exenatide on smoking craving and cigarette-cue reactivity in the ex-smokers that could strengthened the case for the use of GLP-1 analogues as an anti-smoking therapy in the future.

CHAPTER 5:

EFFECT OF DAG ON EATING BEHAVIOUR IN DIETING ADULTS WITH OBESITY AND EX-SMOKERS

5.0 ABSTRACT

Introduction: The ghrelin system is an attractive target for novel anti-obesity therapies. Acyl ghrelin (AG), the only known orexigenic gut hormone, increases with meal initiation and weight loss and decreases in obesity. The ghrelin system can modulate central reward networks for towards food and nicotine in preclinical and human studies. Desacyl ghrelin (DAG), another ghrelin peptide, can act as a functional antagonist to AG and appears to induce weight loss and reduce hyperphagia in obesity and Prader-Willi syndrome.

Aims: In this study, the effects of acute administration of DAG was explored in dieting adults with obesity, or in abstinent nicotine-dependence (in a double blind randomised placebo controlled cross-over design), on food cue responsivity using fMRI in reward-processing regions, food intake, food reward and appetite.

Results: In dieting group with obesity, DAG increased BOLD signal to high-energy (HE) food pictures in the frontal pole, middle frontal gyrus, superior frontal gyrus (SFG), paracingulate gyrus (PCG), orbitofrontal cortex and anterior cingulate cortex, possibly indicating an increase in inhibitory control and attention. In contrast, in ex-smokers, DAG decreased BOLD signal to HE food pictures in the mesolimbic reward-processing regions (caudate, putamen, thalamus, amygdala) and prefrontal cortex regions (SFG, frontal pole and PCG), suggesting a reduction in anticipatory food reward with a concomitant decrease in executive control. However, there was no effect of DAG on HE food picture appeal ratings, food intake, food reward behaviour and appetite. DAG tended to reduce food craving scores in both groups.

Conclusion: To the best of my knowledge, this is the first human fMRI study examining the effects of DAG on food cue reactivity. The novel fMRI findings in the ex-smokers supports the hypothesis that DAG attenuates anticipatory food reward. Interestingly in dieting group with obesity, there was an increase in BOLD signal to HE food pictures in prefrontal regions with DAG. This study findings are in accord with the possibility that DAG and other DAG analogues could be used as a treatment option for prevention of smoking cessation weight gain.

5.1 INTRODUCTION

Obesity, and its associated comorbidities, is a growing epidemic with serious consequences for the individual and society at large. An attractive target in the search of novel anti-obesity drugs is the ghrelin system [256, 267, 337, 472]. Acyl-Ghrelin (AG), the only peripheral orexigenic hormone currently known, acts via the growth hormone secretagogue receptor-1a (GHSR-1a) to exert its effects on food intake, body weight and glucose metabolism [302, 317, 321]. In non-obese adults, AG acutely enhances activation of brain reward regions to food cues in fMRI studies, mimicking the effects of acute fasting [176, 177, 473].

The recently characterised endogenous GHSR antagonist, liver-enriched antimicrobial peptide-2 (LEAP2), prevents AG activation of arcuate orexigenic NPY neurons [342], thereby suppressing AG-induced food intake in mice [474]. Furthermore, in both mice and humans, plasma AG and LEAP2 are reciprocally regulated with fasting and weight loss (AG increases, LEAP2 decreases), or

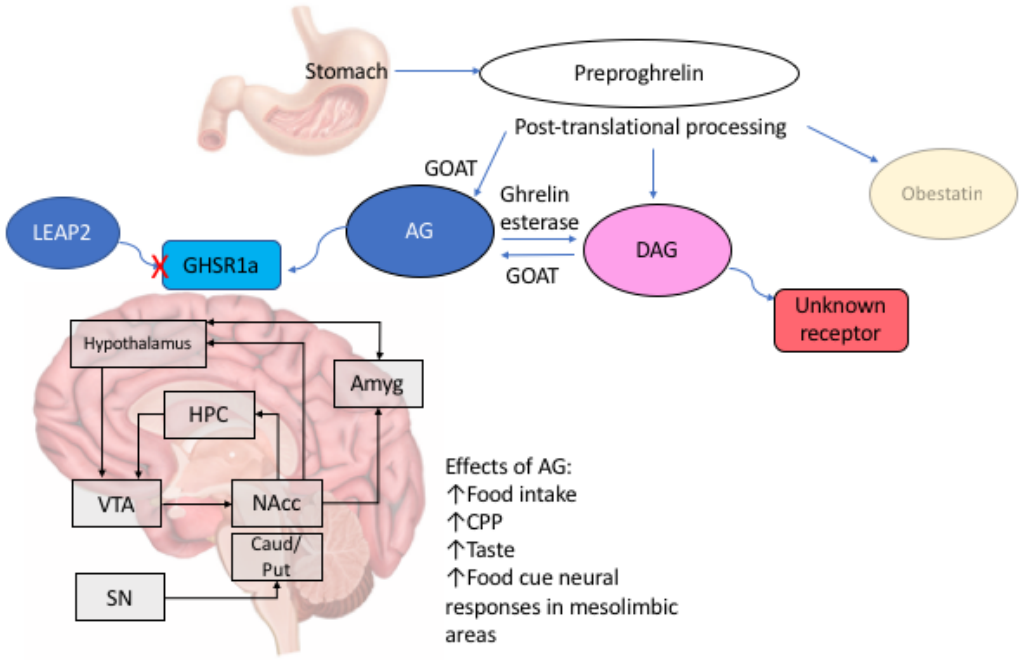


Figure 5.1. Ghrelin system in food reward

Ghrelin precursors are secreted in the stomach, one of which is preproghrelin. This peptide undergoes post-translational processing to produce AG, DAG and obestatin. AG exerts its effects through GHSR1a and LEAP2 is an antagonist of GHSR1a. Brain areas associated with AG effects are shown and arrows denote the neural connectivity amongst them. Both receptors of DAG and obestatin remain unknown. Arrows indicate neural connections. Abbreviations: AG, acyl-ghrelin; Amyg, amygdala; Caud, caudate; CPP, conditioned place preference; DAG, desacyl ghrelin; GHSR1a, growth hormone secretagogue receptor 1a; GOAT, ghrelin O-acyl transferase; HPC, hippocampus; LEAP2, liver-expressed antimicrobial peptide 2; NAcc, nucleus accumbens; Put, putamen; SN, substantia nigra; VTA, ventral tegmental area.

food intake and obesity (AG decreases, LEAP2 increases) [475]. Similarly, exogenous GHSR-1a antagonists reduce food intake, body weight and fat tissue mass in preclinical studies [340, 341]. Furthermore, inhibition of ghrelin-O-acyltransferase (GOAT), the enzyme which acylates DAG into AG, reduced food intake in rats by reducing meal frequency [344]. However, a limited number of antagonists/inverse agonists of GHSR1a and GOAT inhibitors are only just becoming available for clinical studies.

Another mature form of ghrelin peptide is des-acyl ghrelin (DAG), which is more abundant in circulation and can also be converted from AG via serum and tissue esterase enzymes, such as butyrylcholinesterase [476]. DAG has only in recent years been recognised to possess biological functions after being initially regarded as an innate by-product of AG due to the lack of an acyl side chain required for full agonism of GHSR [350, 477]. The target receptor and pathway through which DAG exerts its actions remains unknown. Whilst it does not function as an antagonist at the GHSR1a at physiological levels, DAG can directly counteract the actions of AG on neural activity in hypothalamus, glucose metabolism and lipolysis [361, 478, 479] when co-administered together. DAG when infused alone improved glucose tolerance and insulin sensitivity in lean and people with obesity and T2DM [376, 388] although not consistently demonstrated [363, 480]. The few preclinical studies into the effects of DAG on food intake have shown contradictory results, with some demonstrated an anorexic effect and others reported no effect on food intake [357-362] (Table 1.3). DAG-induced reduction in food intake was associated with the activation of neurons in the paraventricular nucleus of the hypothalamus and was not mediated by the vagal afferents [362]. Furthermore, DAG bound to a subset of hypothalamic arcuate nucleus (ARC) neurons in a GHSR-independent manner [347] and suppressed AG-induced neuronal activity in the hypothalamic ARC of rats thereby abolishing induction of food intake [360]. Nevertheless, the physiology of DAG on food intake and reward processing remains unclear.

To date, there are only a few clinical studies examining the effects of exogenous DAG or DAG analogues on food intake or body weight. DAG infusion in humans decreased glucose and fructose consumption when compared to saline although it did not have an effect of overall food intake [363]. A DAG analogue, AZP-531 (Livoletide), has shown promising results in people with obesity, T2DM and Prader-Willi syndrome (PWS) [364, 365] after demonstrating it prevented development of prediabetic metabolic state in mice on high-fat diet [481]. Livoletide, unlike

endogenous DAG, is protected from peptidase degradation and has an improved bioavailability [482, 483]. It decreased body weight when compared to baseline and improved glycaemic control in T2DM after 2 weeks [364]. In addition, Livoletide reduced waist circumference, fat mass, postprandial glucose and hyperphagia questionnaire and appetite scores in PWS over a 2-week period [365]. Given DAG's potential as an anti-obesity therapy, it will be important to explore the neurohormonal effects of DAG on eating behaviour and its underlying mechanisms.

Furthermore, another group who are at risk of gaining weight are smokers who have given up cigarettes. The relationship between smoking, abstinence and ghrelin levels is unclear as studies have reported contradictory results. Exposure to acute cigarette smoke was suggested to decrease AG concentrations in some studies [484, 485] whereas in another study ghrelin levels increased [486]. Early abstinence from smoking was associated with higher ghrelin levels [487] while other studies did not find an association [488]. Smokers have lower levels of DAG than non-smokers after 2 weeks of abstinence, but similar AG levels [489].

The neural correlates underpinning reward processing in smokers following smoking cessation are still being delineated. Specifically, a better understanding of how DAG can modulate brain neurocircuitry to food and influence intake and eating behaviour in this group, as well as in a group with obesity on a weight management program, may inform more effective therapeutic option for adults with obesity who are seeking treatment and also help prevent smoking cessation weight gain.

5.2 HYPOTHESES

In dieting adults with obesity and in ex-smokers,

1. DAG will attenuate the appeal of and cue reactivity in mesolimbic brain regions to HE food pictures in both groups of participants.
2. DAG will reduce appetite and food intake.
3. DAG will reduce food reward behaviour, including motivation to receive a food reward and approach bias.
4. DAG will improve glucose homeostasis.
5. BMI and eating behavioural traits influence the impact of DAG on food cue reactivity, and in ex-smokers the severity of nicotine dependence or duration of abstinence.

5.3 AIMS

To investigate the effects of acute infusion of DAG on eating behaviour in dieting adults with obesity who are dieting, or in abstinent nicotine-dependence, in a double-blind randomised placebo-controlled cross-over design, on:

1. BOLD response in reward system during a HE food picture evaluation fMRI task.
2. appetite, food appeal, taste, and intake.
3. eating behavioural, namely motivation to eat using a progressive ratio task (PRT) and attentional bias to food using an approach-avoidance task (AAT).
4. plasma metabolites and hormones, including glucose and insulin.

   And to:
5. explore influence of BMI and eating behaviours associated with altered overeating (dietary restraint, uncontrolled eating, emotional eating, susceptibility to overeating from hunger cues and binge eating symptoms) on the HE food cue reactivity response to DAG, and in ex-smokers with severity of nicotine dependence (Fagerstrom Test for Nicotine Dependence) and duration of cigarette abstinence.

5.4 RESULTS

Summary statements of the findings are given in bold at the start of each section. In this section, data was available for n=25 dieting participants with obesity and n=24 ex-smokers for analysis of behavioural tasks. One participant with obesity had to be excluded from fMRI analysis due to frontal pole signal drop-out, leaving a final n=24 in each group for fMRI analysis.

5.4.1 Food Evaluation fMRI task

Summary: In dieting group with obesity, DAG increased BOLD signal to high-energy (HE) food pictures in the frontal pole, OFC, MFG, SFG, paracingulate gyrus and ACC (Figure 5.3). In contrast, in ex-smoker group, DAG decreased BOLD signal to HE food pictures in the caudate, thalamus, putamen, amygdala, SFG, frontal pole and paracingulate gyrus (Figure 5.6). However, there was no effect of DAG on relative food appeal ratings during the task in both groups (Figure 5.2). At the saline visit, ex-smokers had a higher average BOLD signal to HE food pictures in all fROI than in dieting group with obesity (Figure 5.10), but no difference in food appeal rating.

5.4.1.1 Food appeal rating

To examine the effect of DAG on food hedonics, a repeated measures ANOVA was performed including both groups, with visit (saline, DAG) as within subject factor, and group (obese, ex-smokers) as between subject factor. For HE food picture appeal rating (relative to neutral pictures), there was no significant interaction effect for visit*group [$F(1,47)=1.99$, $P=0.17$], nor an overall effect of visit [$F(1,47)=0.51$, $P=0.48$] (Figure 5.2).

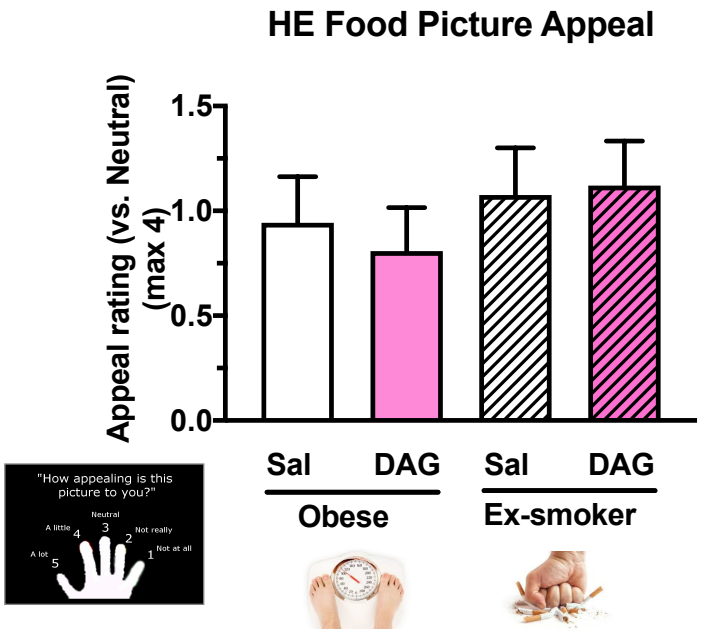


Figure 5.2. No effect of DAG on appeal rating of high-energy (HE) foods in picture evaluation fMRI task. Comparison of appeal rating of HE foods (vs. neutral) between saline (Sal) and DAG visits. Data presented as mean ± SEM, n=24-25.

5.4.1.2 fMRI food cue reactivity: whole brain analysis

5.4.1.2a Dieting group with obesity

In dieting group with obesity, DAG increased BOLD signal to HE food pictures in two frontal clusters, one on the left involving the frontal pole and OFC, and the other on the right involving the middle frontal gyrus (MFG), superior frontal gyrus (SFG), paracingulate gyrus and anterior cingulate gyrus (ACC)(Figure 5.3; Table 5.1). However, there was no associated reduction in BOLD signal in other reward areas, such as the striatum or amygdala.

By contrast, this effect of DAG was only seen in the dieting group with obesity and not in the ex-smokers. Using the same clusters from the dieting group with obesity as ROIs, in ex-smokers there was a decrease in BOLD signal to food cues in these frontal clusters in the ex-smokers with DAG compared to saline (Figure 5.4). This was evident from a significant visit*group interaction [$F(1,46)=23.92$, $P<0.001$], driven by a higher BOLD signal to HE food pictures with DAG vs. saline in dieting group with obesity [effect size mean ± SEM 0.07 ± 0.02, (95% CI 0.40, 0.10), $P<0.001$, Cohen's $d=0.97$], and a lower BOLD signal to HE food pictures with DAG vs. saline in ex-smokers [effect size mean ± SEM -0.03 ± 0.02, (95%CI -0.07, -0.004), $P=0.028$, Cohen's $d=0.46$] (Figure 5.4).

A

Contrast High-energy food pictures	Cluster	Number of voxels	Z statistic	x	y	z	Side	Brain region
DAG > Saline	1	674	3.71	-40	42	4	L	Frontal pole
			3.11	-36	42	14	L	Frontal pole
			3.10	-12	28	4	L	White matter
			3.05	-44	48	2	L	Frontal pole
			3.04	-30	38	0	L	OFC/frontal pole
			3.03	-22	30	-4	L	White matter
	2	723	3.30	38	30	32	R	MFG
			3.18	12	28	38	R	Paracingulate gyrus
			3.16	24	16	32	R	White matter
			3.09	8	26	16	R	ACC
			3.03	24	24	38	R	SFG/MFG
			3.03	24	32	12	R	White matter
Saline > DAG		nil significant						

B

Contrast High-energy food pictures	Cluster	Number of voxels	Z statistic	x	y	z	Side	Brain region
DAG > Saline		nil significant						
Saline > DAG	1	1606	3.87	12	44	14	R	Paracingulate gyrus
			3.87	12	40	16	R	Paracingulate gyrus
			3.48	8	20	56	R	SFG
			3.32	8	50	38	R	SFG
			3.13	14	44	30	R	Frontal pole
			3.07	18	10	60	R	SFG
	2	1676	4.20	22	14	8	R	Putamen
			4.01	20	8	12	R	Caudate
			3.76	26	16	-2	R	Putamen
			3.73	20	8	-2	R	Putamen
			3.32	16	0	-16	R	Amygdala
			3.28	-6	0	12	L	Caudate/thalamus

Table 5.1. Whole brain analysis for effect of DAG on HE food evaluation fMRI task in dieting group with obesity and ex-smokers.

Stereotactic coordinates (x, y, z in standard MNI space) for peak voxel of group activation for (A) obese (n=24) and (B) ex-smokers (n=24), for HE food vs. neutral pictures at DAG compared to saline visits. Cluster-wise threshold Z>2.3, family wise error (FWE) P<0.05. Abbreviations: ACC, anterior cingulate cortex; IFG, inferior frontal gyrus; SFG, superior frontal gyrus.

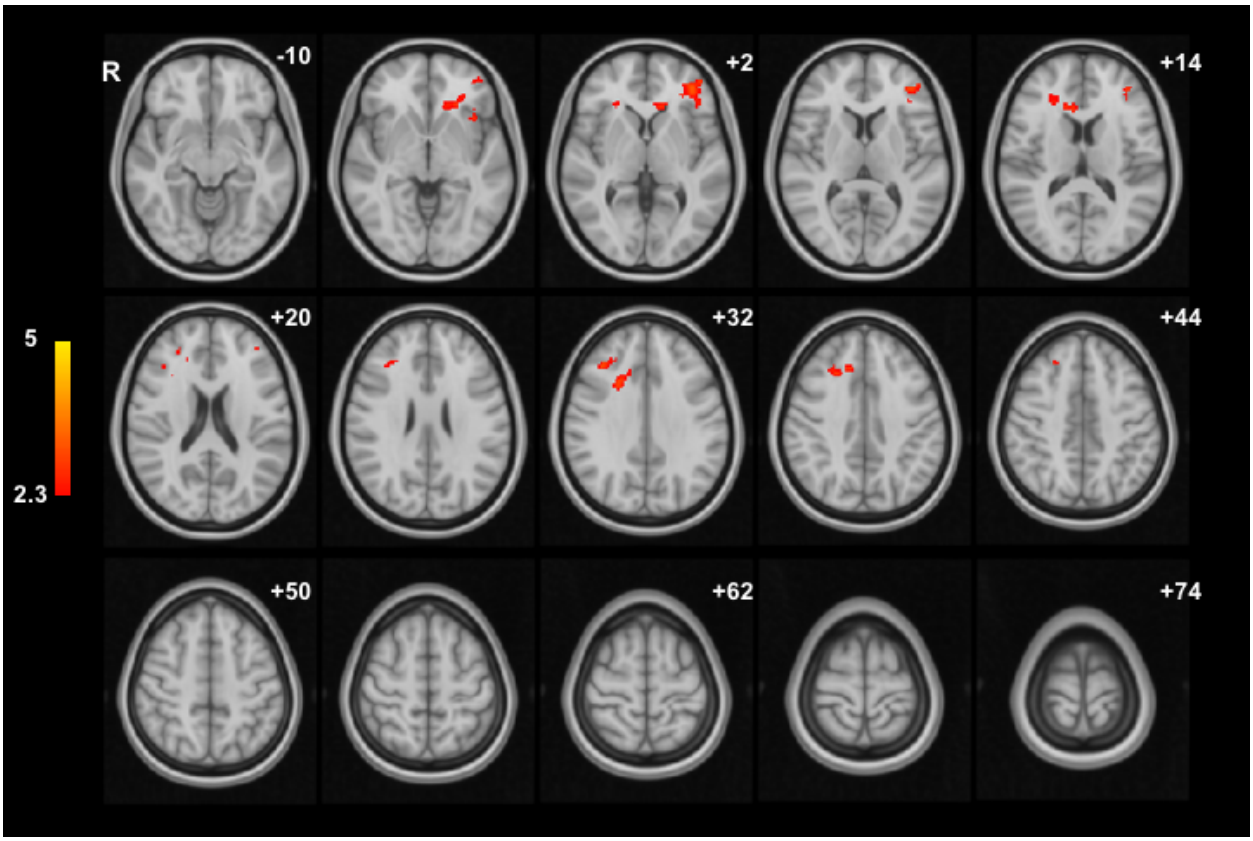


Figure 5.3. In dieting group with obesity, DAG increased BOLD signal to HE food pictures in frontal cluster.
Group activation map for HE food > neutral picture for DAG > saline in dieting adults with obesity (n=24). Colour bar indicates Z score. Cluster-wise threshold Z>2.3, family-wise error (FWE) P<0.05. z co-ordinates given in Montreal Neurological Institute (MNI) space. Abbreviations: R, right. See Table 5.1 for MNI co-ordinates.

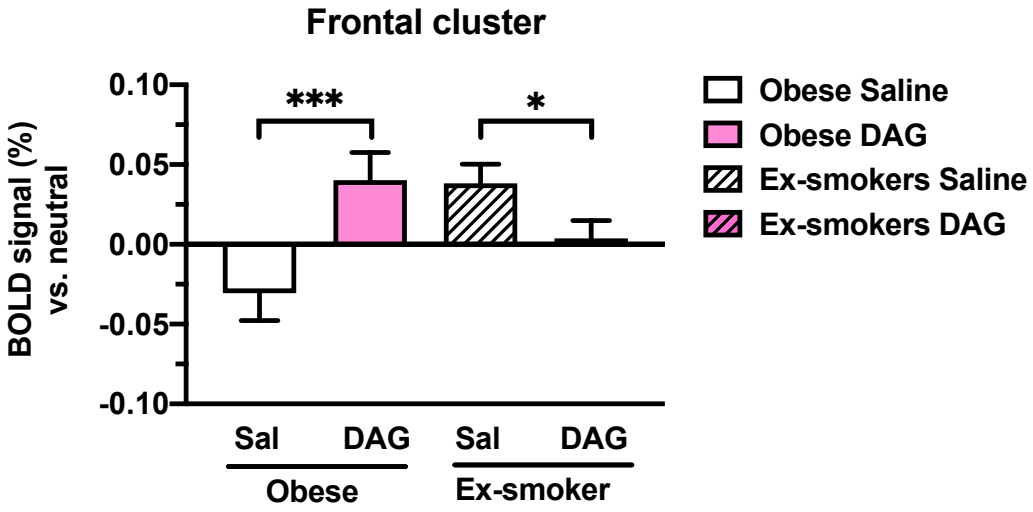


Figure 5.4. Increased BOLD signal to HE foods with DAG frontal clusters in dieting group with obesity but not ex-smokers.
Comparison of BOLD signal to HE food pictures (vs. neutral) in frontal clusters (see Figure 5.2) between saline and DAG visits for dieting group with obesity and ex-smokers. Data presented as mean ± SEM, n=24. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor and visit (saline, DAG) as within subject factor, with post-hoc Fisher LSD test: *P<0.05, **P<0.01. See Table 5.1 for MNI co-ordinates of cluster.

Correlation of effects of DAG in food evaluation fMRI task with BMI and eating behaviour

To test whether changes in BOLD signal in these frontal clusters were related to any eating behaviour measures or traits, a correlation between the median BOLD signal to HE food pictures at saline visit and BMI, individual scores from DEBQ (restraint, emotional, external), TFEQ (restraint, hunger, disinhibition) and BES was undertaken (Table 5.2). There were no significant correlation between these variables and median BOLD signal to HE food pictures at saline visit.

Furthermore, to examine if DAG-induced BOLD signal changes to HE food pictures in this frontal cluster was influenced by BMI or eating behavioural traits, a correlation between change in BOLD signal to HE food pictures at DAG (relative to saline) visit and the aforementioned questionnaire scores was performed (Table 5.2). However, there was no significant correlation between these variables and DAG-induced BOLD signal changes to HE food pictures in the frontal clusters in dieting group with obesity.

OBESE	BMI	DEBQ-emotional	DEBQ-external	DEBQ-restaint	TFEQ-restraint	TFEQ-hunger	TFEQ-disinhibition	BES
BOLD at saline								
Correlation Coefficient	0.22	0.17	-0.002	-0.07	0.20	0.08	0.18	0.36
P value	0.31	0.44	0.99	0.76	0.37	0.70	0.41	0.080
DAG-induced changes in BOLD								
Correlation Coefficient	-0.24	-0.14	0.18	0.15	0.10	0.04	-0.05	-0.12
P value	0.26	0.52	0.39	0.48	0.66	0.86	0.83	0.59

Table 5.2. Correlation of food cue reactivity with eating behaviour and BMI in dieting group with obesity
Spearman correlation coefficient in group with obesity (n=24) for BOLD signal to HE food pictures in frontal clusters (Figure 5.2) at saline visit and changes in BOLD signal induced by DAG (relative to saline) visit with BMI, and eating behaviour using DEBQ, TFEQ and BES questionnaires, demonstrating a trend for higher BOLD signal to HE foods at saline visit in frontal clusters with higher BES scores. Abbreviations: BES, Binge Eating Scale; BOLD, blood oxygen level dependent DEBQ, Dutch Eating Behavioural Questionnaire; MRI, magnetic resonance imaging; TFEQ, Three Factor Eating Questionnaire.

5.4.1.2b Ex-smokers

In keeping with the hypothesis, in the ex-smokers, DAG reduced BOLD signal to HE food pictures in brain areas around the frontal and striatal regions (Figure 5.5; Table 5.1). These included right paracingulate gyrus, right SFG, right frontal pole, right putamen, right caudate, right amygdala and left caudate/thalamus. Of note, this effect of DAG was only seen in ex-smokers and not in dieting group with obesity. Using the same frontal and dorsal striatal clusters as ROIs, in dieting

group with obesity there was an increase in BOLD signal to HE food pictures in the corresponding frontal and striatal clusters with DAG in dieting group with obesity (Figure 5.6).

For the frontal cluster, this was evident from a significant visit*group interaction [$F(1,46)=22.73$, $P<0.001$], driven by a lower BOLD signal to HE food pictures in ex-smokers with DAG [effect size mean ± SEM -0.10 ± 0.02, (95% CI -0.14, -0.05), $P<0.001$, Cohen's $d=0.92$], but a higher BOLD signal with DAG vs. saline in dieting group with obesity [effect size mean ± SEM 0.05 ± 0.02, (95% CI 0.006, 0.09), $P=0.028$, Cohen's $d=0.47$] (Figure 5.6A).

For the dorsal striatal cluster, there was a significant visit*group interaction [$F(1,46)=19.10$, $P<0.001$], driven by a lower BOLD signal to HE food pictures in ex-smoker group with DAG [effect size mean ± SEM -0.11 ± 0.03, (95% CI -0.17, -0.06), $P<0.001$, Cohen's $d=0.85$], but a higher BOLD signal with DAG vs. saline in dieting group with obesity [effect size mean ± SEM 0.06 ± 0.03, (95%CI 0.001, 0.11), $P=0.047$, Cohen's $d=0.42$] (Figure 5.6B).

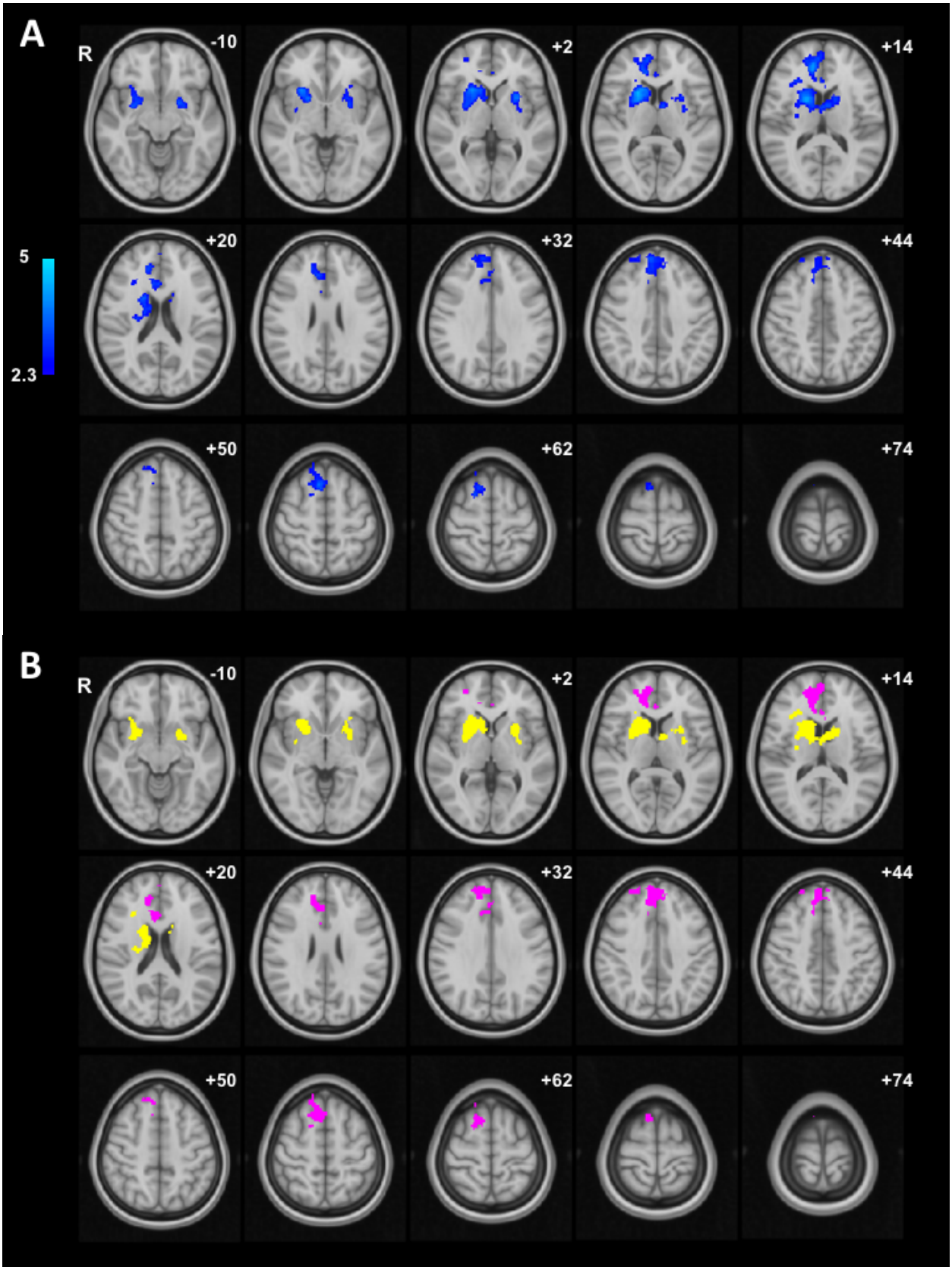


Figure 5.5. In ex-smokers, DAG reduced BOLD signal to HE foods in prefrontal cortex and dorsal striatum. Group activation map for HE food > neutral pictures for saline > DAG visit in ex-smokers (n=24) in (A) whole brain analysis, colour bar indicates Z scores; (B) separate clusters in pre-frontal cortex (paracingulate gyrus, SFG, frontal pole cluster; pink) and dorsal striatum (caudate, putamen; yellow). Cluster-wise threshold Z>2.3, family-wise error (FWE) P<0.05. z co-ordinates given in Montreal Neurological Institute (MNI) space. Abbreviations: R, right. See Table 5.1 for MNI co-ordinates.

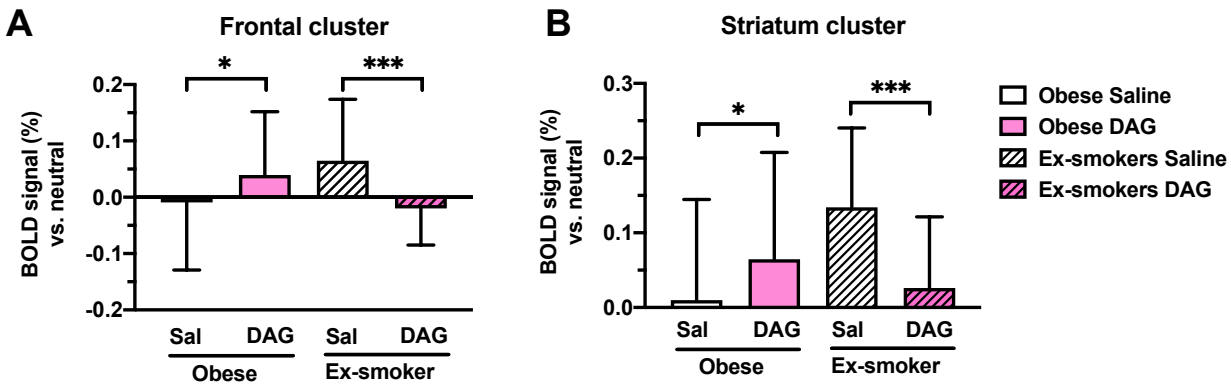


Figure 5.6. Decreased BOLD signal to HE food in PFC and dorsal striatum by DAG in ex-smokers, but an opposite effect in dieting group with obesity.

Comparison of BOLD signal to HE food pictures in (A) frontal and (B) dorsal striatum clusters (See Figure 5.5B) between saline and DAG visits in dieting group with obesity and ex-smokers. Data presented as mean ± SEM, n=24. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor and visit (saline, DAG) as within subject factor, with post-hoc Fisher LSD. *P<0.05 ***P<0.001 See Table 5.1 for MNI co-ordinates of cluster.

Correlations with BMI, eating behaviour and nicotine dependence

As with the dieting group with obesity, examination was made as to whether BOLD signal to HE food pictures in these frontal and dorsal striatal clusters for the ex-smokers was related to degree of overweight or eating behaviour traits. Thus correlations were performed between the individual BOLD signal to HE food pictures in the clusters as ROIs at either (i) the saline visit alone or (ii) the effect of DAG (DAG minus saline visit) and (iii) BMI or (iv) individual scores from the DEBQ (restraint, emotional, external), TFEQ (restraint, hunger, disinhibition) and BES (Table 5.3). Furthermore, correlations were made with (v) retrospective FTND score, as a marker of severity of previous nicotine dependence, and (vi) duration of cigarette abstinence in ex-smokers (Table 5.3).

Correlation with BOLD signal to HE foods at saline visit:

For the frontal cluster, there were no significant correlations of any of these variables to the BOLD signal to HE food pictures at saline visit in ex-smokers (Table 5.3).

For the dorsum striatum cluster, there was a negative correlation between BMI and baseline BOLD signal to HE food pictures in dorsal striatal cluster (Table 5.3, Figure 5.7A). This indicates that contrary to current literature, the higher the BMI, the lower the BOLD signal to food cues in this cluster, which usually is implicated in motivating reward behaviour.

Correlation with effect of DAG on BOLD signal to HE foods:

For the frontal cluster, there were no significant correlations of any variables to the DAG-induced decrease in BOLD signal to HE food pictures in ex-smokers (Table 5.3).

For the dorsal striatum cluster, there was a positive correlation between BMI and change in BOLD signal to food cues in dorsal striatal cluster at DAG compared to saline visit in ex-smoker group (Table 5.3, Figure 5.7B). This indicates the higher the BMI, the less the DAG-induced reduction of BOLD signal to food cues in this cluster. However, this may be reflective of a floor effect whereby in individuals with a high BMI, the lower BOLD signal at saline visit limits in the degree to which it can be lowered further with DAG.

EX-SMOKERS: FRONTAL	BMI	FTND	Abstinence	DEBQ-emotional	DEBQ-external	DEBQ-restaint	TFEQ-restraint	TFEQ-hunger	TFEQ-disinhibition	BES
BOLD at saline										
Correlation Coefficient	-0.24	-0.15	-0.007	0.25	-0.15	0.02	0.14	-0.26	0.002	-0.23
P value	0.26	0.47	0.97	0.22	0.47	0.91	0.51	0.21	0.99	0.27
DAG-induced changes in BOLD										
Correlation Coefficient	0.31	0.35	0.07	0.01	0.14	-0.007	-0.009	0.30	0.13	0.32
P value	0.14	0.099	0.76	0.95	0.52	0.97	0.97	0.15	0.55	0.13
EX-SMOKERS: STRIATAL										
BOLD at saline										
Correlation Coefficient	-0.52	-0.13	-0.04	0.29	0.08	-0.36	-0.32	-0.02	0.03	-0.02
P value	**0.0095**	0.53	0.84	0.15	0.71	0.078	0.12	0.92	0.90	0.94
DAG-induced changes in BOLD										
Correlation Coefficient	0.58	0.30	-0.12	0.10	0.16	0.22	0.15	0.29	0.15	0.40
P value	**0.0027**	0.15	0.58	0.64	0.45	0.30	0.48	0.17	0.49	0.054

Table 5.3. Correlations of BMI, eating behaviour, severity of nicotine dependence and duration of abstinence with BOLD signal to HE foods in frontal and dorsal striatum clusters at saline visit or effects of DAG.
Spearman correlation in ex-smokers (n=24) of BOLD signal to HE food pictures in frontal and dorsal striatum clusters (see Figure 5.5B) at saline visit or effect of DAG (relative to saline visit) with BMI, FTND, duration of cigarette abstinence (weeks), DEBQ, TFEQ and BES scores. Analysis reveals that BMI negatively correlated with BOLD signal to HE foods at saline visit, and also negatively correlates with ability of DAG to reduce BOLD signal to HE foods, in dorsal striatum. Abbreviations: BES, Binge Eating Scale; BOLD, blood oxygen level dependent DEBQ, Dutch Eating Behavioural Questionnaire; FTND, Fagerstrom Test for Nicotine Dependence; TFEQ, Three Factor Eating Questionnaire.

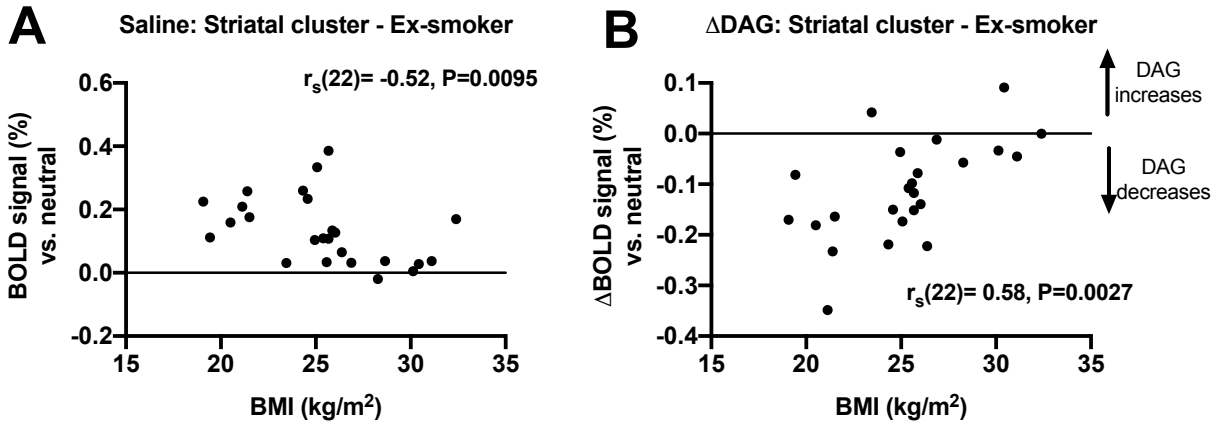


Figure 5.7. In ex-smokers, BMI was positively correlated with BOLD signal to HE food pictures in dorsal striatal cluster at saline visit and effects of DAG.

In ex-smoker group (n=24), correlation between BMI and (A) BOLD signal to HE food pictures in dorsal striatal cluster (Figure 5.5B) at saline visit and (B) change in BOLD signal to HE food pictures in same striatal cluster at DAG visit relative to saline. r_S represents Spearman correlation.

5.4.1.3 fMRI food cue reactivity: functional ROI analysis

To assess the effects of DAG on BOLD signal during evaluation of HE food pictures vs. neutral in the *a priori* functional ROIs (nucleus accumbens, caudate, putamen, orbitofrontal cortex, amygdala, hippocampus, anterior insula, ventral anterior cingulate cortex, dorsolateral prefrontal cortex, Figure 2.6, Table 2.2), a repeated measures ANOVA was performed including both groups, with fROI and visit (saline, DAG) as within subject factors, and group (obese, ex-smokers) as between subject factor.

For BOLD signal during evaluation of HE food pictures vs. neutral pictures, there was no significant interaction effect for: (i)visit*ROI*group $F(4.61,211.82)=2.00$, $P=0.086$ with Greenhouse-Geisser (GG) correction], (ii)visit*ROI [$F(4.61,211.82)=0.72$,$P=0.60$ with GG correction], nor an effect of (iii) visit [$F(1,46)=0.02$, $P=0.90$], in repeated measures ANOVA (Figure 5.9).

Nevertheless, there was a significant interaction effect of: visit*group [$F(1,46)=7.21$, $P=0.010$]. This was driven by a significantly higher BOLD signal (averaged across all fROI), to HE food pictures in the ex-smokers at the saline visit compared to dieting group with obesity [effect size mean ± SEM 0.075 ± 0.031, (95%CI 0.011, 0.138), $P=0.022$, Cohen's $d=0.70$], that was not seen for DAG visit. This indicates that ex-smokers had a higher BOLD signal to food pictures averaged across all fROI than the dieting group with obesity, that was lost with DAG administration.

In dieting group with obesity, DAG tended to increase the BOLD signal averaged across all fROI to HE food pictures compared to saline but this did not reach significance [effect size mean ± SEM 0.048 ± 0.024, (95%CI -0.001, 0.097), $P=0.053$, Cohen's $d=0.40$)] as well as a trend for a decrease of BOLD signal to HE food pictures in the ex-smoker group at DAG visit compared to saline [effect size mean ± SEM -0.044 ± 0.024, (95%CI -0.092, 0.005), $P=0.077$, Cohen's $d=0.37$] (Figure 5.8).

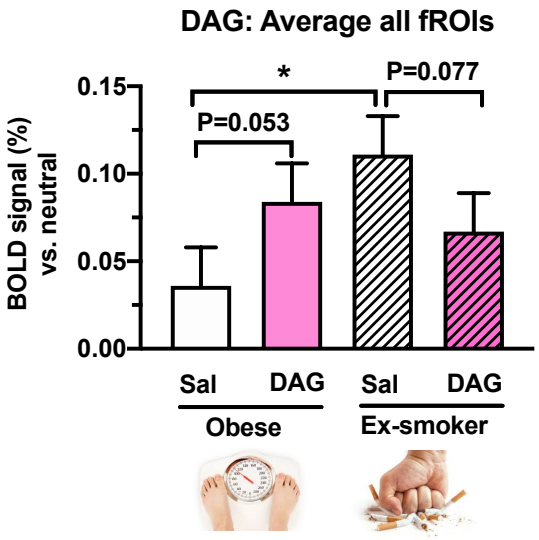


Figure 5.8. Ex-smokers have higher BOLD signal to HE food pictures across all fROI than in dieting group with obesity at saline but not DAG visits.

Comparison of BOLD signal during evaluation of food pictures (vs. neutral pictures) averaged across all functional regions of interest (fROI: nucleus accumbens, caudate, putamen, orbitofrontal cortex, amygdala, hippocampus, anterior insula, ventral anterior cingulate cortex, dorsolateral prefrontal cortex) between saline and DAG visits for obese and Ex-smoker. Data presented as mean ± SEM, n=24. Statistics from repeated measures ANOVA, with group (obese, Ex-smoker) as between subject factor and visit (saline, DAG) as within subject factor, post-hoc Fisher LSD test: *P<0.05.

Whilst the interaction effect for visit*group*ROI did not reach significance [F(4.61, 211.82)=2.00, P=0.086 with Greenhouse-Geisser correction], exploratory analysis revealed a general pattern of increase in BOLD signal to HE food pictures with Exenatide in most fROI, but this was only significant in the putamen and vACC (Figure 5.9A). In contrast, in ex-smoker group, there was a general pattern of reduction in BOLD signal to HE food pictures with Exenatide across most fROI, only significant in caudate, putamen, vACC and dlPFC (Figure 5.9B).

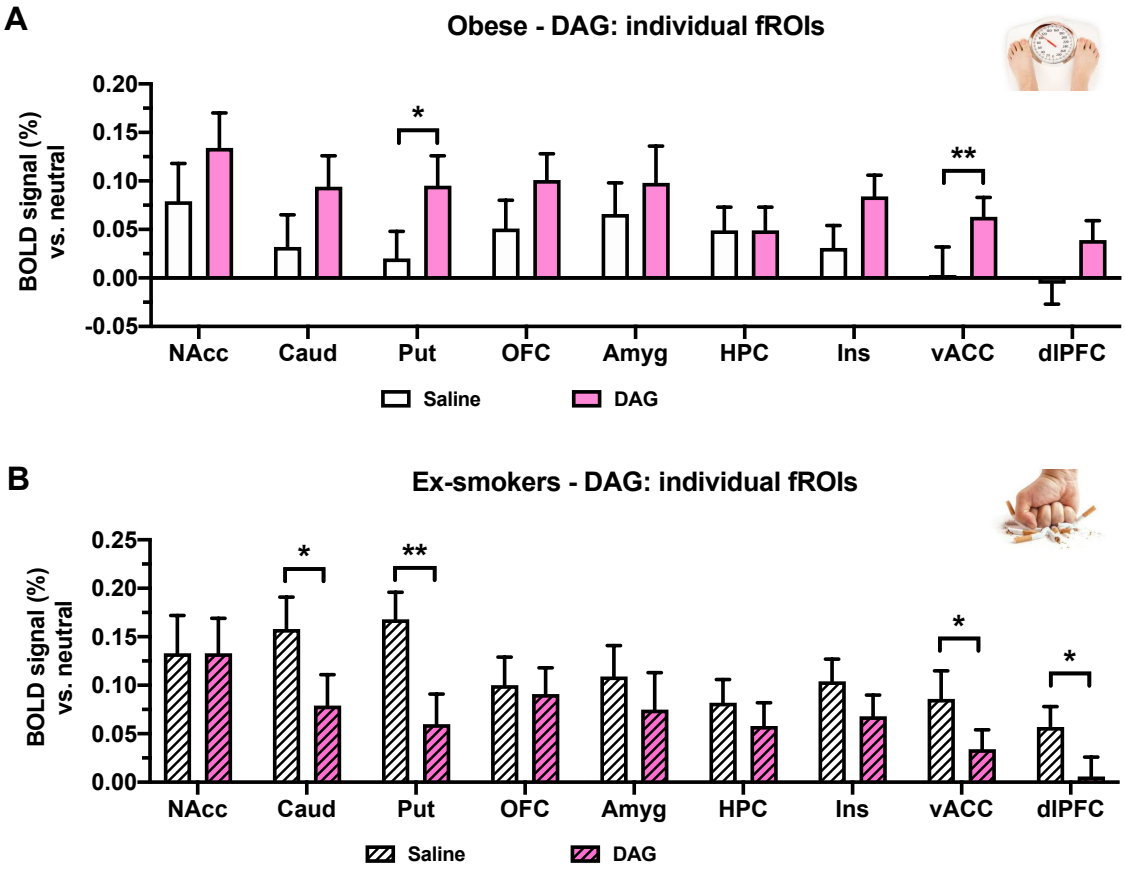


Figure 5.9. Effect of DAG on BOLD signal to food pictures in individual fROI in dieting group with obesity and ex-smokers.

Comparison of BOLD signal during evaluation of food pictures (vs. neutral pictures) in all functional regions of interest [fROI: nucleus accumbens (NAcc), caudate (Caud), putamen (Put), orbitofrontal cortex (OFC), amygdala (Amyg), hippocampus (HPC), anterior insula (Ins), ventral anterior cingulate cortex (vACC), dorsolateral prefrontal cortex (dlPFC)] between saline and DAG visits in (A) obesity and (B) Ex-smokers. Data presented as mean ± SEM, n=24. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor and visit (saline, DAG), ROI (NAcc, Caud, Put, OFC, Amyg, HPC, Ins, vACC, dlPFC) as within subject factors, with posthoc Fisher LSD test *P<0.05 **P<0.01.

Adjusting for Covariates

To assess whether the interaction effect for visit*group was altered when adjusting for co-variates, the visit order was included in separate repeated measures ANCOVA analyses.

Visit order: The interaction effect for visit*group after adjusting for difference in visit order between visits (i.e. visit number of DAG minus saline visits) was F(1,45)=8.02, P=0.007. This was driven by the higher average BOLD signal to HE food pictures at saline in ex-smokers compared to dieting group with obesity [effect size mean ± SEM 0.08 ± 0.03, (95% CI 0.01, 0.14), P=0.020], that was not seen in the DAG visit [effect size mean ± SEM -0.02 ± 0.03, (95%CI -0.08, 0.04), P=0.55]. Also, there was an increase in average BOLD signal to HE food pictures with DAG in the dieting group with obesity [effect size mean ± SEM 0.05 ± 0.02, (95% CI 0.002, 0.10), P=0.042], that was not seen in ex-smokers [effect size mean ± SEM -0.05 ± 0.02, (95%CI -0.09, 0.002), P=0.063].

5.4.2 Potential confounding factors for visit outcome variables

Summary: There were no significant differences in confounding factors that may account for differences in BOLD responses to food pictures or other behavioural outcomes (Table 5.4; Figure 5.10).

5.4.2.1 Confounds for food evaluation fMRI task

Several behavioural and state factors may confound comparison between visits. However, when assessing specific behavioural measures in the fMRI task, there were no significant interaction effects for visit*group*picture, visit*group, visit*picture, nor an overall effect of visit for:

(i) *rating accuracy* (defined as the percentage of recorded picture evaluation responses averaged across four picture types for a study visit);

(ii) *rating reaction time* (defined as the average of the duration between each picture appearing and recorded evaluation response in msec);

and no significant interaction effect for visit*group, nor an overall effect of visit for:

(iii) *relative motion per scan volume;*

(iv) *visit order* (defined as the visit number of DAG minus that of saline, performed to assess appropriate randomisation of study visits);

(v) *day of menstrual cycle* for women (Women who had cycles of more than 3 months were excluded from this analysis as the accuracy of date of last menstrual period was deemed questionable).

5.4.2.2 Confounds for other outcomes

The following variables were measured at each study visit to assess differences in mood state: (i) *Profile of Mood States (POMS-2)* which included fatigue, tension, vigour, depression, anger and confusion, (ii-iii) *Positive affect and Negative Affect Schedule (PANAS)* as a measure of positive and negative mood, and (iv) *Spielberger State Anxiety Inventory (SSAI)* as a measure of state anxiety.

However, there were no significant visit*group interactions nor overall effect of visit for:

(i) *Profile of Mood States (POMS-2):*

(ii) *Positive affect (PANAS)*

(iii) *Negative affect (PANAS)*

(iv) For *Spielberger State Anxiety Inventory (SSAI)* there was no significant visit*group interaction nor overall effect of visit. However, there was a trend for a significant interaction effect for visit*group [F(1,47)=3.56, P=0.065]. This was driven by lower anxiety scores in the dieting group with obesity compared to ex-smoker group at DAG visit [effect size mean ± SEM -4.94 ± 1.69, (95%CI -8.34, -1.54), P=0.005, Cohen's d=1.33], not seen at the saline visit [effect size mean ± SEM -1.76 ± 1.81, (95%CI -5.41, 1.89), P=0.34]. Nonetheless, this was unlikely to be attributed to DAG as the questionnaires were performed just after the start of infusion. Moreover, this was unlikely to have caused an impact on the assessments between visits since the absolute change was clinically small relative to the maximum rating in this questionnaire (max 80). In addition, analysis of anxiety VAS throughout the visits showed no difference between groups nor visits (Figure 5.17).

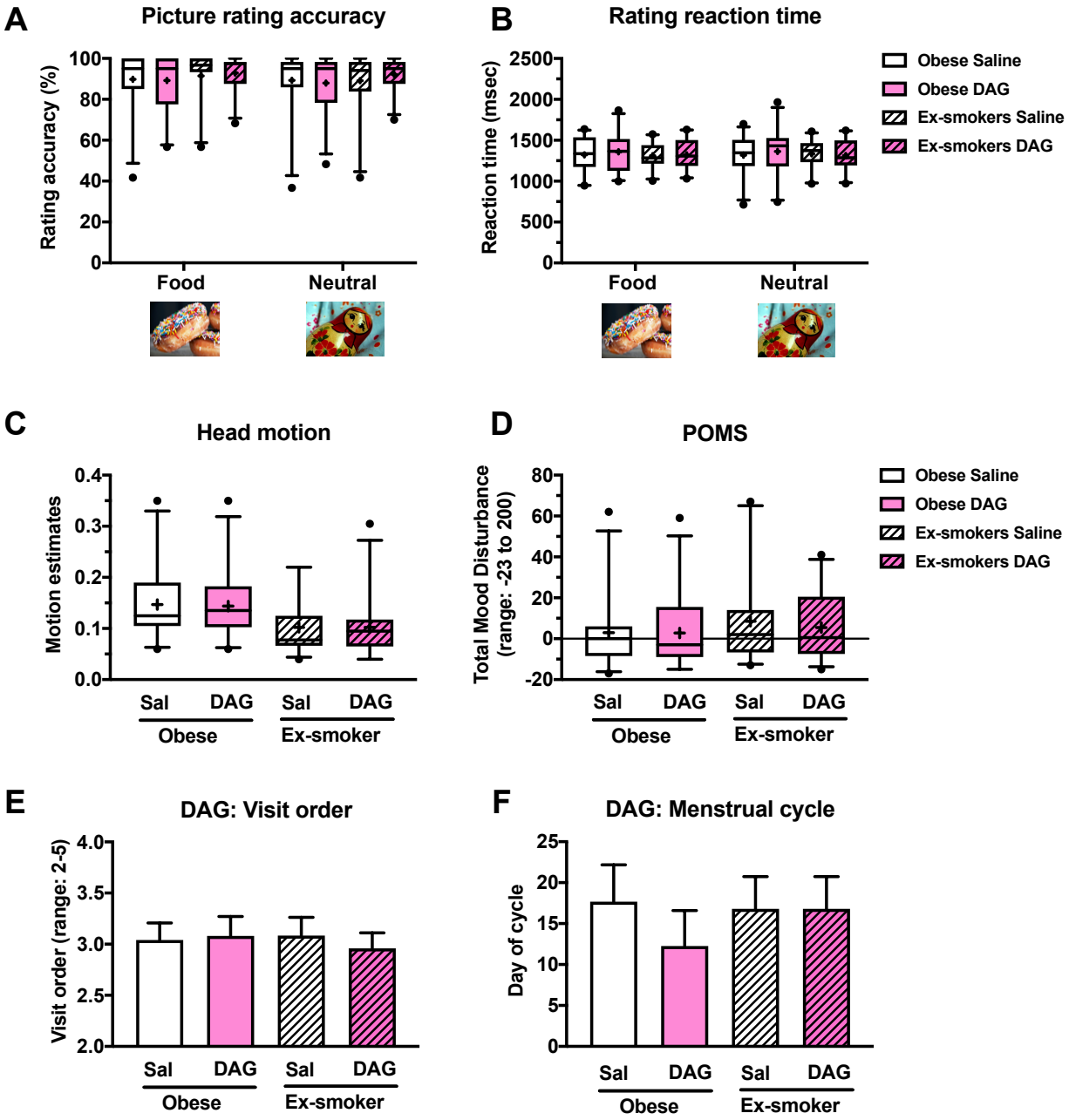


Figure 5.10. No significant within-subject differences in confounding factors of picture evaluation fMRI task. Comparison of (A) rating accuracy (%), (B) rating reaction time (msec), (C) head motion artefact (mm/TR) during the picture evaluation fMRI task, (D) Profile of Mood States-2 (POMS), (E) visit order and (F) day of menstrual cycle (n=11-11) between saline (white) and DAG (pink) visits for obese (clear) and ex-smoker (hatched) groups. (A-D) Data presented as box plots with median as line, mean as +, box 25th and 75th percentiles and bars 5th and 95th percentiles, n=24-25. (E-F) Data presented as bar graphs (mean) and SEM error bars. Abbreviations: DAG, Desacyl ghrelin; POMS, Profile of mood states 2; Sal, saline; TR, time to repeat.

	Picture type	Obese		Ex-smoker		Interactions	F	P
		Saline	DAG	Saline	DAG			
Rating accuracy[a] (%)	Food	89.8 ± 14.0	89.2 ± 13.3	91.8 ± 12.9	92.8 ± 8.3	Visit	0.50	0.48
						Visit*picture	1.03	0.31
	Neutral	89.3 ± 15.4	88.0 ± 14.3	89.5 ± 14.9	92.2 ± 8.2	Visit*group	0.49	0.49
						Visit*group*picture	1.21	0.28
Reaction time (msec)	Food	1323 ± 215	1360 ± 242	1312 ± 147	1322 ± 181	Visit	0.86	0.36
						Visit*picture	1.13	0.29
	Neutral	1317 ± 240	1363 ± 286	1335 ± 163	1314 ± 189	Visit*group	0.16	0.70
						Visit*group*picture	0.64	0.43
Motion[a] (mm/TR)	All	0.147±0.067	0.144 ± 0.062	0.104±0.052	0.102±0.055	Visit	0.001	0.97
						Visit*group	0.006	0.94
POMS (range: -23 to 200)		2.92 ± 3.81	2.80 ± 3.31	8.54 ± 3.89	5.42 ± 3.37	Visit	0.46	0.50
						Visit*group	0.39	0.53
SSAI (max 80)		27.24 ± 1.27	25.64 ± 1.18	29.00 ± 1.30	30.58 ± 1.21	Visit	<0.001	>0.99
						Visit*group	3.56	0.065
PA (max 50)		35.04 ± 1.22	35.64 ± 1.32	32.58 ± 1.25	32.83 ± 1.34	Visit	0.32	0.57
						Visit*group	0.06	0.82
NA (max 50)		12.88 ± 0.93	13.56 ± 0.95	14.79 ± 0.95	14.58 ± 0.97	Visit	0.10	0.76
						Visit*group	0.34	0.56

Table 5.4. Potential confounding factors for HE food picture evaluation task.

Analyses looked for differences in rating accuracy and reaction time between groups using repeated measures ANOVA with visit (saline, DAG) and picture type (Food, Neutral) as within subject factors, and group (obese, ex-smoker) as between subject factor. Results from repeated measures ANOVA for state mood questionnaires with visit (saline, DAG) as within subject factor and group (obese, ex-smoker) as between subject factor Data presented as mean ± SEM, n=24-25. [a] Data used in repeated measures ANOVA was normalised to log10 values. Abbreviations: NA, Negative Affect; PA, Positive Affect; POMS, Profile of Mood States; SSAI, Spielberger State Anxiety Inventory

5.4.3 Lunch Taste Visual Analogue Scale Ratings

Data was available for n=25 dieting participants with obesity and n=24 ex-smokers for analysis of initial visual analogue scale (VAS) taste ratings, and overall consumption (see next section), of the individual dishes at the *ad libitum* test lunch meal after scanning. All participants had the tomato soups on their study visits, apart from one in the ex-smoker group who had the chicken soups. Two participants from the ex-smoker group had to be excluded from the analysis of energy intake of meal to avoid a ceiling effect as they completely finished a dish on one of their study visits. In these analyses, a single repeated measures ANOVA was performed across both groups and all 4 dishes, with visit (saline, DAG), dish sweetness (savoury, sweet), dish fat content (low fat, high fat) as within subject factors, and group (obese, ex-smoker) as between subject factor, primarily to look at interactions and overall effect of visit.

Summary: DAG increased ideal creaminess rating of cream soup and reduced that of broth independent of groups (Figure 5.11D, Table 5.5).

5.4.3.1 Liking and pleasantness

For rating of liking and pleasantness, there was no significant interaction effect for: (i) visit*sweet*fat*group, (ii) visit*sweet*fat, (iii) visit*fat*group, (iv) visit*fat, (v) visit*sweet*group, (vi) visit*sweet, (vii) visit*group, nor an overall effect of (viii) visit (Figure 5.11AB & Table 5.5).

5.4.3.2 Creaminess intensity

For rating of creaminess intensity, there was no significant interaction effect for: (i) visit*sweet*fat*group, (ii) visit*sweet*fat, (iii) visit*fat*group, (iv) visit*fat, (v) visit*sweet*group, (vi) visit*sweet, (vii) visit*group, nor an overall effect of (viii) visit (Figure 5.11C & Table 5.5).

5.4.3.3 Ideal creaminess

For rating of ideal creaminess, there was a significant interaction effect for: visit*sweet*fat [$F(1,47)=4.38$, $P=0.042$] independent of group. This is driven by a significant reduction of ideal creaminess rating of broth with DAG [39.04 ± 1.86 to 34.65 ± 2.28; effect size mean ± SEM -4.39 ± 2.04, (95%CI -8.50, -0.29), $P=0.037$, Cohen's $d=0.31$] and a significant increase in ideal creaminess rating of cream soup with DAG [60.71 ± 2.45 to 66.63 ± 2.33; effect size mean ± SEM

5.93±2.52, (95%CI 0.86, 11.00), P=0.023, Cohen's d=0.34] independent of group. This suggests that DAG increased ideal creaminess rating of cream soup and reduced that of broth (Figure 5.11D & Table 5.5).

5.4.3.4 Sweetness intensity

For rating of sweetness intensity of the desserts, there was a significant interaction effect for visit*fat, independent of group [F(1,47)=4.79, P=0.034]. This is driven mainly by the higher ratings of ice-cream compared to yogurt at the saline visit [effect size mean ± SEM 24.82 ± 4.57, (95%CI 15.63, 34.00), P<0.001, Cohen's d=0.78], and to a lesser extent the DAG visit [effect size mean ± SEM 15.68 ± 4.37, (95%CI 6.89, 24.47), P=0.001, Cohen's d=0.51].

There was no significant interaction effect for: (i) visit*fat*group, (ii) visit*group nor an overall effect of (iii) visit (Figure 5.11E & Table 5.5).

5.4.3.5 Ideal sweetness

For rating of ideal sweetness for desserts, there was no significant interaction effect for: (i) visit*fat*group, (ii) visit*fat, (iii) visit*group nor an overall effect of (iv) visit (Figure 5.11F & Table 5.5).

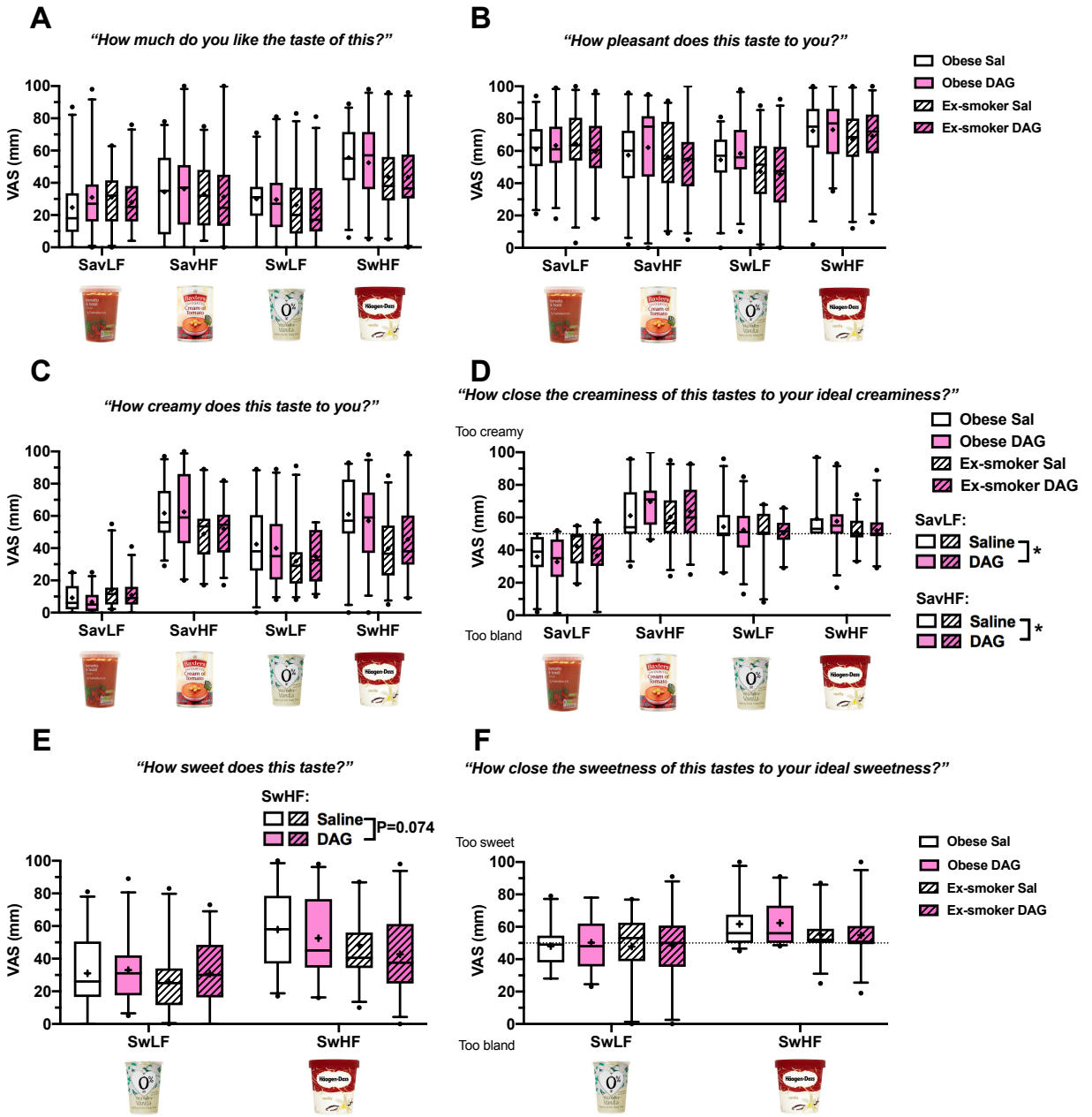


Figure 5.11. Effects of DAG on taste ratings of lunch dishes in dieting group with obesity and ex-smokers.
Comparison of visual analogue scale ratings of (A) liking, (B) pleasantness, (C) creaminess intensity, (D) ideal creaminess, (E) sweetness intensity, (F) ideal sweetness, tasted at the start of the *ad libitum* test lunch meal, for (A-D) individual dishes (savoury LF broth, savoury HF cream soup, sweet LF yogurt, sweet HF ice-cream), or (E,F) just desserts (yogurt, ice-cream), between visits (saline, DAG) and group (obese, ex-smoker). Data presented as boxplots showing median (line), mean (cross), interquartile range (box) and 5^{th} – 95^{th} percentile (bars), n=24-25. Statistics from repeated measures ANOVA for taste measures, with group (obese, ex-smoker) as between subject factor, and dish fat content (low fat, high fat), dish sweetness (savoury, sweet) and visit (saline, DAG) as within subject factors. , with post hoc Fisher LSD test: *P<0.05

Interactions: Liking	***df***	***F***	***P***	***Interactions: Pleasantness***	***df***	***F***	***P***
Visit	(1,47)	<0.0001	0.98	Visit	(1,47)	0.13	0.72
Visit*group	(1,47)	0.35	0.56	Visit*group	(1,47)	1.83	0.18
Visit*sweet	(1,47)	1.08	0.31	Visit*sweet	(1,47)	0.28	0.60
Visit*sweet*group	(1,47)	1.06	0.31	Visit*sweet*group	(1,47)	1.14	0.29
Visit*fat	(1,47)	0.20	0.66	Visit*fat	(1,47)	0.50	0.49
Visit*fat*group	(1,47)	1.81	0.19	Visit*fat*group	(1,47)	0.86	0.36
Visit*sweet*fat	(1,47)	0.03	0.86	Visit*sweet*fat	(1,47)	0.36	0.55
Visit*sweet*fat*group	(1,47)	0.02	0.89	Visit*sweet*fat*group	(1,47)	0.56	0.46
Interactions: Creaminess				***Interactions: Ideal creaminess***			
Visit	(1,47)	<0.0001	>0.99	Visit	(1,47)	0.002	0.96
Visit*group	(1,47)	1.35	0.25	Visit*group	(1,47)	0.09	0.77
Visit*sweet	(1,47)	0.17	0.69	Visit*sweet	(1,47)	0.56	0.46
Visit*sweet*group	(1,47)	2.02	0.16	Visit*sweet*group	(1,47)	2.72	0.11
Visit*fat	(1,47)	1.27	0.27	Visit*fat	(1,47)	5.73	**0.021***
Visit*fat*group	(1,47)	0.57	0.46	Visit*fat*group	(1,47)	0.08	0.78
Visit*sweet*fat	(1,47)	0.55	0.46	Visit*sweet*fat	(1,47)	4.38	**0.042***
Visit*sweet*fat*group	(1,47)	0.01	0.91	Visit*sweet*fat*group	(1,47)	0.12	0.74
Interactions: Sweetness				***Interactions: Ideal sweetness***			
Visit	(1,47)	0.19	0.67	Visit	(1,47)	0.39	0.54
Visit*group	(1,47)	0.13	0.72	Visit*group	(1,47)	0.26	0.61
Visit*fat	(1,47)	4.79	**0.034***	Visit*fat	(1,47)	0.29	0.60
Visit*fat*group	(1,47)	0.17	0.68	Visit*fat*group	(1,47)	0.003	0.96

Table 5.5. Repeated measures ANOVA results for effect of DAG on taste ratings of *ad libitum* meal.
Results from repeated measures ANOVA for taste measures (liking, pleasantness, creaminess, ideal creaminess) for the soups and desserts as well as sweet and ideal sweetness for the desserts with group (obese, ex-smoker) as between subject factor and fat (High-fat, Low-fat), sweet (Savoury, Sweet) and visit (saline, DAG) as within subject factors. Significant results are in bold. *P<0.05, n=24-25

5.4.4. Ad libitum meal intake

Summary: DAG did not affect total energy intake but tended to reduce energy intake from low-fat foods (broth and yogurt) independent of group (Figure 5.12, Table 5.6).

At the *ad libitum* test meal, DAG did not affect the total energy intake in both groups of participants (both for absolute amount and as % of estimated 24 hour resting energy expenditure, calculated using Cunningham equation) (Figure 5.12). When examining individual dishes, there were no significant interaction effects for: (i) visit*sweet*fat*group, (ii) visit*sweet*fat, (iii) visit*fat*group, (iv) visit*fat, (v) visit*sweet*group, (vi) visit*sweet, (vii) visit*group, nor an overall effect on (viii) visit. This indicates that DAG did not affect energy intake from individual dishes in both participant groups (Figure 5.12A).

However, there was a trend for a significant interaction effect for visit*fat [energy intake %REE: $F(1,45)=3.77$, $P=0.059$], independent of sweet/savoury and group. This was driven by the greater energy intake from the high-fat compared low-fat food, at DAG [effect size mean ± SEM 10.99 ± 1.52, (95%CI 7.93, 14.05), $P<0.001$, Cohen's $d=1.05$] and to a lesser extent, saline visit [effect size mean ± SEM 9.29 ± 1.41, (95%CI 6.38, 12.04), Cohen's $d=0.96$]. Indeed, there was also a reduction in energy intake from low-fat foods (broth and yogurt) at the DAG visit independent of group [effect size mean ± SEM -0.71 ± 0.27, (95%CI -1.26, -0.16), $P=0.013$, Cohen's $d=0.38$] (Figure 5.12C).

Interactions: Energy intake by dishes (kcal)	***df***	***F***	***P***	***Interactions: Energy intake by dishes (%REE)***	***df***	***F***	***P***
Visit	(1,45)	0.15	0.70	Visit	(1,45)	0.16	0.69
Visit*group	(1,45)	0.02	0.88	Visit*group	(1,45)	0.004	0.95
Visit*sweet	(1,45)	0.47	0.50	Visit*sweet	(1,45)	0.76	0.39
Visit*sweet*group	(1,45)	0.97	0.33	Visit*sweet*group	(1,45)	0.65	0.42
Visit*fat	(1,45)	3.98	0.052	Visit*fat	(1,45)	3.77	0.059
Visit*fat*group	(1,45)	0.45	0.50	Visit*fat*group	(1,45)	0.82	0.37
Visit*sweet*fat	(1,45)	0.94	0.34	Visit*sweet*fat	(1,45)	1.24	0.27
Visit*sweet*fat*group	(1,45)	0.09	0.77	Visit*sweet*fat*group	(1,45)	0.02	0.89

Table 5.6. Repeated measures ANOVA results for effect of DAG on energy intake of *ad libitum* meal.
Results from repeated measures ANOVA for energy intake [absolute, or as a percentage of estimated 24 hour resting energy expenditure (REE)] for the individual dishes, with group (obese, ex-smoker) as between subject factor, and visit (saline, DAG), dish sweetness (savoury, sweet) and dish fat content (LF, HF) as within subject factors. N=22-25 per group.

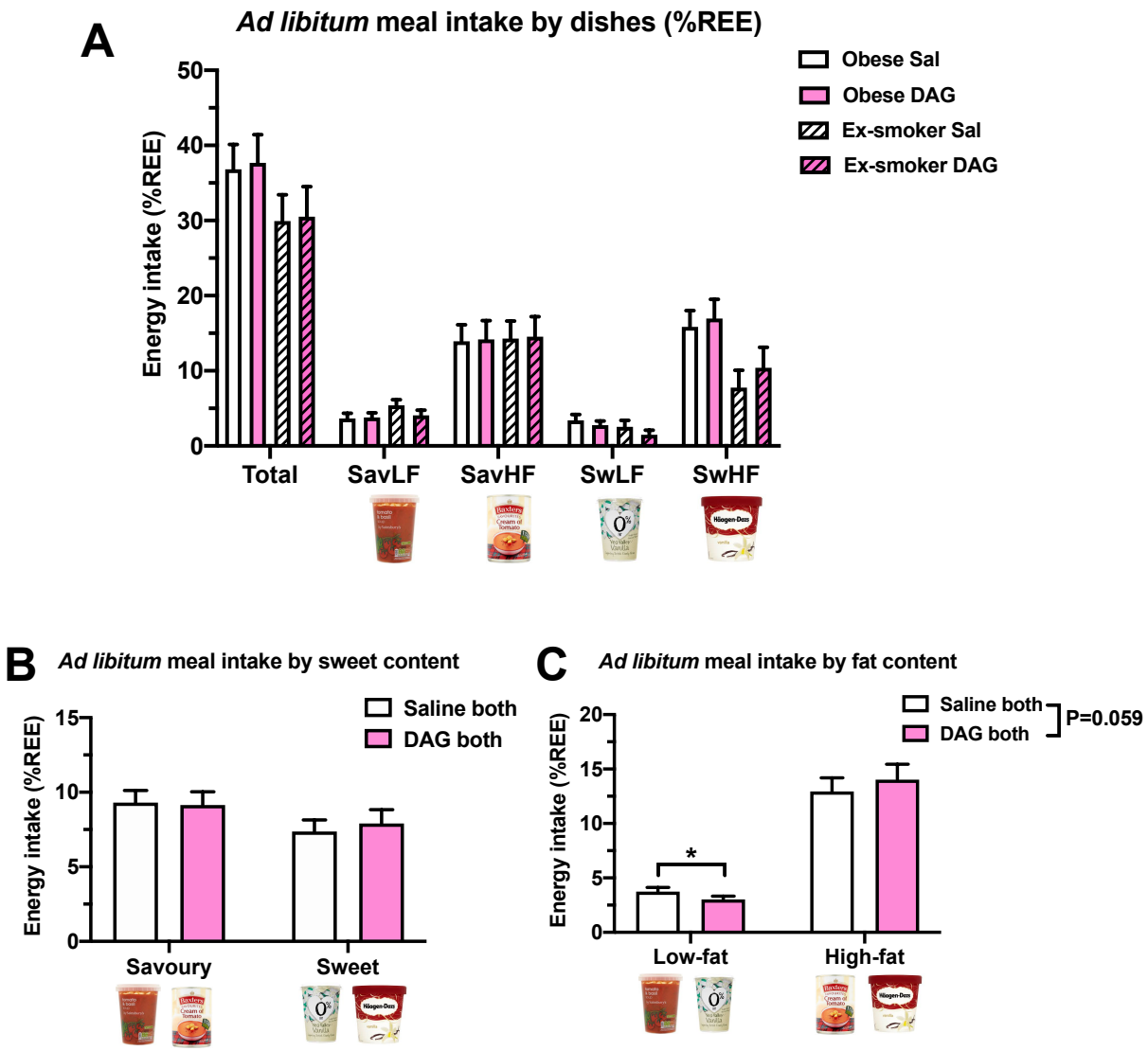


Figure 5.12. Effects of DAG on energy intake at *ad libitum* test meal.

Comparison of energy intake for: (A) total food and individual dishes [(SavLF) savoury low-fat broth; (SavHF) savoury high-fat cream soup; (SwLF) sweet low-fat yogurt; (SwHF) sweet high-fat ice-cream] as percentage of estimated 24 hour resting energy expenditure (REE), and (B,C) energy intake as %REE by dish (B) sweetness and (C) fat content, between saline and DAG visits in (A) in obese and ex-smokers separately, or (B-C) independent of group. Data presented as mean ± SEM, n=22-25 per group. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor, and visit (saline, DAG), dish sweetness (savoury, sweet) and dish fat content (LF, HF) as within subject factors, post-hoc Fisher LSD: *P<0.05.

5.4.5 Progressive Ratio Task

Summary: DAG had no effect on the motivation to receive a chocolate using the PRT in either group (Figure 5.13).

Data was available for PRT in n=25 in dieting group with obesity and n=24 in ex-smokers. However, 1 participant with obesity had to be excluded as they disliked M&Ms and declined to do the task. Another 6 participants with obesity and 1 ex-smoker had to be excluded as they did not understand the task instructions or there was a discrepancy of more than 2 chocolates between number of chocolates eaten and the number of completed ratios on software records on either DAG or saline infusion visit, resulting in a final n=18 dieting participants with obesity and n=23 ex-smokers. Data was normalised by $\log_{10}$ transformation for statistical analysis given the exponential nature of the progressive ratio design.

Outcome measures of PRT

For the total number of completed clicks and the breakpoint (last completed click to earn an M&M), there were no significant interaction effects for: (i) visit*group [F(1,38)=0.01, P=0.92; F(1,38)=0.03, P=0.87 respectively] nor overall (ii) visit [F(1,38)= 2.16, P=0.15; F(1,38)=1.13, P=0.30 respectively]. This indicates that DAG did not affect motivation for a chocolate in either group.

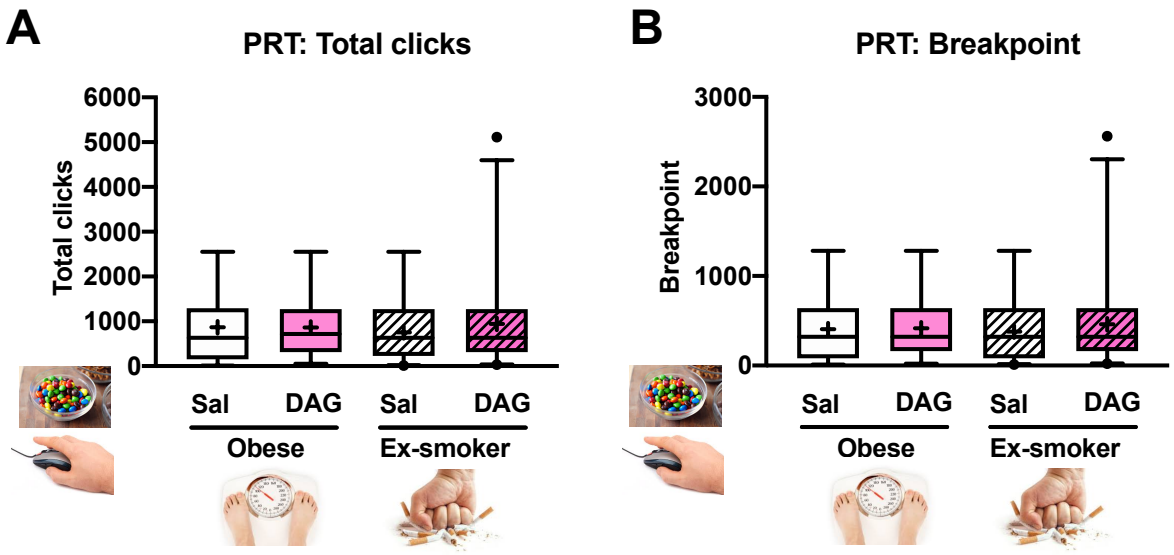


Figure 5.13. DAG had no effect of total clicks or breakpoint in dieting group with obesity or ex-smokers in progressive ratio task.

Comparison of [A] total number of clicks and [B] breakpoint (last completed click) to receive an M&M chocolate between saline (Sal) and DAG visits in the dieting group with obesity and ex-smokers. Data presented as boxplots showing median (line), mean (cross), interquartile range (box) and 5^{th} – 95^{th} percentile (bars), n=18-23. Data normalised by log transformation for [A-B] outcome measures in task for statistical analysis. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor and visit (saline, DAG) as within subject factors.

5.4.6 Approach Avoidance Task

Summary: DAG had no effect on the approach bias to food pictures in the AAT in either group (Figure 5.14).

Data was available for AAT in n=25 dieting participants with obesity and n=23 in ex-smokers. However, only participants achieving an average accuracy of 75% and over for each visit were included, giving a final n=22 dieting participants with obesity and n=19 ex-smokers.

Outcome measures of AAT

For the relative food bias (median pull reaction time of food pictures subtracted from median push reaction time of food pictures compared to that of neutral pictures), there were no significant interaction effects for: (i)visit*group [F(1,39)=0.94, P=0.34] nor overall effect of (ii)visit [F(1,38)=0.003, P=0.95], indicating there was a similar approach bias to food pictures in both saline and DAG visits. This remained non-significant after adjusting for difference in visit order (saline visit number subtracted from DAG visit number). Data was normalised using log transformation for statistical analysis.

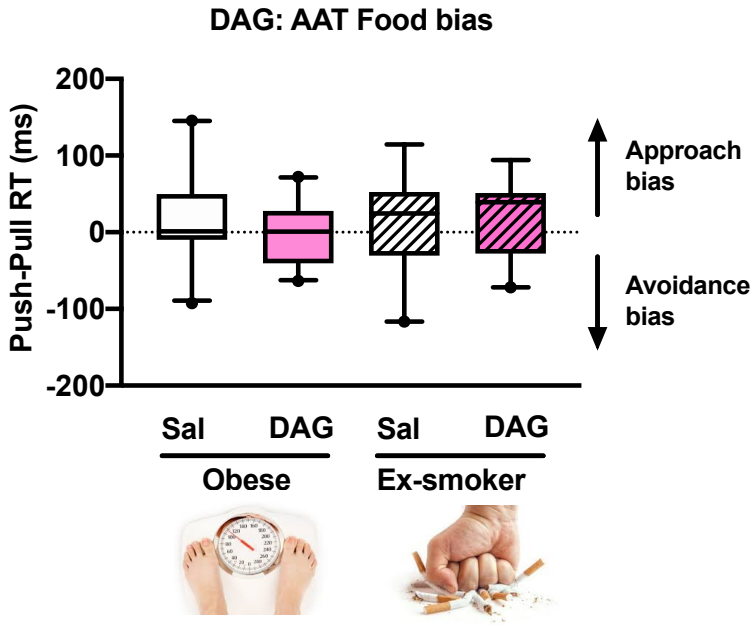


Figure 5.14. No effect of DAG on HE food approach bias in dieting group with obesity or ex-smokers.
Comparison of relative food bias (vs. neutral) [i.e. median pull reaction time subtracted from median push reaction time to food pictures vs. that of neutral pictures] during the task between saline and DAG visits in the dieting group with obesity and ex-smokers. Data presented as boxplots showing median (line), mean (cross), interquartile range (box) and 5^{th} – 95^{th} percentile (bars), n=19-22. Data normalised by log transformation for outcome measures in task for statistical analysis. Statistics from repeated measures ANOVA, with group (obese, ex-smoker) as between subject factor and Visit (DAG, saline) as within subject factors.

5.4.7. Visual analogue scale (VAS) ratings of appetite and confounding factors

Summary: DAG did not significantly affect appetite and food craving in both groups (Figure 5.15-5.16). Also, DAG reduced sleepiness rating independent of group but DAG did not increase nausea ratings in participants (Figure 5.17-5.20).

Data was available for n=25 in dieting group with obesity and n=24 in ex-smokers.

5.4.7.1. Composite appetite VAS rating

There was no significant interaction effect for: visit*time*group and visit*time, visit*group, nor an overall effect for visit for (Figure 5.15, Table 5.7):

(i) appetite VAS rating for whole visit

Neither was there a significant interaction effect for visit*group, nor an overall effect for visit for (Figure 5.15, Table 5.7):

(ii) AUC of appetite VAS rating from T=-10mins to T=-315mins

(iii) ΔAUC of appetite VAS rating from T=-10mins to T=-315mins

This indicates there was no effect of DAG on appetite VAS rating in both participant groups.

5.4.7.2. Composite food craving VAS rating

There was no significant interaction effect for: visit*time*group and visit*time, visit*group, nor an overall effect for visit for (Figure 5.16, Table 5.7):

(i) Food craving VAS rating for whole visit

Neither was there a significant interaction effect for visit*group, nor an overall effect for visit for (Figure 5.16, Table 5.7):

(ii) AUC of food craving VAS rating from T=-10mins to T=-315mins

(iii) ΔAUC of food craving VAS rating from T=-10mins to T=-315mins

However, for the ΔAUC of food craving VAS rating from T=-10mins to T=-315mins, there was a trend for a significant overall effect of visit [$F(1,47)=2.88$, $P=0.096$]. This was driven by a reduction in ΔAUC for food craving with DAG infusion [effect size mean ± SEM -171.96 ± 101.31, (95%CI -375.78, 31.86), $P=0.096$, Cohen's $d=0.24$] (Figure 5.16F). This suggests DAG tended to reduce food craving ratings independent of group.

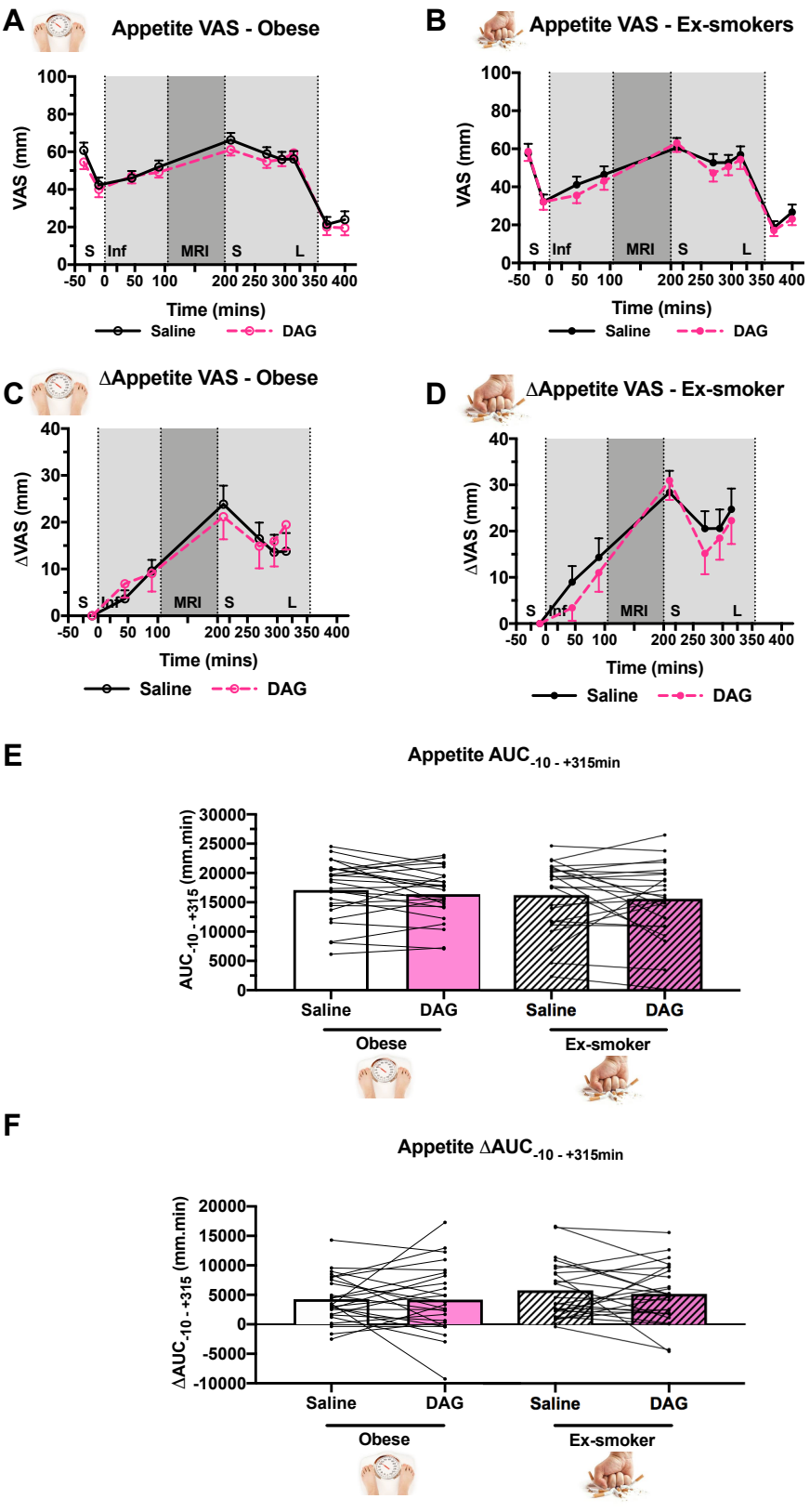


Figure 5.15. There was no effect of DAG on appetite visual analogue scale (VAS) ratings.
Comparison of composite appetite [A-B] VAS ratings (max 100mm), [C-D]delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and DAG visits in the [A,C,E,F] obese and [B,D,E,F] ex-smoker groups. Data presented as [A-D] mean ± SEM or [E,F] mean (bar), n=24-25. [A-D] VAS ratings plotted against time in [A,C] group with obesity and [B,D] ex-smoker during infusion of saline (black line) and DAG (pink dotted line). Light grey shaded area denotes time of infusion (T=0 to +355min), darker grey shaded area denotes period of fMRI scan (T=+105 to +205min). S indicates time of snacks and L indicates time of *ad libitum* test meal. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 5.7.

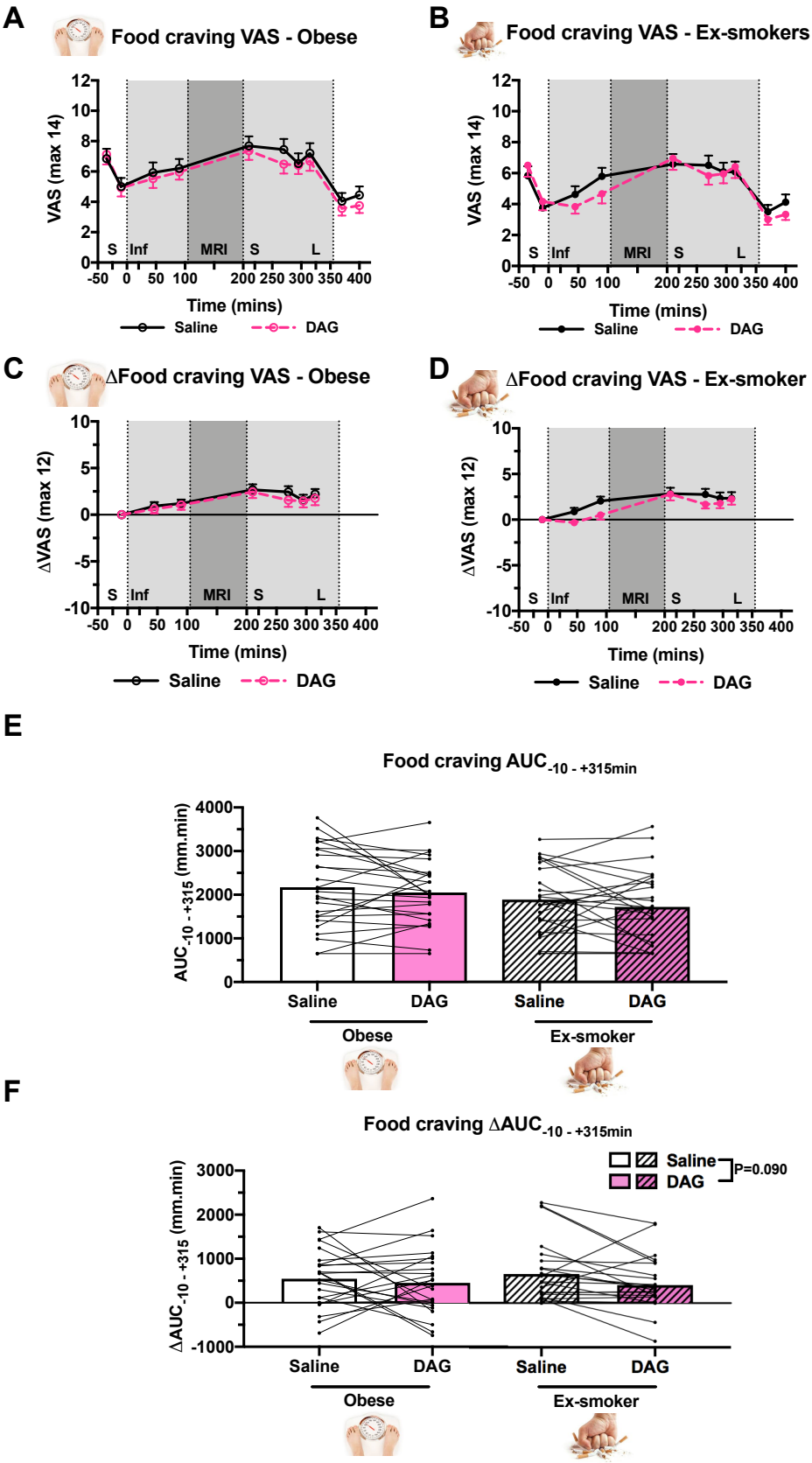


Figure 5.16. DAG tended to decrease food craving VAS ΔAUC (T=-10 to +315min) independent of group.
Comparison of composite food craving [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and DAG visits in the [A,B,E,F] obese and [C,D,E,F] ex-smoker groups. Data presented as [A-D] mean ± SEM or [E,F] mean (bar), n=24-25. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 5.7.

Interactions: Appetite VAS	***df***	***F***	***P***	***Interactions: Food craving VAS***	***df***	***F***	***P***
Visit	(1,47)	1.76	0.19	Visit	(1,47)	1.47	0.23
Visit*group	(1,47)	<0.001	0.99	Visit*group	(1,47)	0.08	0.78
Visit*time (GG)	(6.01, 282.41)	0.50	0.81	Visit*time (GG)	(6.58, 309.27)	1.55	0.15
Visit*time*group (GG)	(6.01, 282.41)	1.09	0.37	Visit*time*group (GG)	(6.58, 309.27)	0.56	0.78
Interactions: Appetite AUC -10 - +315				***Interactions: Food craving AUC -10 - +315***			
Visit	(1,47)	1.51	0.23	Visit	(1,47)	1.46	0.23
Visit*group	(1,47)	0.03	0.86	Visit*group	(1,47)	0.001	0.98
Interactions: Appetite ΔAUC -10 - +315				***Interactions: Food craving ΔAUC -10 - +315***			
Visit	(1,47)	0.22	0.64	Visit	(1,47)	2.88	0.096
Visit*group	(1,47)	0.13	0.72	Visit*group	(1,47)	0.60	0.44

Table 5.7. Repeated measures ANOVA results for effect of DAG on appetite and food craving VAS ratings.
Results from repeated measures ANOVA for appetite and food craving VAS ratings with group (obese, ex-smoker) as between subject factor and visit (DAG, saline), time (timepoints 1-10) as within subject factors. Results from repeated measures ANOVA for AUC (T=-10mins to T=+315mins) and ΔAUC (T=-10mins to T=+315mins) for appetite and food craving with group (obese, ex-smoker) as between subject factor and visit (DAG, saline) as within subject factors. Abbreviations: GG Greenhouse-Geisser correction.

Interactions: Nausea VAS	***df***	***F***	***P***	***Interactions: Anxious VAS***	***df***	***F***	***P***
Visit	(1,47)	0.32	0.58	Visit	(1,47)	0.05	0.83
Visit*group	(1,47)	0.27	0.61	Visit*group	(1,47)	0.10	0.76
Visit*time (GG)	(5.01, 235.56)	1.13	0.34	Visit*time (GG)	(4.10, 192.47)	0.70	0.60
Visit*time*group (GG)	(5.01, 235.56)	1.21	0.30	Visit*time*group (GG)	(4.10, 192.47)	1.56	0.19
[a] Interactions: Nausea AUC -10 - +315				***[a] Interactions: Anxious AUC -10 - +315***			
Visit	(1,47)	0.60	0.44	Visit	(1,47)	0.20	0.65
Visit*group	(1,47)	4.34	**0.043***	Visit*group	(1,47)	0.24	0.63
[a] Interactions: Nausea ΔAUC -10 - +315				***[a] Interactions: Anxious ΔAUC -10 - +315***			
Visit	(1,47)	0.47	0.50	Visit	(1,47)	0.97	0.33
Visit*group	(1,47)	1.65	0.21	Visit*group	(1,47)	1.34	0.25
Interactions: Stress VAS	***df***	***F***	***P***	***Interactions: Sleepy VAS***	***df***	***F***	***P***
Visit	(1,47)	0.04	0.85	Visit	(1,47)	6.99	**0.011***
Visit*group	(1,47)	0.46	0.50	Visit*group	(1,47)	0.39	0.54
Visit*time (GG)	(4.66, 218.89)	1.58	0.17	Visit*time (GG)	(6.10, 286.83)	1.51	0.18
Visit*time*group (GG)	(4.66, 218.89)	1.13	0.35	Visit*time*group (GG)	(6.10, 286.83)	1.02	0.41
[a] Interactions: Stress AUC -10 - +315				***[a] Interactions: Sleepy AUC -10 - +315***			
Visit	(1,47)	0.40	0.53	Visit	(1,47)	4.73	**0.035***
Visit*group	(1,47)	0.19	0.67	Visit*group	(1,47)	0.93	0.34
[a] Interactions: Stress ΔAUC -10 - +315				***[a] Interactions: Sleepy ΔAUC -10 - +315***			
Visit	(1,47)	0.81	0.37	Visit	(1,47)	1.37	0.25
Visit*group	(1,47)	1.32	0.26	Visit*group	(1,47)	0.91	0.34

Table 5.8. Repeated measures ANOVA results for effect of DAG on nausea, anxiety, stress and sleepiness VAS ratings.
Results from repeated measures ANOVA for nausea, anxiety, stress and sleepiness VAS ratings with group (obese, ex-smoker) as between subject factor and visit (DAG, saline), time (timepoints 1-10) as within subject factors. Results from repeated measures ANOVA for AUC (T=-10mins to T=+315mins) and ΔAUC (T=-10mins to T=+315mins) for nausea, anxiety, stress and sleepiness with group (obese, ex-smoker) as between subject factor and visit (DAG, saline) as within subject factors. Significant results are in bold. [a] All data used in repeated measures ANOVA was normalised to log10 values. Abbreviations: GG Greenhouse-Geisser correction.

5.4.7.3. Nausea VAS rating

For nausea VAS rating throughout the whole study visit, there were no significant interaction effects for: (i) visit*time*group, (ii) visit*time, (iii) visit*group, nor overall effect of (iv) visit (Figure 5.17A-B).

For AUC of nausea VAS rating from T=-10mins to T=-315mins, there was a significant interaction effect for visit*group [F(1,47)=4.34, P=0.043]. This was driven by a trend for DAG to reduce nausea AUC in ex-smokers [effect size mean ± SEM -0.39 ± 0.19, (95%CI -0.78, 0.002), P=0.051] and a trend for higher nausea AUC at saline visit in ex-smokers compared to dieting group with obesity [effect size mean ± SEM 0.52 ± 0.27, (95%CI -0.02, 1.06), P=0.059] (Figure 5.17E). This is unlikely to have caused an impact on the outcomes of the study visits as the absolute change is clinically small.

For the ΔAUC of nausea VAS rating from T=-10mins to T=-315mins, there were no significant interaction effects for visit*group nor overall effect of visit (Figure 5.17F).

5.4.7.4. Anxiety VAS rating

There was no significant interaction effect for: visit*time*group and visit*time, visit*group, nor an overall effect for visit for (Figure 5.18, Table 5.8):

(i) Anxiety VAS rating for whole visit

Neither was there a significant interaction effect for visit*group, nor an overall effect for visit for (Figure 5.18, Table 5.8):

(ii) AUC of anxiety VAS rating from T=-10mins to T=-315mins

(iii) ΔAUC of anxiety VAS rating from T=-10mins to T=-315mins

This indicates there was no difference in anxiety VAS rating between saline and DAG visits in both participant groups.

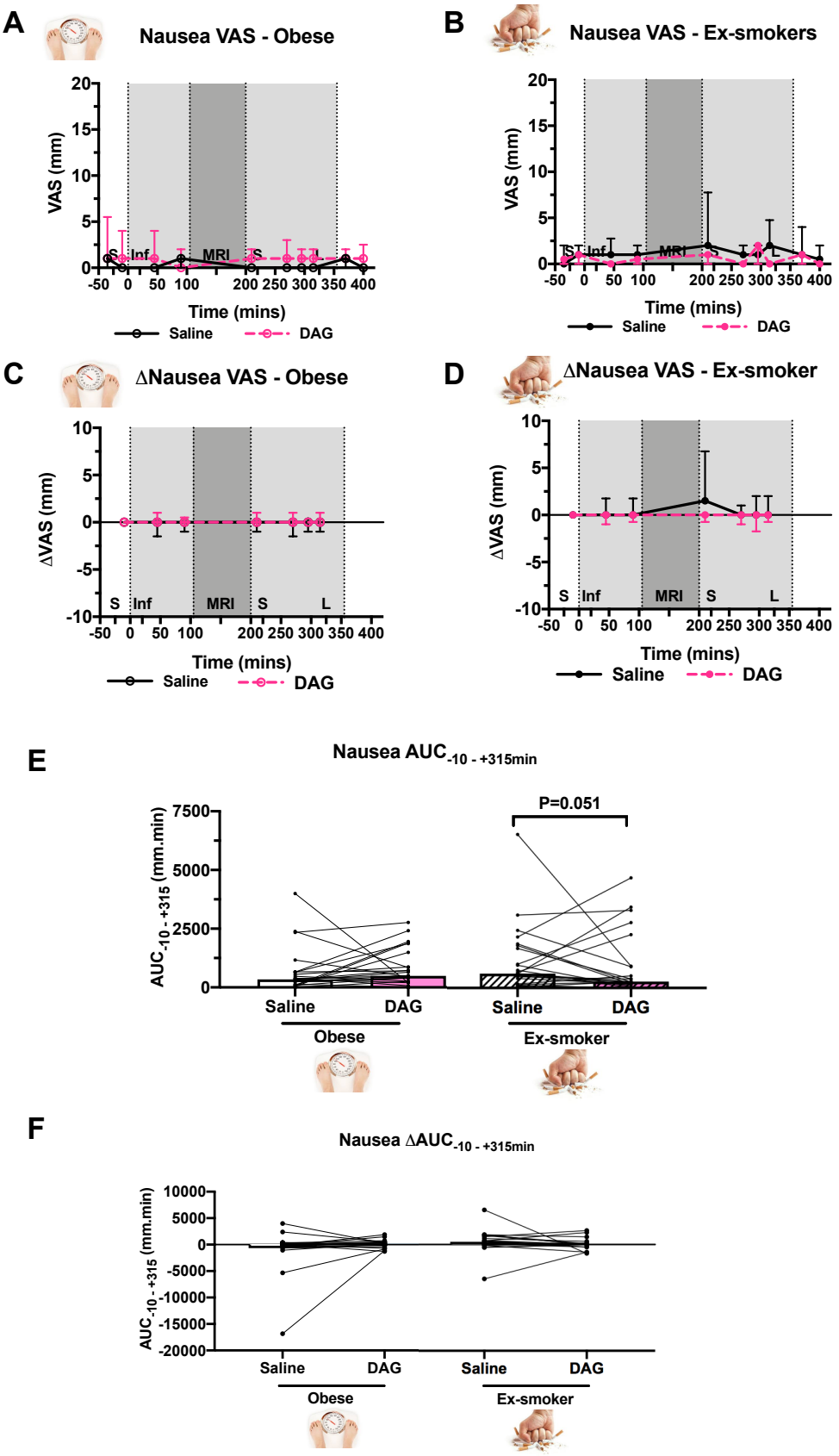


Figure 5.17. DAG tended to reduce nausea VAS AUC (T=-10 to +315min) in ex-smokers.
Comparison of nausea [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and DAG visits in the [A,C,E,F] obese and [B,D,E,F] ex-smokers. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=24-25. AUC data normalised by log transformation for statistical analysis. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 5.8.

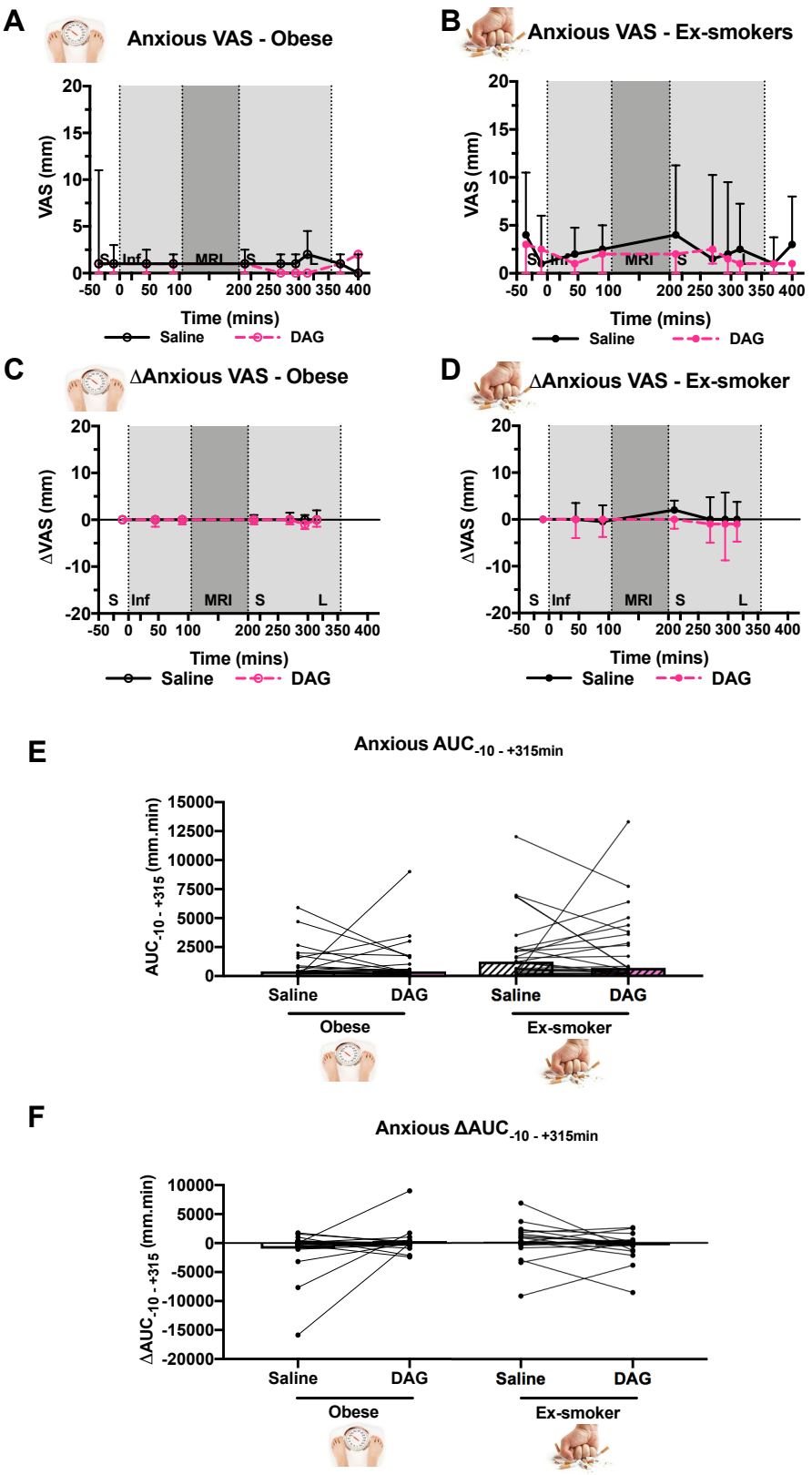


Figure 5.18. There was no effect of DAG on anxiety VAS ratings.
Comparison of anxiety [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and DAG visits in the [A,C,E,F] obese and [B,D,E,F] ex-smokers. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=23-24. AUC data normalised by log transformation for statistical analysis. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 5.8.

5.4.7.5. Stress VAS rating

There was no significant interaction effect for: visit*time*group and visit*time, visit*group, nor an overall effect for visit for (Figure 5.19, Table 5.8):

(i) Stress VAS rating for whole visit

Neither was there a significant interaction effect for visit*group, nor an overall effect for visit for (Figure 5.19, Table 5.8):

(ii) AUC of stress VAS rating from T=-10mins to T=-315mins

(iii) ΔAUC of stress VAS rating from T=-10mins to T=-315mins

This indicates there was no difference in stress VAS rating between saline and DAG visits in both participant groups.

5.4.7.6. Sleepy VAS rating

For sleepy VAS rating throughout the whole study visit, there were no significant interaction effects for: (i) visit*time*group, (ii) visit*time and (iii) visit*group. However, there was a significant overall effect of (iv) visit [F(1,47)=6.99, P=0.011]. This was driven by decrease in sleepy VAS ratings at the DAG visit independent of group [effect size mean ± SEM -7.01 ± 2.65, (95%CI -12.34, -1.68), P=0.011, Cohen's d=0.38] (Figure 5.20A-B, Table 5.8).

For AUC of sleepy VAS rating from T=-10mins to T=-315mins, there was no significant interaction effect for visit*group but an overall effect of visit [F(1,47)=4.73, P=0.035]. This was driven by a reduction in sleepy VAS AUC at the DAG visit independent of group [effect size mean ± SEM -0.19 ± 0.09, (95%CI -0.37, -0.01), P=0.035, Cohen's d=0.31] (Figure 5.20E). This indicates that DAG reduced sleepiness independent of group.

For the ΔAUC of sleepy VAS rating from T=-10mins to T=-315mins, there were no significant interaction effects for visit*group nor overall effect of visit (Figure 5.20F).

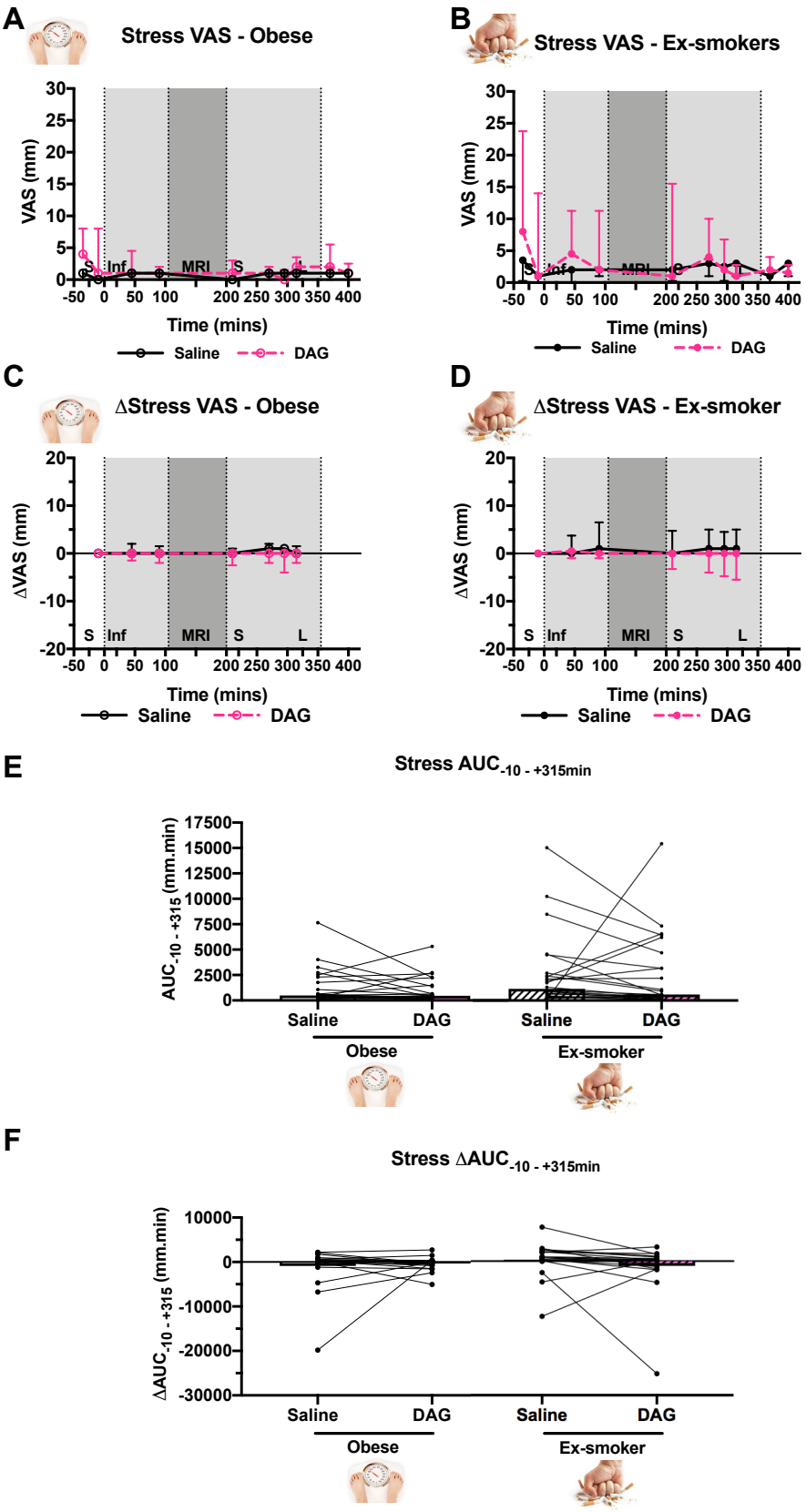


Figure 5.19. There was no effect of DAG on stress VAS ratings.

Comparison of stress [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and DAG visits in the [A,C,E,F] obese and [B,D,E,F] ex-smoker groups. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=23-24. AUC data normalised by log transformation for statistical analysis. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 5.8.

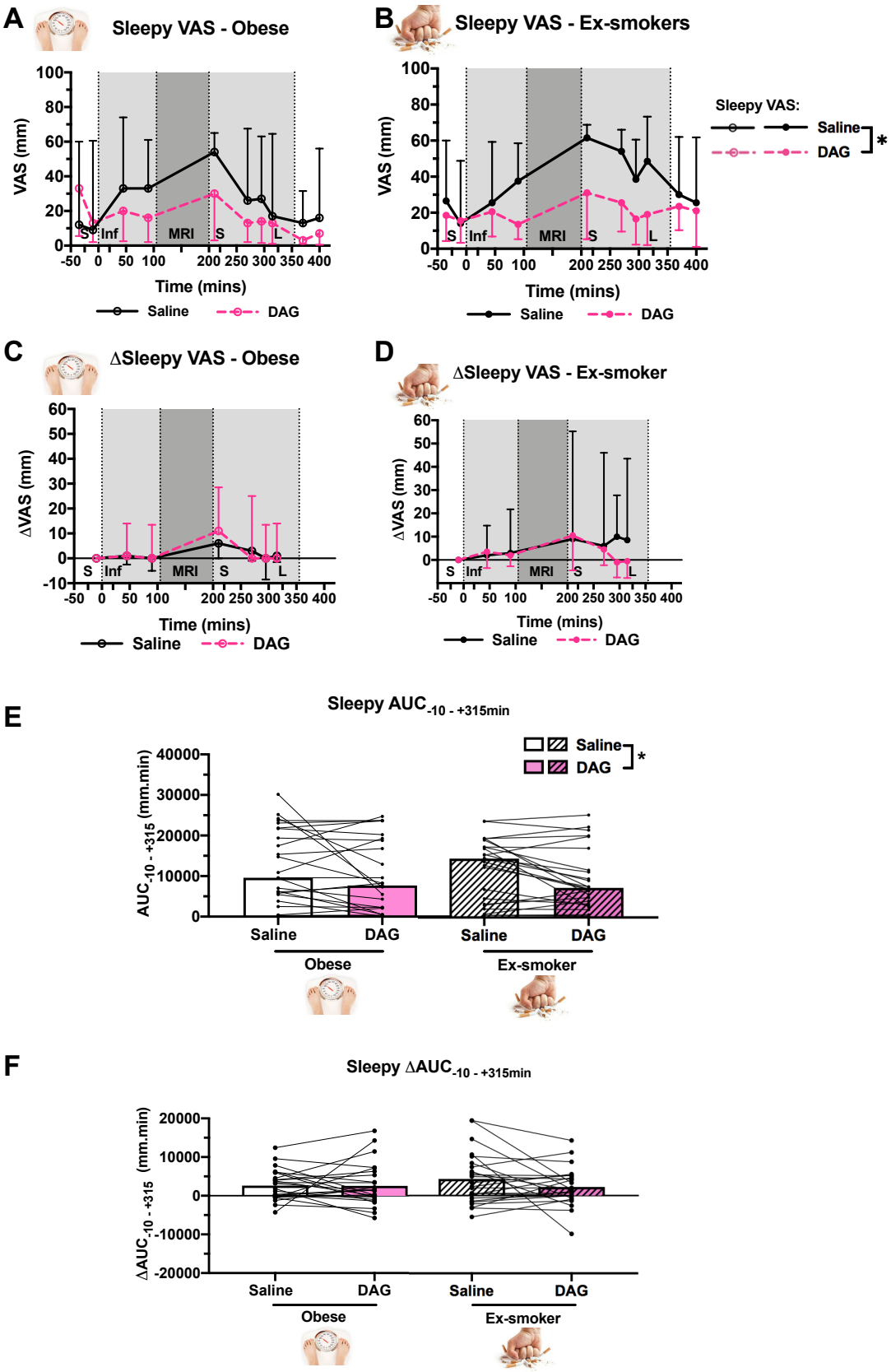


Figure 5.20. DAG reduced sleepiness VAS ratings in both groups.

Comparison of sleepiness [A-B] visual analogue scale (VAS) ratings, [C-D] delta VAS from baseline (T=-10min), [E] area under the curve (AUC) (T=-10mins to T=+315mins), [F] ΔAUC (T=-10mins to T=+315mins) from baseline, between saline and DAG visits in the [A,C,E,F] obese and [B,D,E,F] ex-smoker groups. Data presented as [A-D] median ± IQR or [E,F] mean (bar), n=23-24. AUC data normalised by log transformation for statistical analysis. Repeated measures ANOVA with post-hoc Fisher's LSD test: *P<0.05. For ANOVA results see Table 5.8.

5.4.8. Plasma Glucose, Hormones and Lipid Profile

Summary: DAG had no effect on plasma glucose, serum insulin, GH, cortisol, prolactin in both groups (Figure 5.21-5.25).

Results were combined into a single linear mixed model ANOVA analysis. This resulted in blood data across six timepoints being available for n=17-21 in dieting group with obesity and n= 21-24 in ex-smokers. Furthermore, blood AUC data was available for n=16-21 in dieting group with obesity and n=20-22 in ex-smokers.

5.4.8.1 Plasma Glucose

Summary: DAG did not affect plasma glucose concentrations in both groups.

There was no significant interaction effect for: visit*time*group and visit*time, visit*group, nor an overall effect for visit for (Figure 5.21, Table 5.9):

(i) Plasma glucose concentrations from T=-35mins to T=-315mins

(ii) Change in glucose from baseline (Δglucose from T=-35)

Neither was there a significant interaction effect for visit*group, nor an overall effect for visit for (Figure 5.21, Table 5.9):

(iii) AUC $_{-35\text{-}315}$ for plasma glucose from T=-35mins to T=-315mins

(iv) Δ AUC $_{-35\text{-}315}$ for plasma glucose from T=-35mins to T=-315mins

GLUCOSE	*Visit*		*Group*		*Group*visit*		*Visit*time*		*Group*visit*time*	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
glucose	(1,475.97)=0.31	0.58	(1,44.31)=1.25	0.27	(1,475.97)=0.50	0.48	(5,464.32)=0.14	0.98	(5,464.32)=0.37	0.87
Δglucose	(1,393.83)=0.15	0.70	(1,40.95)=1.78	0.19	(1,393.83)=0.03	0.86	(4,374.33)=0.12	0.98	(4,374.33)=0.65	0.63
glucose AUC	(1,37.48)=0.96	0.34	(1,44.47)=1.46	0.23	(1,37.48)=0.02	0.89				
glucose ΔAUC	(1,42.50)=0.17	0.68	(1,45.16)=2.59	0.12	(1,42.50)=0.35	0.56				
INSULIN										
insulin	(1,473.00)=1.79	0.18	(1,45.47)=7.64	**0.008**	(1,473.00)=3.96	**0.047**	(5,456.70)=0.34	0.89	(5,456.70)=0.40	0.85
Δinsulin	(1,396.39)=1.30	0.26	(1,44.38)=3.07	0.26	(2,396.39)=0.11	0.74	(4,373.35)=0.28	0.89	(4,373.35)=0.42	0.80
insulin AUC	(1,38.01)=0.60	0.44	(1,44.44)=10.43	**0.002**	(1,38.01)=2.56	0.12				
insulin ΔAUC	(1,80.00)=0.29	0.59	(1,80.00)=2.04	0.16	(1,80.00)=0.05	0.83				
GROWTH HORMONE										
growth hormone	(1,466.70)=0.19	0.67	(1,45.75)=5.70	**0.021**	(1,466.70)=0.05	0.83	(5,445.92)=0.09	>0.99	(5,445.92)=0.10	>0.99
Δgrowth hormone	(1,376.17)=0.03	0.87	(1,45.00)=0.26	0.61	(1,376.17)=0.008	0.93	(4,362.08)=0.13	0.97	(4,362.08)=0.09	0.99
growth hormone AUC	(1,36.85)=0.21	0.65	(1,41.64)=4.83	**0.034**	(1,36.85)=0.08	0.78				
growth hormone ΔAUC	(1,39.65)=0.17	0.68	(1,41.89)=0.17	0.69	(1,39.65)=0.06	0.81				
CORTISOL										
cortisol	(1,476.26)=0.51	0.48	(1,44.74)=0.71	0.40	(1,476.26)=0.48	0.49	(5,453.82)=0.43	0.83	(5,452.82)=0.47	0.80
Δcortisol	(1,379.42)=0.001	0.97	(1,42.92)=1.72	0.20	(1,379.42)=0.38	0.54	(4,366.85)=0.23	0.92	(4,366.85)=0.31	0.87
cortisol AUC	(1,40.12)=0.85	0.36	(1,44.66)=3.75	0.059	(1,40.12)=0.49	0.49				
cortisol ΔAUC	(1,40.10)=0.41	0.53	(1,43.28)=0.65	0.43	(1,40.10)=0.40	0.53				
PROLACTIN										
prolactin	(1,463.80)=13.57	**<0.001**	(1,45.24)=0.55	0.46	(1,463.80)=12.00	**0.001**	(5,456.23)=0.32	0.90	(5,456.23)=0.39	0.86
Δprolactin	(1,44.87)=0.83	0.37	(1,375.90)=1.78	0.18	(1,375.90)=1.97	0.16	(4,370.92)=0.44	0.78	(4,370.92)=0.36	0.84
prolactin AUC	(1,39.49)=3.14	0.084	(1,44.93)=0.21	0.65	(1,39.49)=3.26	0.079				
prolactin ΔAUC	(1,37.86)=0.71	0.40	(1,44.17)=1.47	0.23	(1,37.86)=1.03	0.32				

Table 5.9. Mixed model ANOVA results for effect of DAG on plasma glucose and hormones.

Results from fixed effects mixed model ANOVA for plasma glucose and serum insulin with group as between subject factor (obese, ex-smoker) and visit (saline, DAG) and time (T=-35, +45, +90, +210, +270, +315) as within subject factors. Significant results are in bold.

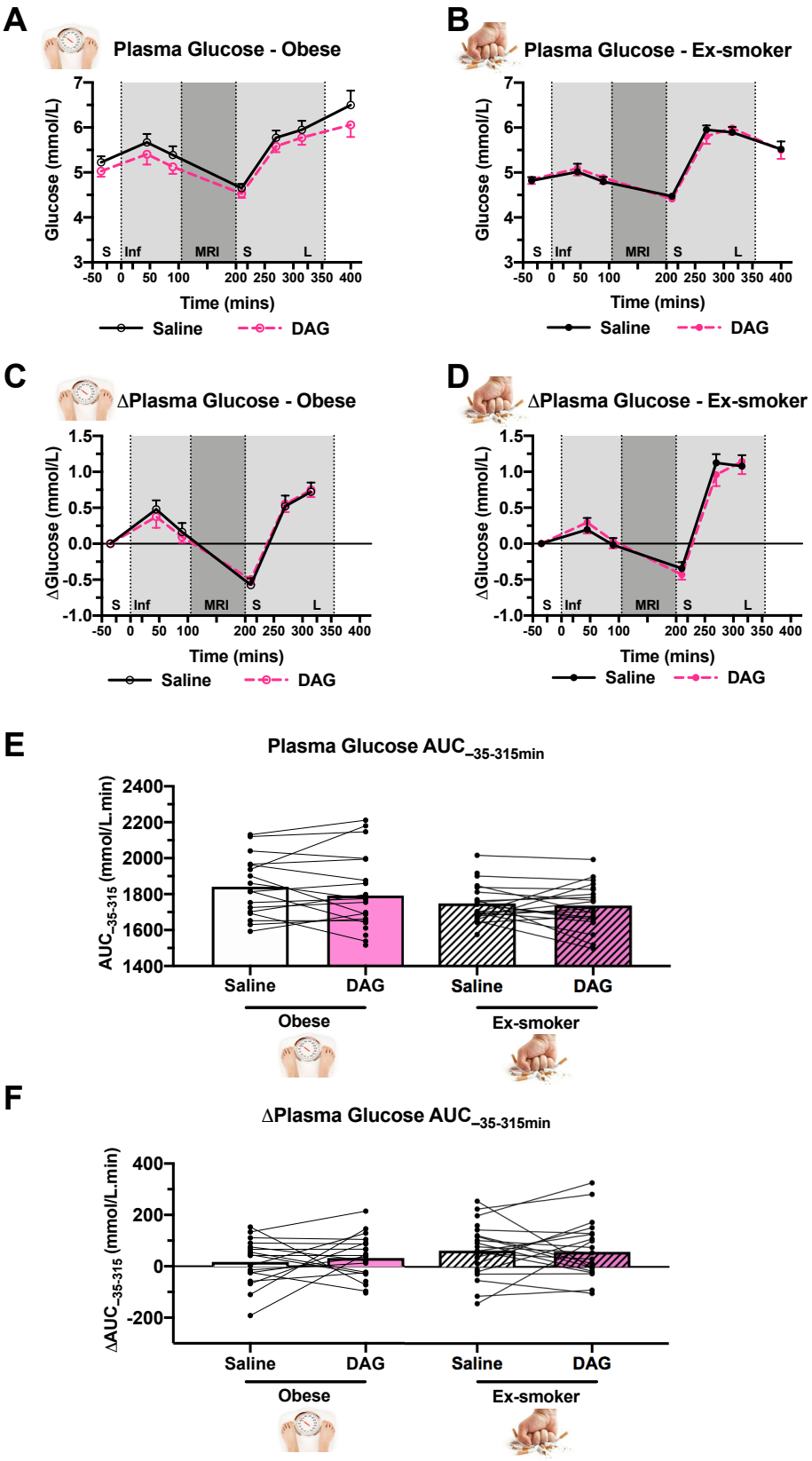


Figure 5.21. DAG did not affect plasma glucose concentrations in both groups.

(A, C) Glucose concentrations (mmol/L) across seven timepoints, (B,D) change in glucose from baseline, (E) AUC glucose (T=-35 to 315min) and (F) Δ AUC glucose (T=-35 to 315min) for (A,B) obese or (C,D) ex-smokers or (E,F) both groups at the saline and DAG visits. Data presented as (A-D) mean ± SEM, n=18-22 or (E,F) before-after with bar graph (mean), n=17-21. Fixed effects mixed model ANOVA with post-hoc LSD test. For ANOVA results see Table 5.9.

5.4.8.2 Serum insulin

Summary: There was no effect of DAG on insulin concentrations but there were higher insulin concentrations at DAG visit in dieting group with obesity than ex-smokers (Figure 5.22).

For serum insulin, there were no significant interaction effects for (i) visit*time*group, (ii) visit*time nor overall effect of (iii) visit. However, there was a significant interaction effect for visit*group [F(1, 473.00)=3.96, P=0.047]. This was driven by an increase in insulin at the DAG visit in the dieting group with obesity [effect size mean ± SEM 3.68 ± 1.62, (95%CI 0.50, 6.87), P=0.024, Cohen's d=0.72] and a higher concentration of insulin in the dieting group with obesity compared to the ex-smokers at the DAG visit [effect size mean ± SEM 9.24 ± 2.76, (95%CI 3.73, 14.75), P=0.001], but not significantly at saline visit [effect size ± SEM 4.84 ± 2.80, (95%CI -0.75, 10.42), P=0.089] (Figure 5.22, Table 5.9).

However, for change in insulin from baseline (Δinsulin from T=-35), there were no significant interaction effects for (i) visit*time*group, (ii) visit*group, (iii) visit*time nor overall effect of (iv) visit. This suggest that DAG did not affect insulin concentrations in both groups.

There were no significant interaction effects for visit*group nor overall effect of visit for (Figure 5.22):

(i) $AUC_{-35-315}$ between T= -35 and T=315min for insulin

(ii) $\Delta AUC_{-35-315}$ between T= -35 and T=315min from baseline for insulin

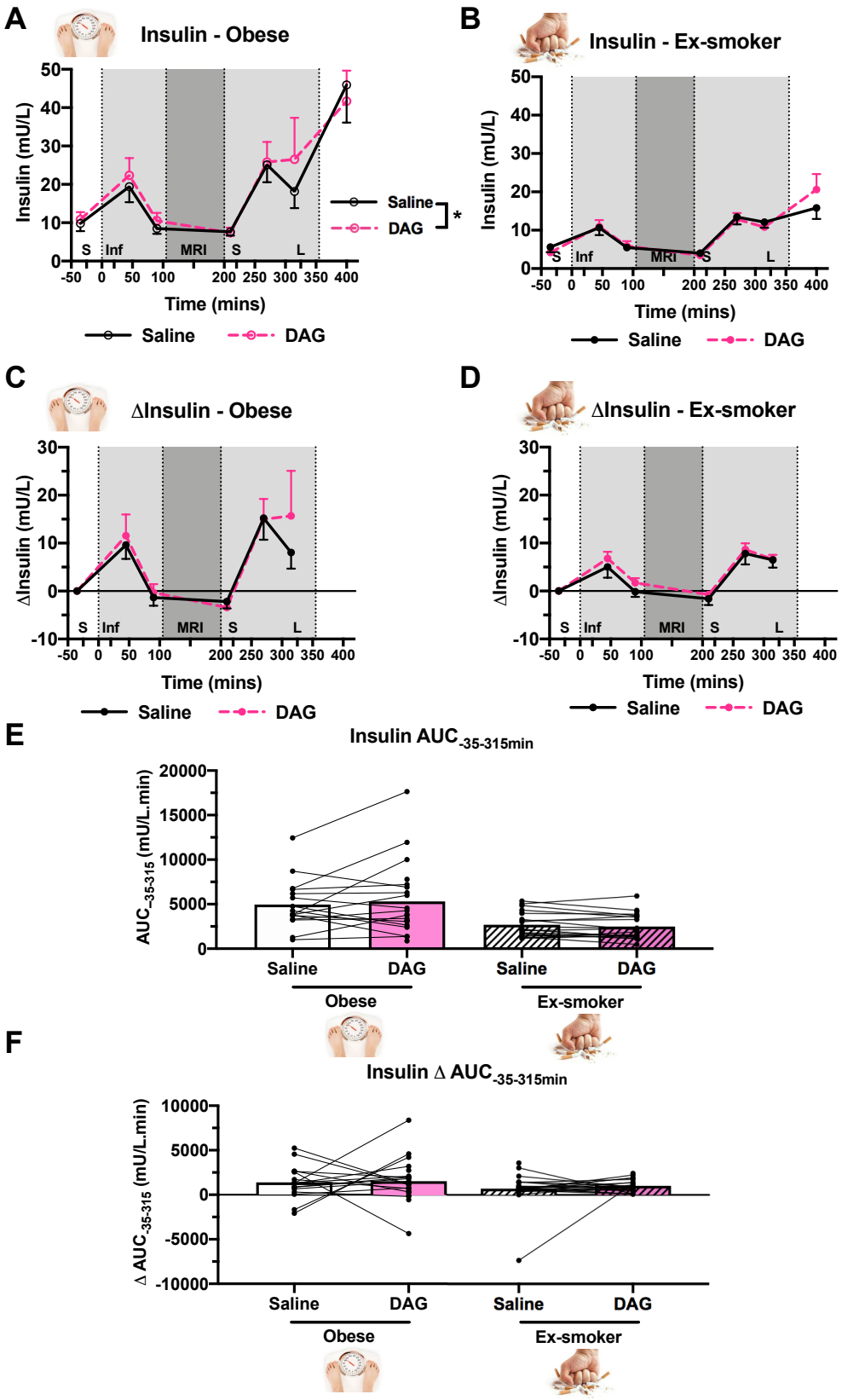


Figure 5.22. DAG did not affect serum insulin concentrations in both groups.

(A, C) Insulin concentrations (mU/L) across seven timepoints, (B,D) change in insulin from baseline, (E) AUC insulin (T=-35 to 315min) and (F) Δ AUC insulin (T=-35 to 315min) for (A,B) obese or (C,D) ex-smokers or (E,F) both groups at the saline and DAG visits. Data presented as (A-D) mean ± SEM, n=19-22 or (E,F) before-after with bar graph (mean), n=16-21. Fixed effects mixed model ANOVA with post-hoc LSD test. For ANOVA results see Table 5.9.

5.4.8.3 Growth hormone (GH)

Summary: DAG did not affect GH concentrations in both groups (Figure 5.23; Table 5.9).

There was no significant interaction effect for: visit*time*group and visit*time, visit*group, nor an overall effect for visit for (Figure 5.23, Table 5.9):

(i) GH concentrations from T= -35mins to T=-315mins

(ii) Change in GH from baseline (ΔGH from T= -35min)

Neither was there a significant interaction effect for visit*group, nor an overall effect for visit for (Figure 5.23, Table 5.9):

(iii) AUC $_{-35\text{-}315}$ for GH from T=-35mins to T=-315mins

(iv) Δ AUC $_{-35\text{-}315}$ for GH from T=-35mins to T=-315mins

5.4.8.4 Cortisol

Summary: DAG did not affect cortisol concentrations in both groups (Figure 5.24; Table 5.9).

There was no significant interaction effect for: visit*time*group and visit*time, visit*group, nor an overall effect for visit for (Figure 5.24, Table 5.9):

(i) cortisol concentrations from T= -35mins to T=-315mins

(ii) Change in cortisol from baseline (ΔGH from T= -35min)

Neither was there a significant interaction effect for visit*group, nor an overall effect for visit for (Figure 5.24, Table 5.9):

(iii) AUC $_{-35\text{-}315}$ for cortisol from T=-35mins to T=-315mins

(iv) Δ AUC $_{-35\text{-}315}$ for cortisol from T=-35mins to T=-315mins

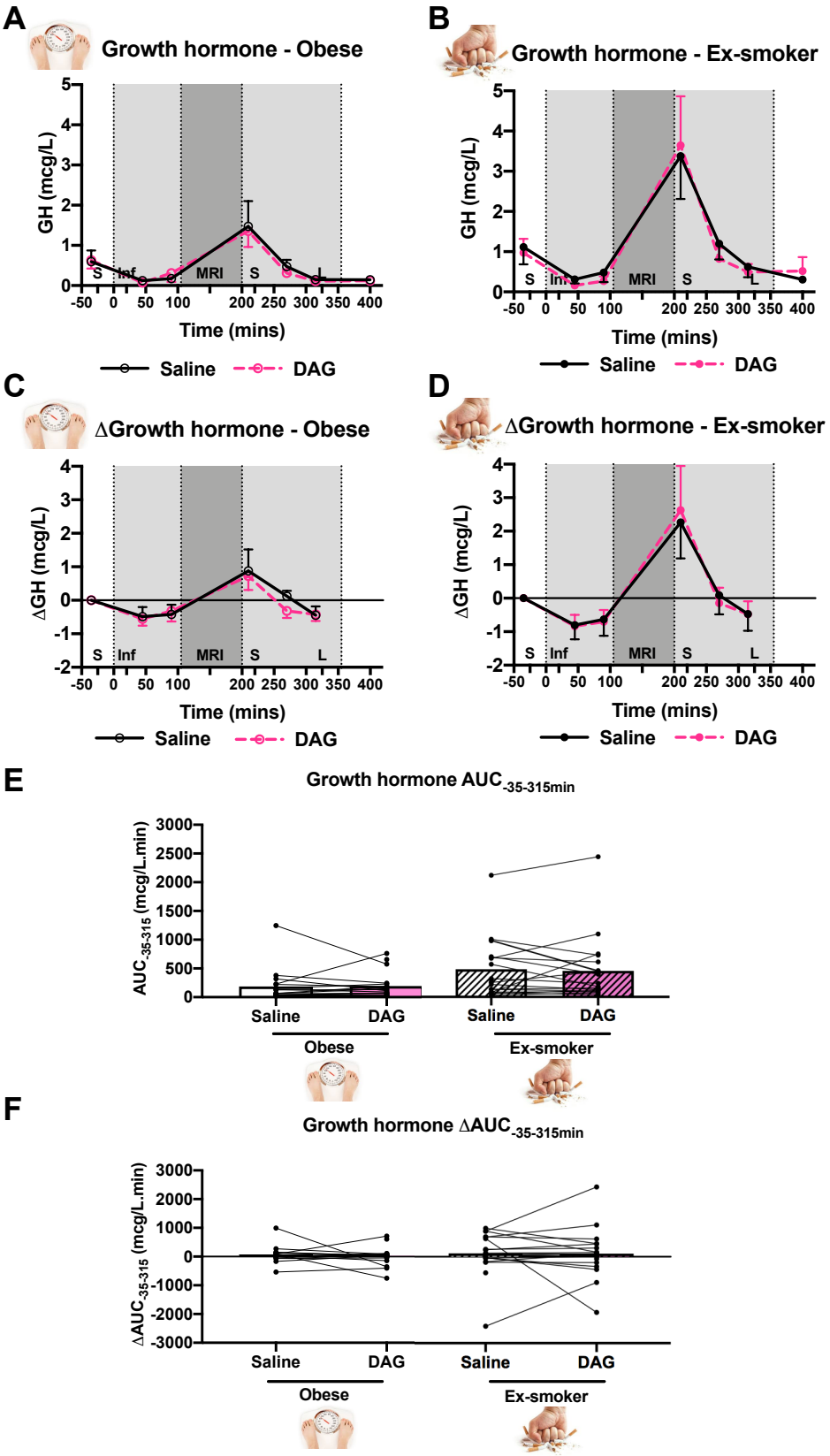


Figure 5.23. DAG did not affect GH concentrations in both groups.

(A, C) GH concentrations (mcg/L) across seven timepoints, (B,D) change in GH from baseline, (E) AUC GH (T=-35 to 315min) and (F) Δ AUC GH (T=-35 to 315min) for (A,B) obese or (C,D) ex-smokers or (E,F) both groups at the saline and DAG visits. Data presented as (A-D) mean ± SEM, n=19-22 or (E,F) before-after with bar graph (mean), n=17-20. Fixed effects mixed model ANOVA with post-hoc LSD test. For ANOVA results see Table 5.9.

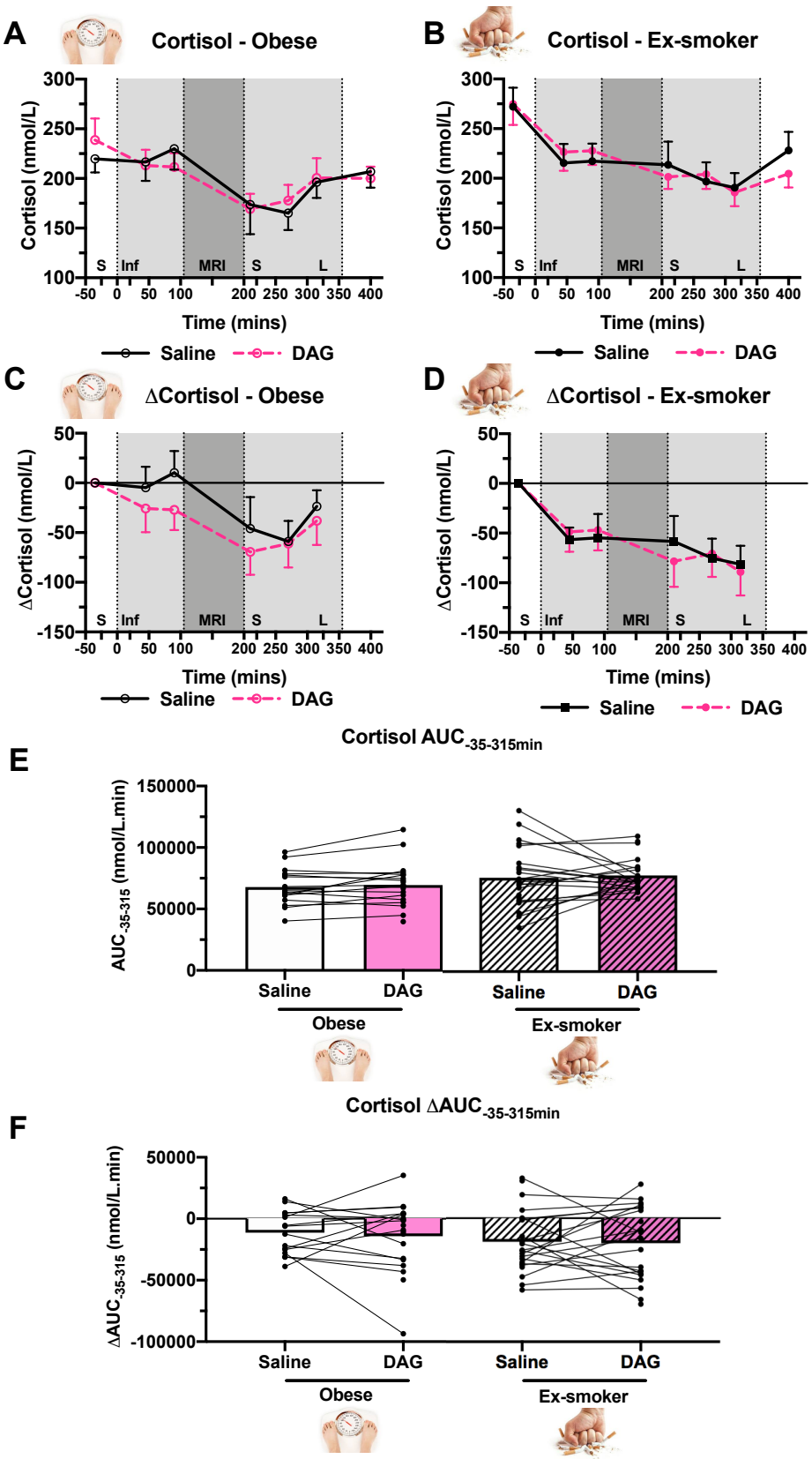


Figure 5.24. DAG did not affect cortisol concentrations in both groups.
(A, C) Cortisol concentrations (nmol/L) across seven timepoints, (B,D) change in cortisol from baseline, (E) AUC cortisol (T=-35 to 315min) and (F) Δ AUC cortisol (T=-35 to 315min) for (A,B) obese or (C,D) ex-smokers or (E,F) both groups at the saline and DAG visits. Data presented as (A-D) mean ± SEM, n=18-22 or (E,F) before-after with bar graph (mean), n=17-22. Fixed effects mixed model ANOVA with post-hoc LSD test. For ANOVA results see Table 5.9.

5.4.8.5 Prolactin

Summary: There was no effect of DAG on prolactin concentrations in both groups (Figure 5.25; Table 5.9).

For prolactin concentrations, there were no significant interaction effects for (i) visit*time*group, and (ii) visit*time. However, there was a significant effect for group*visit [F(1,463.80)=12.00, P=0.001]. This was driven by lower prolactin concentrations at the DAG visit compared to saline in ex-smokers [effect size mean ± SEM -36.33 ± 6.73, (95%CI -49.56, -23.10), P<0.001, Cohen's d=0.58], and not in dieting group with obesity [effect size mean ± SEM -1.12 ± 7.62, (95% -16.09, 13.85), P=0.88].

However, for change in prolactin from baseline (Δprolactin from T=-35min), there were no significant interaction effects for (i) visit*time*group, (ii) visit*group, (iii) visit*time nor overall effect of (iv) visit. This suggests that DAG did not affect the change in prolactin concentrations from baseline.

For area under the curve between T=-35 and T=315 ($AUC_{-35-315}$) for prolactin, there were no significant interaction effects for (i) visit*group nor overall effect of (ii) visit.

For the change in area under the curve between T=-35 and T=315 ($\Delta AUC_{-35-315}$) from baseline for prolactin, there were no significant interaction effects for (i) visit*group nor overall effect of (ii) visit.

In spite of the significant reduction in prolactin levels seen at the DAG visit in ex-smoker group, the non-significant interaction effects in Δprolactin and $\Delta AUC_{-35-315}$ prolactin levels collectively suggest that this was likely because prolactin levels started off lower at the DAG visit and not attributed to an effect of DAG.

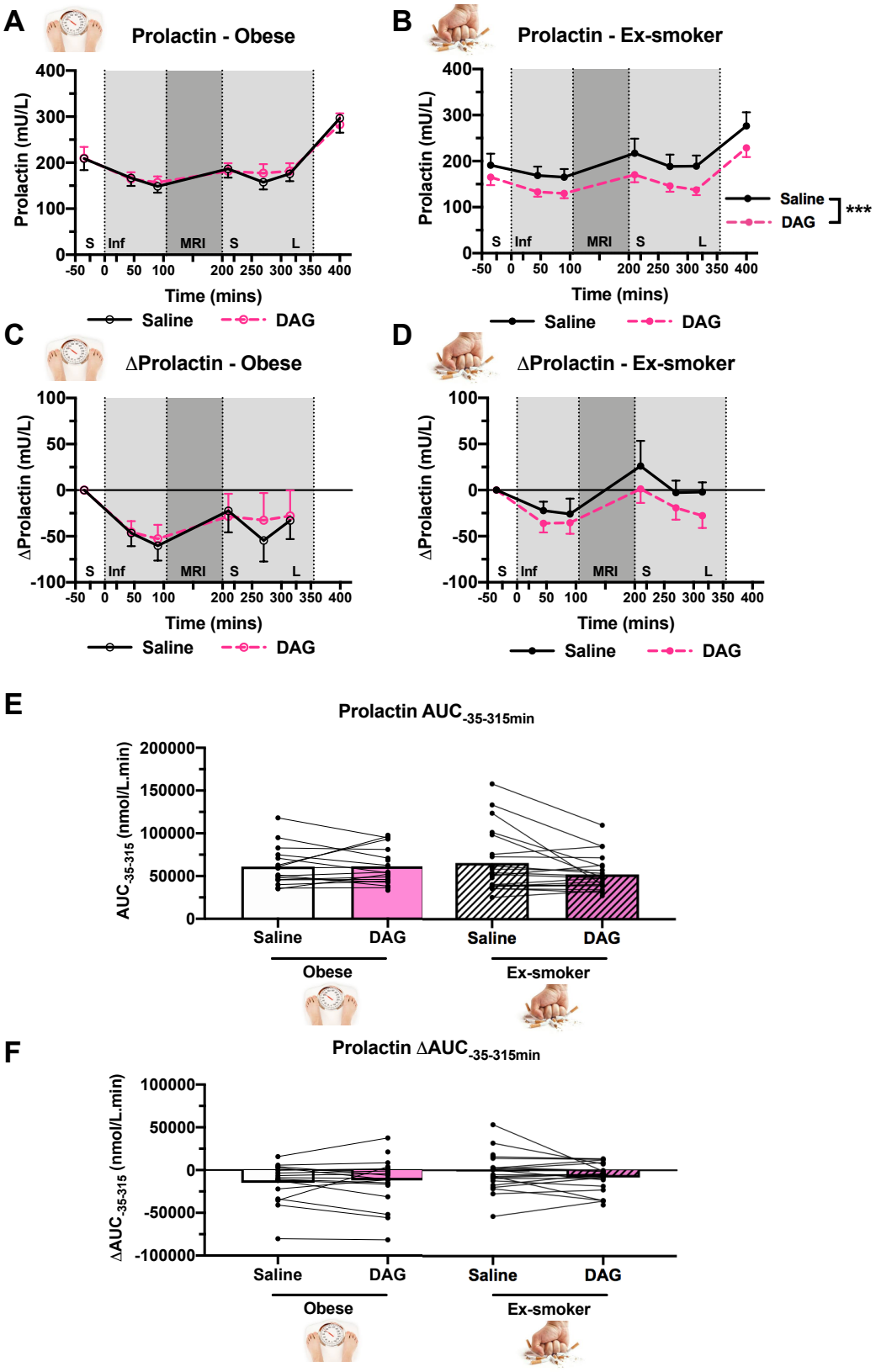


Figure 5.25. DAG did not affect prolactin concentrations in both groups.

(A, C) Prolactin concentrations (nmol/L) across seven timepoints, (B,D) change in prolactin from baseline, (E) AUC prolactin (T=-35 to 315min) and (F) Δ AUC prolactin (T=-35 to 315min) for (A,B) obese or (C,D) ex-smokers or (E,F) both groups at the saline and DAG visits. Data presented as (A-D) mean ± SEM, n=18-22 or (E,F) before-after with bar graph (mean), n=16-22. Fixed effects mixed model ANOVA with post-hoc LSD test. For ANOVA results see Table 5.9

5.5 DISCUSSION

To the best of my knowledge, this is the first human fMRI study reporting effects of DAG infusion on eating behaviour. The main findings from analysis of the HE food evaluation fMRI task are that DAG differentially modulated HE food cue reactivity in dieting adults with obesity and ex-smokers. In dieting group with obesity, there was a general pattern of enhanced BOLD signal to HE food pictures, in particular in the PFC, with DAG. On the contrary, in ex-smokers, there was a reduction in BOLD signal to food pictures with DAG in the PFC, thalamus, amygdala and striatum (See table 5.10 for summary).

Measure	Obese	Ex-smoker
Saline		
fMRI: average fROI	Ex-smoker > obese	
DAG vs. saline		
fMRI: WBA	↑ SFG/FP/MFG/	↓ DS/Amyg & ↓ SFG/FP/PCG
fMRI: average fROI	(↑)	(↓)
Food appeal	→	→
Taste VAS		
Liking	→	→
Pleasantness	→	→
Creaminess	→	→
Ideal creaminess	↑ SavHF & ↓SavLF	↑ SavHF & ↓SavLF
Sweetness	(↓SwHF)	(↓SwHF)
Ideal sweetness	→	→
Food intake		
Total	→	→
Savoury	→	→
Sweet	→	→
High-fat	→	→
Low-fat	(↓)	(↓)
PRT	→	→
AAT	→	→
VAS rating		
Appetite	→	→
Food craving	(↓)	(↓)
Confounding VAS		
Nausea	→	(↓)
Anxiety	→	→
Stress	→	→
Sleepiness	↓	↓

Table 5.10 Summary table of effects of DAG on food-related fMRI and behavioural outcome measures

Abbreviations: AAT, approach avoidance task; ACC, anterior cingulate cortex; Amyg, amygdala; DAG, desacyl ghrelin; DS, dorsal striatum (caudate/putamen); FP, frontal pole; fROI, functional region of interest (including nucleus accumbens, caudate, putamen, orbitofrontal cortex, amygdala, hippocampus, anterior insula, ventral anterior cingulate cortex, dorsolateral prefrontal cortex); MFG, middle frontal gyrus; OFC, orbitofrontal cortex; PCG, paracingulate gyrus; PRT, progressive ratio task; SavHF, savoury high-fat (cream soup); SavLF, savoury low-fat (broth); SFG, superior frontal gyrus; SwHF; sweet high-fat (ice-cream); VAS, visual analogue scale; WBA, whole brain analysis. Trends are denoted in parentheses.

In addition, DAG tended to reduce energy intake from low-fat foods (broth and yogurt) and increased ideal creaminess rating for cream soup and decreased that for broth, independent of group. Despite an effect of DAG on the anticipatory BOLD signal to HE food pictures in both groups, DAG did not change approach bias to food cues in AAT nor motivation for a chocolate sweet (M&M) in PRT. DAG tended to reduce food craving independent of group, whereas it had no effect on appetite VAS ratings. On the other hand, DAG had no effect on plasma glucose, serum insulin, cortisol, GH and prolactin.

Hypothesis: DAG will attenuate the appeal of and BOLD response in mesolimbic regions to HE food pictures in both groups.

Result: There was no effect of DAG on HE food picture appeal in both groups.

This finding is not in accordance with the hypothesis. In spite of BOLD signal changes in this fMRI task, there was no effect of DAG on appeal ratings. In this current study with Exenatide (Chapter 4), changes in food cue reactivity was accompanied by reduction in food appeal ratings. In the absence of a change in HE food appeal, this suggests that neuronal measures of eating behaviour may be more sensitive to effects of DAG than this behavioural measure. Another possible explanation is that the effect of DAG on food appeal may be smaller than that of Exenatide, therefore with a relatively small number of participants, no significant difference was observed.

Dieting group with obesity

Result: In dieting group with obesity, DAG increased BOLD signal to HE food pictures in the PFC and tended to do so across all fROI.

Contrary to the hypothesis, in the whole brain analysis for the dieting group with obesity, DAG increased BOLD signal to HE food pictures in many brain regions in the PFC, namely the frontal pole, OFC, MFG, SFG, paracingulate gyrus and ACC, when compared to saline. This was consistent in the fROI analysis for dieting group with obesity in which DAG tended to enhance BOLD signal to HE food pictures across all fROI although exploratory post-hoc analysis showed the increase to be significant only in the putamen and vACC.

The interpretation of DAG response on food cue reactivity in the dieting group with obesity depends on the role of these PFC brain regions. The prefrontal cortex is large heterogenous region and there are many variations in anatomical and functional subdivisions of prefrontal area in different studies. As discussed in Chapter 4, the SFG and frontal pole lies within the PFC, which is involved in executive

control, value-encoding towards reward stimulus and attention. However, the frontal clusters in dieting group with obesity that demonstrated an increased BOLD signal to HE foods with DAG, covered a large region and therefore it is unclear as to the implications on functional changes. DAG-induced BOLD signal changes in the frontal clusters did not have a correlation with dietary restraint scores nor other eating behavioural traits.

One possible explanation is the increase in food cue reactivity in the PFC regions in dieting group with obesity could represent an enhanced inhibition of a habitual response to the HE food picture cues. There was an increased BOLD activation in the ACC, paracingulate gyrus, lateral OFC, insula and IFG when inhibiting a habitual motor response to a stimulus in lean participants [490]. Supporting this, enhancement of BOLD signal to food cues in related PFC regions, including the lateral PFC and superior/medial PFC, was associated with an improved response inhibition towards food cues in patients after bariatric surgery and in binge-eating disorders [491, 492]. This would be in accordance to the discussion in Chapter 4, where the increase in BOLD signal to HE foods in PFC could indicate heightened inhibitory control towards HE foods.

On the other hand, another possible interpretation is that the increase in BOLD signal to HE foods with DAG in the frontal clusters in dieting group with obesity represents enhanced cognitive evaluation of HE food pictures and a concomitant increase in saliency of the HE food pictures. The OFC and paracingulate gyrus appear to have a role in value-encoding of reward stimulus and attention towards salient cues [35, 493]. In obesity, the increased food cue reactivity in dorsomedial PFC, parahippocampal gyrus, precentral gyrus, ACC and SFG/IFG and decreased food cue reactivity in the dlPFC and insula, was interpreted as a weakened cognitive control over appetite and pay greater attention to motivation reward of food than lean people [55]. With AG administration, an increase in BOLD signal to HE food pictures in OFC, amygdala, insula and striatum in non-obese volunteers, along with higher food appeal, indicated a stimulatory effect of AG on food hedonics [176, 382]. It could be plausible that some of the DAG-induced effects on anticipatory neural food response was a consequence of the activation of GHSR1a ghrelin system by supraphysiological plasma concentrations of DAG, or local acylation of DAG to AG by GOAT. Supporting this, in rodent studies, there is evidence of local acylation of AG by GOAT in the hippocampus [494].

Unexpectedly in the fROI analyses, there was also a trend for DAG to increase the BOLD signal in non-frontal areas involved in reward processing in the dieting group with obesity, including NAcc, caudate, putamen, insula, hippocampus and amygdala. Some of these reward processing brain regions coincide with those known to have an effect of AG [176, 382]. The increase in food cue reactivity could be a consequence of the activation of GHSR1a ghrelin system as discussed above. Interestingly, this increase in food cue reactivity in the reward processing regions with DAG is similar to that observed with Exenatide. This could also be linked to the lower BOLD signal to HE foods in obesity at saline visit, thus giving rise to a floor effect (Further discussion in section 6.1).

Ex-smokers

Result: In ex-smokers, DAG reduced BOLD signal in caudate, putamen, amygdala, thalamus, SFG, frontal pole and paracingulate gyrus.

In line with the hypothesis, DAG attenuated the BOLD signal to food cues in brain areas, namely caudate, putamen, thalamus and amygdala that are responsible for reward saliency and reward-related emotions. Consistent with this, in the fROI analysis for ex-smoker group, DAG tended to attenuate BOLD signal to HE food pictures across most of the fROI and exploratory post-hoc analysis showed the decrease to be significant in the caudate, putamen, vACC and dlPFC. The caudate and putamen are responsible for anticipation of rewards and motivation in rewarding behaviour. The thalamus is responsible for relaying sensory and motor signals to the cerebral cortex. Moreover, the amygdala performs a crucial role in processing emotions, anxiety and memory. Closely connected to these areas, the vACC is also involved in reward anticipation and modulating emotional responses. Nicotine dependence desensitises the mesolimbic dopaminergic reward network which is a common pathway for processing food-related cues, resulting in a blunted striatal response to food cues (Further discussion in section 1.5.3)[215, 219]. However, following abstinence, neural responses to HE food pictures in ex-smokers is greater than dieting group with obesity in the corticolimbic regions. By reducing the food cue reactivity, this supports the assumption that DAG and DAG analogues, could be used in prevention of smoking cessation weight gain by reducing anticipatory food reward.

On the other hand, the DAG-induced attenuation of BOLD signal to food cues in the PFC cluster comprising of SFG, frontal pole and paracingulate gyrus, may signify a concomitant decrease in cognitive evaluation of the food pictures and reduction in executive control. This may stem from the

diminished anticipatory food reward to HE food pictures and consequently a decrease in need for top-down inhibitory control and attention to food. Taken together, DAG appears to reduce corticolimbic reward-cognitive system activation during food evaluation in ex-smokers.

This is in direct contrast to the effect of AG enhancing the food hedonic response by stimulating the corticolimbic network [176, 382]. The overlap in the brain regions of the 2 ghrelin peptides (AG and DAG) responses point towards a common pathway of action, which may be via the GHSR1a. The AG:DAG ratio may be a key factor in modulating the effect of ghrelin peptides. Plasma AG:DAG ratio is associated with development of hyperphagia in the genetic obesity PWS and with incidence of metabolic syndrome [495-497]. It is likely that in ex-smokers, DAG levels are diminished whereas AG levels may be unchanged or elevated compared to non-smokers [489]. The AG:DAG ratio, usually in the ratio of 1:2-1:9 [373, 498], may be pertinent in determining the baseline activity of the ghrelin system and consequently influence the central effect of exogenous ghrelin peptides. Similarly, a different AG:DAG ratio may also explain why DAG differentially modulates food cue reactivity in dieting group with obesity and ex-smokers.

In obesity, AG levels are typically lower or unchanged compared to in lean population, but there are conflicting findings regarding DAG levels in obesity, with some reporting lower concentrations but not others [480, 496, 499, 500]. It may be possible that DAG works by counteracting the endogenous AG effects, so in obesity where AG concentrations are low, DAG has limited effects on eating behaviour. Conversely, in PWS, where AG concentrations are high, DAG analogue has shown promising results in decreasing hyperphagia [365]. Results of ghrelin peptide concentrations, along with the other gut hormone profiles, are awaited and would hopefully provide more clarity to this conundrum.

Hypothesis: BMI and eating behavioural traits can influence the DAG response on food cue reactivity in both groups.

Result: In dieting group with obesity, BMI and eating behavioural traits did not influence DAG response to HE foods.

In dieting group with obesity, there were no significant associations with eating behavioural constructs, including dietary restraint, emotional eating and disinhibition, and DAG response on HE food cue reactivity. As discussed in section 4.7, this could be because the frontal clusters may not be involved in regulating these facets of eating behaviour therefore no influence on the DAG response in the frontal

clusters was observed. Furthermore, the frontal clusters covered many different anatomical brain regions of the PFC likely reflecting heterogeneity in its function and therefore a close association with any specific eating behaviour is not evident.

Notably, there were no associations with BMI and DAG-induced increases in frontal cluster in dieting group with obesity. As discussed in section 4.7, BMI may not be an appropriate measure in this analysis as there are a heterogeneity of causes for obesity. Moreover, there may have been recent change in BMI from dieting that may have disrupted any pre-existing correlations. Furthermore, the small groups may be underpowered to find any correlations.

Result: In ex-smokers, BMI positively correlated with DAG-induced changes of BOLD signal to HE foods in striatal cluster.

This partially supports the hypothesis as stated above. This suggests that in ex-smokers, the higher the BMI, the less DAG reduced food cue reactivity. Interestingly, DAG appears to reduce the food cue reactivity in the striatal cluster within the overweight range but may paradoxically increase BOLD signal to food pictures at higher BMI ranges (Refer to Figure 5.7B). In addition, BMI in ex-smokers was found to be positively correlated to the BOLD signal to HE food pictures in this striatal cluster. Collectively, it may imply that to achieve maximal reduction in food-cue responsivity in striatum, DAG or DAG analogues may have to be commenced at the start of nicotine abstinence to prevent smoking cessation weight gain to achieve the best results.

Similarly, binge eating scale (BES) scores tended to be positively correlated to DAG-induced changes in striatal cluster in ex-smokers. This suggests the greater the prevalence of binge eating symptoms, the less DAG reduces food cue reactivity. However, this association should be interpreted with caution as it could be just a reflection of the correlational relationship between BMI and BES scores in ex-smokers ($r_s(23)=0.40$, P=0.056, Table 3.3). Nevertheless, no correlation was seen between DAG-induced BOLD signal changes to HE foods and dietary restraint, susceptibility to overeating from hunger cues or disinhibition, which all had a significant positive relationship with BMI in ex-smokers.

Hypothesis: DAG will reduce appetite and food intake in both groups of participants.

Result: DAG did not reduce appetite ratings but tended to reduce food craving ratings and energy-intake from low-fat foods in both groups. DAG also increased ideal creaminess to cream soup but decreased that of broth.

In contrast to the hypothesis, DAG did not alter appetite ratings in both groups. In line with previous findings, DAG had no effect on appetite in non-obese participants [363] but DAG analogue was found to reduce hyperphagia and appetite scores in PWS [365].

On the other hand, DAG tended to reduce food craving ratings independent of group. Here, the food craving VAS rating comprised of these 2 questions: (i) 'All I want right now is something to eat', and (ii) 'Nothing would be better than eating something right now'. They were modified from the Questionnaire of Smoking Urges-Brief (QSU-Brief) and Alcohol Urge Questionnaire (AUQ) which were both utilised in this study. They are well-validated research questionnaires measuring the of cravings for cigarettes and alcohol respectively [501-503]. So, it is plausible that DAG modulates brain regions that govern food cravings but not appetite. These 2 different measures of eating behaviour (appetite and food cravings) have different sensitivities but as discussed previously, the small numbers in this study may be underpowered to detect small changes.

DAG did not affect overall energy intake in *ad libitum* meal, contrary to the hypothesis. In agreement with previous findings, DAG did not reduce total energy intake in non-obese participants but reduced energy intake from glucose and fructose [363]. This was not seen in this current study although DAG tended to reduce energy intake from low-fat foods in both groups.

This may be in part attributed to the change in taste of broth with DAG. DAG diminished the ideal creaminess of broth, resulting in ratings being closer to "far too bland" which could lead to a reduction in intake. However, there was also a corresponding increase with DAG in ideal creaminess rating with cream soup, resulting in ratings being close to "far too creamy" and a tendency to diminish sweetness intensity of ice-cream. These subtle changes in taste of high-fat cream soup and ice-cream did not lead to any changes in the energy intake of the high-fat foods. This was in spite of the attenuation of food cue reactivity in striatum to HE food pictures in ex-smokers. Nevertheless, this is largely in line with previous literature in which DAG did not affect overall energy consumption in *ad libitum* test meals in

humans.

However, in obesity, weight reduction was still seen with DAG without affecting food intake in clinical and preclinical studies [364, 481]. After 2 weeks of DAG analogue, Livoletide, group with obesity had a greater weight reduction compared to placebo even though they had fixed meal intake throughout the study [364]. This may represent an effect of Livoletide on energy expenditure or fat oxidation, which is a different mechanism of weight loss. There are important differences in study protocol and participant characteristics, that would confound any direct comparison, between this current and previous study in that their participants:

(i) were treated with a DAG analogue, Livoletide instead of DAG peptide;
(ii) were treated for a duration of 2 weeks instead of an acute infusion;
(iii) had fixed meals throughout the 2 weeks stay in the research unit instead of *ad libitum* meals;
(iv) were not actively dieting and their smoking status was unknown.

Hypothesis: DAG will reduce food reward behaviour, including motivation to receive a food reward and approach bias, in both groups.

Result: DAG had no effect on food reward behaviour in both groups.

Contrary to the hypothesis, DAG had no effect on food reward behaviour as measured in the PRT or AAT despite DAG-induced neural changes in food responsivity. This is in contrast to AG, where food motivation for sucrose, in an operant conditioning or progressive ratio task in preclinical studies, was enhanced with AG administration via actions on VTA [504] or lateral septum [505] and attenuated with GHSR antagonism.

As discussed in section 4.7, there was great inter-individual variability in the both PRT and AAT therefore the lack of significant findings may be related to the study being underpowered. With the AAT, there was also great within-subject variability. To elicit a stronger approach bias towards HE foods, it may have been more suitable to instruct participants to pull or push depending on the picture content thus ensuring they process the pictures.

Hypothesis: DAG will improve glucose homeostasis in both groups.

Result: DAG did not affect glucose homeostasis in both groups.

In contrast to the hypothesis, DAG did not have an effect on plasma glucose or serum insulin concentrations. 2 weeks treatment with DAG analogue, Livoletide, demonstrated improvements in glucose concentrations without change in insulin concentrations in obesity and T2DM [364]. Livoletide, unlike endogenous DAG, is protected from peptidase degradation and has an improved bioavailability [482, 483] which may partially explain the difference. Fasting glucose and insulin levels were unchanged with acute DAG administration [363, 373, 389, 479, 480] although it increased fasting glucose concentrations, but not insulin concentrations, in individuals with pituitary insufficiency [478]. The improvement in glucose homeostasis, if any, was observed postprandially [376, 388] and to a greater extent in individuals with impaired glucose tolerance or T2DM [364]. This parallels the evidence in preclinical studies showing the ability of DAG as an insulin secretagogue after IV GTT [506].

In this study, the participants only had light snacks at 2 timepoints, which was followed by an average of 0.5 – 1.5 mmol/L increase in glucose concentrations. It may be that beneficial effects of DAG on glucose homeostasis would be observed with a larger food load such as a fixed test meal. Also, there was only n=1 dieting participant with obesity who had impaired fasting glucose although data for impaired glucose tolerance was not available in both participant groups. According to the other studies, having normoglycaemic participants may minimise any effect of DAG on glucose homeostasis.

Similarly, there was no effect of DAG on the other hormones, GH, prolactin and cortisol which is in line with current literature.

Conclusion

The ghrelin system offers an exciting therapeutic target for the management of obesity, including the prevention of smoking cessation weight gain. After accumulation of years of research, DAG has slowly emerged as the forerunner amongst the ghrelin peptides and its antagonists in this aspect [483]. It showed promising results in PWS, a rare genetic obesity syndrome, and has a good side effect profile [364, 365].

Here, the central effects of an acute infusion of DAG on BOLD signal to food cues in humans were characterised. The novel finding that DAG reduced anticipatory food cue reactivity in abstinent nicotine-dependent participants in brain regions including striatum, amygdala and PFC supports the hypothesis. Furthermore, the contrast in DAG response on anticipatory food cue reactivity between

dieting group with obesity and ex-smokers is particularly intriguing. These results provide evidence that DAG has a modulatory effect on food reward processing networks and are in accord with the possibility that DAG and its analogues could be used as a treatment option for prevention of smoking cessation weight gain.

CHAPTER 6:

GENERAL DISCUSSION

6.1 RECRUITMENT

Despite the high prevalence of obesity, smoking and alcohol-dependence, there were some difficulties faced in recruiting participants at the initial stages. Although previous studies by our group recruited effectively from secondary and tertiary specialist clinics, this proved not to be the case in current study. For instance, individuals with obesity attending obesity clinics, usually considered for bariatric surgery, tended to have a myriad of other metabolic or cardiovascular complications that would have excluded them from the study. Similarly, scarcity of smoking cessation, alcohol-detoxification and alcohol-relapse prevention clinics in the NHS, made it impossible to recruit adequately through such avenues for the study as many patients have complex medical history and met exclusion criteria. Other recruitment methods included liaising with GP practices, advertising in local London newspapers (Metro and Evening Standard), social media and Facebook study accounts. Nonetheless, recruitment was slower than expected with these strategies.

The slow recruitment prompted the implementation of a successful advertising campaign through social media, in particular Facebook. Fortunately, a 1-year grant extension by the Medical Research Council was approved due to unforeseen staffing delays and the slow participant recruitment. As a result, this helped achieve sufficient participant enrolment which allowed the study to be adequately powered without having to compromise on the participant selection criteria (Figure 3.1).

Another challenge encountered during the recruitment was the high drop-out rate. N=15 eligible participants withdrew from the study because of how time-consuming the study was and were therefore unable to accommodate the study visits. The study visits had to be conducted during weekdays and had to be prearranged at least a couple of weeks in advance depending on the MRI availability. Furthermore, there were no direct health benefits to the participants as this was an experimental medicine study, thus compounding the matter. Participants got paid around £250 in total for all 4 visits (screening and study) which was not an incentive for many, especially those who had full-time jobs.

This experience highlights the importance of giving due consideration to the variety of recruitment strategies, in particular social media, during planning of any clinical study and allocating sufficient funds for advertising in social media campaigns when applying for future grants [507]. Importantly, it also highlights how complex and heterogenous these groups of participants are.

6.2 SUMMARY OF RESULTS

In the previous result chapters, the comparisons of participant characteristics, effects of acute administration of Exenatide and DAG and implications of the findings were covered. Here, it would be important to contextualise the current study findings with the published literature.

Effect of Exenatide on eating behaviour

As discussed in Chapter 1.6 and 4.5, in pre-clinical studies Exenatide and other GLP-1 analogues reduces intake of chow, palatable and high-fat foods and decreases rewarding value of food [267, 269, 278]. These effects are mediated in part by the VTA, NAcc, hippocampus and amygdala [90, 278]. Supporting these, fMRI studies have demonstrated GLP-1 or GLP-1 analogues including Exenatide, acutely attenuated food cue reactivity in the insula, putamen, OFC and amygdala in lean adults, with obesity with or without T2DM [141, 158, 271]. In one of these studies, there was also a concomitant reduction in food intake with Exenatide in the groups with obesity with and without T2DM, which positively correlated with the Exenatide-induced reduction of BOLD signal to food cues [141].

In the current study, Exenatide also reduced the total energy intake of test meal and appetite VAS scores in both the dieting group with obesity and ex-smokers, in keeping with the well-known anorexigenic effect of GLP-1 analogues. In addition, Exenatide also attenuated the food cue reactivity in reward processing areas including putamen, caudate and prefrontal cortex, and food appeal rating in ex-smokers. This is a novel finding as no previous studies have examined the effects of GLP-1 analogue in this participant group. Although there was no significant effect of Exenatide on food reward behaviour in this current study, there was a trend for reduction of motivation to earn chocolate sweets in both participant groups in the progressive ratio task. None of the other clinical studies with GLP-1 or analogues have examined such aspects of food reward behaviour therefore a direct comparison cannot be made. Nonetheless, this suggests that in ex-smokers, Exenatide reduces anticipatory food reward, as well as food intake, and could be used as a potential therapy to prevent smoking cessation weight gain.

On the other hand, in the dieting group with obesity, Exenatide increased the food cue reactivity in prefrontal cortex, but had no effect in the mesolimbic brain regions. As the prefrontal cortex has a role in inhibitory control, the findings in the dieting group with obesity could imply Exenatide increases executive control when evaluating food cues. The Exenatide-induced increase in food cue reactivity in

the current dieting group with obesity is inconsistent with the previous literature that suggests Exenatide decreased food cue reactivity in reward processing regions in obesity. However, the differences in results may arise from disparate participant characteristics (dieting vs. non-dieting perhaps with differing degrees of dietary restraint and recent weight change, female/male vs. post-menopausal females) and study design (glucose and somatostatin clamp vs. none, and fasted vs. post-small snack) between previous studies and the current one. Other possible reasons of the discrepancies in results had been discussed in greater detail in Chapter 4.5.

The current study findings of the effect of Exenatide on food cue reactivity in the dieting group with obesity does not preclude the use of Exenatide in this treatment seeking group with obesity. This is because in this study, Exenatide also reduced food appeal rating, food intake, appetite and food craving VAS scores in dieting group with obesity which are all beneficial to prevent weight regain.

Effect of DAG on eating behaviour

As discussed in chapter 1.7 and 5.5, the effect of DAG on appetite and food intake in preclinical and clinical studies have been inconsistent. In a few studies, DAG on its own reduced chow intake in rodents [356, 362, 508], but in others increased intake [358] or had no effect [359, 360]. However, DAG, when combined with AG, reduced AG-induced increase in energy intake in most studies, suggesting a functionally antagonistic effect to that of AG [347]. In the published literature, acute DAG infusion did not have an effect on appetite VAS score or food intake in healthy, lean adults although it selectively decreased the intake of glucose and fructose at an *ad libitum* test meal [363]. The DAG analogue, Livoletide, also decreased appetite VAS score in a group of patients with Prader-Willi syndrome over 2 weeks [365].

This study is the first fMRI study examining the effects of DAG on food cue reactivity in humans. In this current study, DAG had no effect on appetite, food intake or food reward behaviour in both groups of participants in keeping with the aforementioned study. Despite this, in ex-smokers, DAG attenuated food cue reactivity in caudate, putamen, amygdala and prefrontal cortex, suggesting a reduction in food reward and perhaps a concomitant need for inhibitory control. The fMRI findings in the ex-smokers are in accord with the possibility that DAG and other analogues could be used as treatment option for prevention of smoking cessation weight gain.

6.3 COMPARISON BETWEEN DIETING GROUP WITH OBESITY AND EX-SMOKERS

This next section will focus on the: (i) discussion of the differences in neural responses to HE food picture between both participant groups at saline visit, and (ii) comparison of the effects on food cue reactivity of Exenatide alongside DAG, so as to gain some insights regarding possible underlying mechanisms mediating the neural circuitry for food reward.

Differences in BOLD signal to HE food pictures in participant groups at saline visit

Firstly, there was a striking disparity in BOLD signal to HE food pictures at saline visit between the dieting group with obesity and ex-smokers. In fMRI studies, obesity has consistently been shown to have an increased reward response to food cues and an increased motivation to eat [63]. Therefore, the finding that dieting group with obesity had an attenuated average BOLD signal to HE food pictures across all fROI compared to ex-smokers was surprising (Figure 4.8 and 5.9).

A possible explanation for this could be the differences in participant characteristics. It should be highlighted that in this study, the dieting group with obesity was compared to ex-smokers and not lean non-smokers, unlike previous studies where BMI was the only comparator. This dieting group with obesity in the current study was not representative of the population with obesity at large as they were all on a diet and may also have experienced some weight loss prior to the study. As previously described, dieting and weight loss induced changes in neural responsivity to food cues in obesity in brains regions implicated in appetite control and emotional control, including a decrease in BOLD signal to food cues in hypothalamus, amygdala and ventromedial PFC [56, 174]. These changes appear within first 4 weeks of commencing a diet which is consistent with the time frame of the current study inclusion criteria [173].

Notwithstanding, there are many other factors that could influence food cue reactivity, including eating behaviours [126, 129], exercise [164] and dietary intake [167, 509]. As discussed in Chapter 3, the dieting group with obesity had unhealthier eating behaviours, such as greater dietary restraint, emotional eating and uncontrolled eating, when compared to ex-smokers. Supporting this for instance, dietary restraint negatively correlated with BOLD signal to HE foods in OFC, amygdala and caudate [510] but there are inconsistencies with reports of a positive [511] and null relationship [159]. As a result, the unhealthier eating behaviours, dietary adaptions during dieting, as well as possible weight loss, could all have accounted for the lower BOLD signal to HE food pictures in dieting group with

obesity than ex-smokers in the current study.

Furthermore, nicotine alters food cue reactivity. Current smokers have been shown to have attenuated BOLD signal to favourite food cues in caudate, putamen, insula, thalamus and cerebellum compared to non-smokers [219]. It may be that subsequent abstinence from nicotine may have a rebound effect and increase BOLD signal activation to HE foods therefore ex-smokers had a higher average BOLD signal to HE foods across all fROI than dieting group with obesity. The higher food cue reactivity may in fact be one of the underlying mechanisms for smoking cessation weight gain.

Differences in other outcome measures in participant groups at saline visit

Notably at saline visit, there were no differences between the both participant groups in food appeal rating, energy intake at *ad libitum* meal, motivation for a chocolate sweet as measured in progressive ratio task nor approach bias to HE foods as measured in approach-avoidance task. As discussed previously, the number of participants is relatively small per group and may be underpowered to detect any significant differences. Furthermore, these tasks have inter-subject variability, at times intra-subject variability, therefore negatively impacting reliability of the tasks. As a result, interpretation of results should be with caution.

Comparing effects of Exenatide and DAG on BOLD signal to HE food pictures

Notably, the effects of both Exenatide and DAG on neural responses to HE food cues are in some ways similar. To recap, in the dieting group with obesity, there was an increase in BOLD signal to food cues in prefrontal regions generally with both Exenatide and DAG administration. The effect of DAG on BOLD signal in food cues also paralleled that of Exenatide in ex-smokers, where there was a decrease in prefrontal and striatal regions (Table 6.1).

In terms of behaviour tasks, the effect of Exenatide on food cues was more pronounced than DAG, as evident from the reduction in food intake, food appeal, appetite rating in both groups and food craving rating in dieting group with obesity. In addition, Exenatide tended to decrease the motivation for chocolate in both groups. None of these changes were observed in the effects of DAG apart from a trend in reduction of food intake from low-fat foods and food craving rating across both groups of participants.

Outcome	Exenatide		DAG	
	Obesity 	Ex-smoker 	Obesity 	Ex-smoker 
BOLD signal to HE food cues	↑ in FP/SFG	↓ in SFG/PCG/Put/Caud/Thal	↑ in FP/OFC/SFG/MFG/PCG/ACC	↓ in SFG/PCG/FP/Put/Caud/Amyg/Thal
HE food appeal	↓	↓	→	→
Food intake	↓ total ↓ soups/ (↓) HF	↓ total ↓ soups/ (↓) HF	(↓) LF	(↓) LF
Appetite rating	↓	↓	→	→
Food craving rating	↓	→	(↓)	(↓)
PRT	(↓)	(↓)	→	→
AAT	→	→	→	→

Table 6.1. Summary results of Exenatide and DAG effects on neural and behavioural tasks.
Abbreviations: AAT, approach-avoidance task; ACC, anterior cingulate cortex; Amyg, amygdala; BOLD, blood oxygen level dependent; Caud, caudate; FP, frontal pole; HE, high energy; HF, high-fat; LF, low-fat; MFG, middle frontal gyrus; OFC, orbitofrontal cortex; PCG, paracingulate gyrus; PRT, progressive ratio task; Put, putamen; SFG, superior frontal gyrus; Thal, thalamus. Trends are in parentheses.

There are a few points that are worth highlighting from the aforementioned results. Despite different effects of Exenatide and DAG on BOLD responses to HE foods in both groups, they had similar effects on eating behaviour. In humans, central expression of GLP-1 receptor messenger RNA has been reported in the cerebral cortex (occipital, frontal, parietal and temporal), hypothalamus, hippocampus, thalamus, caudate, putamen and globus pallidum [387, 512], so it is plausible that GLP-1 analogue, Exenatide can have a direct effect on these brain regions. However, as to why Exenatide increases neural responses in one and decreases in the other participant group is unclear. Moreover, the opposite effects of Exenatide on BOLD responses to HE foods appeared to be associated with improvements in food-related behaviours in both dieting group with obesity and ex-smokers. In the case of DAG, it is just as intriguing to observe a similar pattern of mismatch of effects on BOLD responses to food cues in both participant groups. The signalling pathway for DAG remains hitherto unknown.

It is possible that what determines the directionality of the hormone neural response to HE foods lies in the activity of the GLP-1 receptor or ghrelin signalling system. There are a few possible factors influencing the activity of these receptors and neurohormone concentrations:

(i) BMI: lower GLP-1, AG and DAG concentrations are associated with obesity compared to lean [496, 513];

(ii) previous nicotine dependence: during smoking cessation, DAG concentration, but not AG, was reduced in healthy non-obese participants [489]. On the other hand, there were no differences in GLP-1 concentrations between male smokers and non-smokers [514].

Currently, gut hormone measurements, including GLP-1, AG, DAG, gastric inhibitory peptide (GIP) and leptin are awaited. Once available, these gut hormone concentrations may help clarify why Exenatide and DAG had opposite effects on BOLD signal to HE foods in both groups. One method could be to investigate if gut hormone concentrations are associated with BOLD signal to HE foods and effect of Exenatide or DAG. For example, in adults with T2DM, greater liraglutide-induced decreases of fasting leptin concentrations resulted in increased BOLD signal to HE foods vs. LE foods in midbrain, precuneus and dlPFC and reduced BOLD signal to thalamus and parietal cortex. In the same study, liraglutide-induced increases of fasting GIP correlated with reduction in BOLD signal to HE foods vs. LE foods in the insula in T2DM [271]. A similar effect of Exenatide on other gut hormone concentrations may be found in this current study that could help explain some of the food cue reactivity in both participant groups.

With DAG, it could also have downstream effects on other gut hormones that could in turn modulate BOLD signal to HE foods differently in both participant groups. Additionally, AG concentrations might influence the effect of DAG. Young adults with obesity had lower AG and much lower DAG concentrations than lean controls [496]. In this current study, AG is likely to be higher in ex-smokers than in dieting group with obesity and this disparity could conceivably cause a greater DAG response in ex-smokers by antagonising AG.

The second inference pertains to the behavioural effects of the two treatments, Exenatide and DAG. Arguably, there were more noticeable improvements in food-related behaviour with Exenatide than DAG, such as a reduction in energy intake and appetite with Exenatide which would result in weight

loss. This could arise from some effects on eating behaviour being peripherally-mediated. For instance, the peripheral effect of GLP-1 and GLP-1 analogues, on gastric emptying and vagal afferent activation [455, 515], could partially explain the reduction in food intake and appetite, which would be consistent with other studies. With DAG, the peripheral metabolic effects are inconsistent in the literature. This inconsistency may stem from differences in AG:DAG ratio of the study participants that have been postulated to, in turn, mediate effects of DAG (See Chapter 5.7 for further discussion).

Finally, there was an overlap between the brain regions that were modulated by Exenatide and DAG in the two groups of participants. This suggests that both treatments may affect these regions directly through their respective receptors or that they converge on the same downstream pathway. Certainly, the pattern of GLP-1 central receptors has been characterised [467, 512] but DAG receptor remains unidentified. Interestingly, emerging evidence of an interaction between the GLP-1 and ghrelin system could be the key to this matter.

Ghrelin and GLP-1 system interactions

There is emerging evidence of the interactions between GLP-1 and ghrelin systems in the gut-brain axis. Reports of ghrelin peptides affecting GLP-1 system is sparse. AG has been reported to increase postprandial GLP-1 concentrations in pre-clinical and human studies [516-518], but in not in others [519, 520].

On the other hand, administration of GLP-1 or analogue suppresses AG and DAG concentrations in rat and human studies, both in stomach and hypothalamus [521-524] and even antagonised the AG effects of metabolic function in preclinical studies via the hypothalamus [525, 526]. It was suggested this functional antagonism of AG by GLP-1 receptor stimulation is through increased insulin concentrations. Furthermore, central GLP-1 analogue administration in a preclinical study inhibited AG-stimulated appetitive reward in an operant responding task for sucrose [527]. Extrapolating from these results, Exenatide may have a greater effect on individuals with higher AG concentrations. It would be therefore be very compelling to investigate if the effect of Exenatide is associated with ghrelin peptide concentrations in this current study.

6.4 STRENGTHS AND LIMITATIONS

This study has several strengths and limitations that warrant discussing. To the best of my knowledge,

this study is the first to examine the acute effects of DAG administration on food-cue neural responses in a dieting group with obesity and in abstinent nicotine dependence, thereby providing a better understanding of the role of DAG in food reward processing in these disorders. As previously discussed, it is also amongst the first studies to examine the acute effects of GLP-1 analogue, Exenatide, in ex-smokers on food cue reactivity. Collectively, these study findings add to the evidence for a clinical therapeutic potential of these gut hormones in obesity and prevention of smoking cessation weight gain.

Another strength of the study is that the study protocol minimised any confounding factors for outcome measures by tightly controlling nutritional state of the participants and time of day. Additionally, the fMRI HE food picture evaluation task was not just a passive task, thereby focusing participants' attention on rating the appeal of the HE foods. A careful examination demonstrated a lack of any differences in important confounds, including mood, motion and response accuracy.

One obvious pitfall of any food-related fMRI study is whether the neuroimaging results will be closely reflective of real-life choices. Being able to see, smell and touch different foods at supermarket or restaurant would presumably influence food choices differently to evaluating food pictures whilst immobile in a narrow MRI scanner. In this current study, this was mitigated by including behavioural measures to substantiate the fMRI findings. Admittedly, the *ad libitum* meal of soups and desserts may not have been individualised however it achieves the purpose of providing a selection of high- / low-fat and savoury /sweet foods. Presenting a whole array of buffet choice for the test meal at each visit is otherwise time-consuming and costly. Alternatively, developing more realistic fMRI paradigms using virtual reality has been proposed to this end [528].

Another caveat to the interpretation of BOLD signal in any fMRI task is the presence of confounding factors which may influence the response in neuromuscular coupling that underpins the BOLD signal, such as reduced vasodilation in ex-smokers. One method of checking for this in this study is by comparing the BOLD signal during a control task (e.g. button press to picture vs. rest in unpleasant images fMRI task) between both groups. Other techniques suggested in the literature include calibrating BOLD signal by measuring effect of vasodilation induced by increasing pCO_2 on BOLD signal [529].

As discussed in Chapter 4.5, another factor that confounded the interpretation of results, particularly

the effects of Exenatide on BOLD signal to food cues, was the difference in blood glucose concentrations between Exenatide and Saline visits. This could have been mitigated by using a glucose clamp to maintain blood glucose at a fixed concentration. In reality, this would have been more cumbersome to implement as it requires additional glucose and insulin infusions, with the need for more study research staff, cannulas and infusion pumps within the MRI scanning room. Additionally, it would introduce exogenous insulin which in itself could have effects on BOLD signal to food cues. Another option for minimising the difference in blood glucose between the visits would be to keep the participants fasted throughout the fMRI tasks and forgo snacks. However, snacks were introduced to prevent hypoglycaemia and minimise hunger so that participants could concentrate on performing the fMRI tasks. More importantly, the current method is a more accurate representation of the effects of Exenatide on BOLD signal to food cues in a real world setting, since in clinical practice they would be used alone.

Notwithstanding, one of the limitations was that the participants in group with obesity were on non-standardised diet plans which were self-reported. This introduced greater variability to the group and could have resulted in a dampened any clinical impact of the gut hormones. Therefore, this may also explain any differences in findings with other obesity studies that include a formal standardised weight management programme. Contributing to this, the cross-sectional design of the study also meant data for change in weight and other eating behaviours was not available. Arguably, however, this sample of participants with obesity may be more representative of the general population of dieting individuals with obesity.

Ideally, BMI-, age-, sex-matched healthy participants who have never smoked would be a more suitable control for the ex-smokers. Similarly, the lean group (excluding those with obesity) could act as a more appropriate control group for the group with obesity in this study. More conclusions could have been drawn about the hormone signalling pathways in healthy adults, obesity and previous nicotine dependence, especially for DAG, with a suitable control group. However, this would have lengthened the recruitment process and increased financial spending of the study. Nonetheless, in future studies, due consideration would be given to include a healthy well-matched control group.

The other limitation of the study is that in female participants, the menstrual cycle phase or method of contraception was not controlled. There are differences in emotions, attention, reward sensitivity

through dopaminergic transmission during the follicular and luteal phases [530, 531]. Some studies recruited only postmenstrual females or arranged fMRI scans to coincide with follicular phase. However, it was impractical in in the current study to do so due to the inherent difficulties with recruitment. In this study, there were significant differences in the menstrual cycle phase in the Exenatide visits between dieting group with obesity (n=11) and ex-smokers (n=10) and a trend for a difference between saline and Exenatide visits in dieting group with obesity which may confound comparisons between-groups, and to a lesser extent, within-group for obesity. In future studies, steps could be taken to mitigate such a difference by taking into consideration their menstrual cycle when planning for study visits.

6.5 FUTURE DIRECTIONS

The focus of this thesis was to study the acute effects of Exenatide and DAG on food-cue reactivity in the two participant groups. Nonetheless, it would be of great interest and clinical relevance to analyse the other outcomes such as cigarette cue-reactivity and cigarette cravings in ex-smokers to determine if there could be a potential added benefit of the gut hormones in the context of smoking cessation. Furthermore, analysis of data in the abstinent alcohol-dependent group of participants could shed light on the role of GLP-1 and DAG in alcohol addiction that could yield promising results in support of an alcohol cessation therapy.

There were other fMRI and behavioural paradigms that were outside the remit of this thesis but would be relevant to analyse as they relate closely to behavioural traits seen commonly in addiction. This larger study, GHADD, was based on the previous Imperial College Cambridge Manchester (ICCAM) experimental study platform, utilising an array of fMRI tasks and questionnaires, to explore in depth the brain mechanisms underpinning reward and relapse circuitry so as to inform future drug development. The fMRI tasks used in GHADD and ICCAM studies included the Monetary Incentive Delay (MID) task which measures non-food monetary reward [532, 533] and unpleasant images task measuring negative emotional reactivity [534, 535]. Studying the effects of Exenatide and DAG on these outcomes will help better understand how they can modulate neural networks and affect behavioural responses.

In this current study, there was a group of never-smoked healthy volunteers (n=43) who attended only one fMRI visit without any infusions. Their data was utilised for the processing of the fROI. Indeed,

future work could analyse their data from fMRI tasks with the dieting group with obesity and ex-smokers but there would be some limitations to these comparisons. They will be confounded by different nutritional state as well as visit order effects. Many of these healthy volunteers were substitutions when study visits were cancelled at short notice hence the variation in duration of fast. To avoid visit order effects, participants in dieting group with obesity and ex-smokers with saline visit as the first visit could be compared with the healthy volunteers but numbers would be approximately a third of the total, thereby underpowering the analysis.

In this study, the fROI analysis was limited to 9 regions involved in reward processing however, it would be compelling to also include the examination of hypothalamus, VTA and habenular nucleus, given their role in mediating food and cigarette intake. With advances in the field of obesity and addiction, there will be additional fROI that are implicated specifically in the context of GLP-1 and ghrelin/DAG signalling which could unravel the interplay of other neural circuits in these conditions.

As discussed, the gut hormones measurements are still awaited and therefore could not be included in this thesis. Analysing the gut hormone concentrations would be paramount to better understanding their role in food reward processing. Undoubtedly, interpreting the effects of Exenatide and DAG in the context of the alterations to gut hormones results would add credence to any drawn conclusions.

Indeed, this study was designed to explore the acute effects of the gut hormones on food cue reactivity and eating behaviours which are merely biomarkers for weight loss. What is not so apparent are the chronic effects of these gut hormone treatments on long term weight loss. It is likely that there are compensatory adaptations to long-term treatment with GLP-1 analogues and DAG analogues so further longer term studies are required to specifically address the feasibility and efficacy of treatment in terms of aiding weight loss or maintenance after a diet and after smoking cessation.

Nonetheless, this experimental medicine proof-of-concept study utilised a platform of fMRI and behavioural tasks to provide pilot data for a larger clinical study. In doing so, it adds to the confidence to trial these gut hormones as potential therapies in obesity and smoking cessation. As this study only examines the acute effects of the gut hormones, it is unable to demonstrate any clinical effect on weight by virtue of the study design. Although this study was not designed to show any relationship between future weight changes and study outcome measures such as HE food picture reactivity,

previous longitudinal studies have demonstrated that changes in food cue reactivity can predict future weight gain or future success of weight loss maintenance [173, 180-183]. This indicates that food cue reactivity can be a reliable biomarker of future weight changes.

A DAG analogue (Livoletide or AZP-531) is currently available and had promising results in obesity and PWS. It caused greater weight loss over 2 weeks in obesity than lean as well as reduced hyperphagia in PWS where the plasma AG:DAG ratio is elevated [365]. A Phase 2b clinical trials for Livoletide in food-related behaviours in PWS is ongoing [483, 536]. The next step would be to do a proof-of-concept study in Livoletide to see if it reduces, in ex-smokers, their weight, body fat, and smoking relapse rates and whether these measures are correlated to food- and smoking-cue reactivity in fMRI. Similarly, the use of GLP-1 analogues in smoking cessation and treatment for alcohol use disorder is being explored [289, 436].

At present, GLP-1 analogues are used in the treatment of obesity as an adjunct to dieting [537]. While the liraglutide studies in obesity report a 4-5% decrease in body weight, it is noted that many participants regained weight after stopping treatment [537]. This suggests that GLP-1 analogues help maintain weight loss during dieting. In the future, fMRI food cue reactivity could potentially be used as a biomarker for selecting patients who would respond well to GLP-1 analogue treatment and possibly guide treatment duration. The efficacy of DAG analogue, a novel therapy, in aiding weight loss in longer term clinical trials remains to be seen. However, if DAG analogue can significantly reduce body weight and food intake over long term, it would be an attractive alternative to GLP-1 analogues as it is better tolerated. There is currently no preventative treatment for smoking cessation weight gain. From this study, both GLP-1 and DAG analogues reduced HE food cue reactivity in ex-smokers hinting at the potential to prevent weight gain although prospective clinical studies will be required to further investigate this. These findings could have far-reaching implications in the future for treatment of obesity, prevention of smoking cessation weight gain, smoking relapse and other forms of substance addiction.

APPENDICES

Appendix 1: Inclusion and Exclusion criteria

INCLUSION CRITERIA
1. Male or female volunteers between the ages of 18 and 60 years.
2. Healthy as determined by a responsible physician, based on a medical evaluation including medical history, physical examination, laboratory tests, cardiac monitoring and a psychiatric evaluation. Any volunteer with a clinical abnormality or laboratory parameters outside the reference range for the population being studied may be included, only if the investigators concur that the finding is unlikely to jeopardize either volunteer safety or study integrity. With regard to liver function, please also see exclusion criterion 16.
3. The subject is capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form.
4. The subject is able to read, comprehend and record information written in English.
5. A signed and dated written informed consent is obtained from the subject.

6. For non-dependent groups:
i) Overweight/obese volunteers with BMI 28.0-50.0 kg/m^2.
ii) Healthy volunteers for pilot testing of main protocol with BMI 18.0-35.0 kg/m^2.

7. For ex-smokers:
iii) Abstinent tobacco dependent individuals who when smoking, had their first cigarette within 60 minutes of waking and had smoked at least 5 cigarettes per day, as measured retrospectively using the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991), and who have been in stable tobacco abstinence for at least 6 weeks. Minor lapses will be allowed but not relapses into dependence.

EXCLUSION CRITERIA
Potential volunteers will NOT be eligible for inclusion in this study if any of the following criteria apply:
1. Previous history of recreational use or abuse of other substances of addiction will be permissible, but there should be no use of any illegal drugs (except cannabis) in the month prior to the Screening Visit or during the course of the study, except where specified for individual groups below.
2. For individual groups:
i) Overweight/obese group and healthy volunteer group: history of or current alcohol abuse or dependence; nicotine use other than “never smoked”, i.e. >100 cigarettes lifetime use; history of dependence, abuse or heavy recreational use of cocaine, cannabis, opiates or other substance of abuse; history of problem gambling. Any previous or current psychiatric diagnosis listed in DSM-5 Axis I, which in the opinion of the clinical team will compromise conduct and interpretability of the study. Participants currently suffering from DSM-5 depressive disorder or on anti-depressant medication will be excluded, though a previous history of depression will be allowed. Screening for Axis I psychiatric diagnoses will be performed using a summarized version of the Mini International Neuropsychiatric Interview for Mini International Neuropsychiatric Interview for DSM-5 (MINI). This interview will be performed by appropriately trained study personnel. The MINI is a short, structured interview requiring “yes” or “no” answers only.
ii) Abstinent tobacco dependent group: history of or current alcohol abuse or dependence; current dependence for cocaine, cannabis, opiates or other substance of abuse, or problem gambling (previous

history will be allowed); taking varenicline, bupropion or other prescription medications for smoking cessation. Any previous or current psychiatric diagnosis listed in DSM-5 Axis I, which in the opinion of the clinical team will compromise conduct and interpretability of the study. Screening for Axis I psychiatric diagnoses will be performed using the MINI Interview for DSM-5. Appropriately trained study personnel will perform this interview.
3. A current or past history of enduring severe mental illness (e.g., schizophrenia, bipolar affective disorder) will not be allowed. Screening for Axis I psychiatric diagnoses will be performed using the MINI Interview for DSM-5. This interview will be performed by appropriately trained study personnel.

For all groups:
4. Cannabis use up to five times in the month prior to the Screening Visit will be allowed, but no use within one week of experimental assessments; no use of any other illegal drugs in the month prior to the Screening Visit or during the course of the study.
5. Intoxication at any of the visits, as manifested by difficulty in walking, slurring of speech, difficulty concentrating or drowsiness (or by the subject volunteering this information directly to the research team). This exclusion criterion would exclude a volunteer from that study day only and not the whole study, at the discretion of the research team.
6. Positive drug/alcohol screens on testing at the screening visit, other than that explicable by other causes (e.g. recent use of opiate containing analgesic, consumption of poppy seeds for positive opiate screen), at the discretion of the research team. A minimum list of drugs that will be screened for include amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines. Positive results for cannabinoids will be allowed given the long half-life of cannabinoid metabolites. If positive at a study visit this exclusion criterion would exclude a subject from that study day only and not the whole study, at the discretion of the research team.
7. The British National Institute for Clinical Excellence (NICE) stipulates that a non-smoker is identified by a breath carbon monoxide reading of less than 10ppm (NHS Stop Smoking Services; Service and Monitoring Guidance 2010/11). Therefore, carbon monoxide levels of =/>10ppm in the healthy volunteer, overweight/obese and abstinent smoker groups at the screening visit will result in exclusion. If positive at a study visit this would exclude a subject from that study day only and not the whole study, at the discretion of the research team.
8. Use of current regular prescriptions (including smoking or alcohol cessation medicines such as Disulfiram, Acamprosate, Naltrexone, Bupropion; weight loss medication including Orlistat, Metformin, GLP-1 agonists, Bupropion, Naltrexone), or over-the-counter medications that in the opinion of the Investigators may affect subject safety or outcome measures.
9. Pulse rate <40 or >100 beats per minute OR systolic blood pressure >160 and <100 and a diastolic blood pressure >95 and <50 in the semi-supine position.
10. Claustrophobia or feels that they will be unable to lay still on their back in the MRI scanner for a period of ~80 minutes.
11. Presence of a cardiac pacemaker or other electronic device or ferromagnetic metal foreign bodies as assessed by a standard pre-MRI questionnaire and radiographer.
12. History or presence of a neurological diagnosis (not limited to but including, for example, stroke, epilepsy, space occupying lesions, multiple sclerosis, Parkinson's disease, vascular dementia, transient ischemic attack, that may influence the outcome or analysis of the scan results).
13. Significant current or past medical or psychiatric history that, in the opinion of the investigators, contraindicates their participation. Screening for Axis I psychiatric diagnoses will be performed using the MINI Interview for DSM-5.
14. Clinically significant head injury (e.g. requiring hospitalisation or surgical intervention) that in the opinion of the investigators may affect subject safety or outcome measures.

15. Unwillingness or inability to follow the procedures outlined in the protocol.
16. Any of the following liver function tests (LFT) abnormalities at screening: Alkaline Phosphatase, AST, ALT or gamma-GT > 4 x upper limit of normal (ULN), INR > 1.5, Albumin <25 g/L, raised bilirubin (other than just isolated i.e. without other liver function tests abnormalities).
17. History of decompensated alcoholic liver disease - i.e. history of variceal bleeding, ascites, jaundice, encephalopathy.
18. History of pancreatitis from any cause.
19. History of type 1 or type 2 diabetes mellitus.
20. ECG abnormality, which in the opinion of the study physician, is clinically significant and represents a safety risk.
21. The volunteer has participated in a clinical trial and has received an investigational product within the following time period prior to the first experimental visit in the current study: 90 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
22. Exposure to more than 3 new investigational medicinal products within 12 months prior to the scan.
23. History of sensitivity to any of the peptides, or components thereof, or a history of drug or other allergy that, in the opinion of the investigators, contraindicates their participation.
24. Diagnosis of endocrine disorder, including uncontrolled hypothyroidism (stable treated hypothyroidism with currently normal thyroid function tests is allowed), history of hyperthyroidism or Cushing's syndrome, which, in the opinion of the investigators, may affect subject safety or outcome measures.
25. History of ischaemic heart disease, heart failure, cardiac arrhythmia or peripheral vascular or cerebrovascular disease.
26. History or presence of significant respiratory, gastrointestinal, hepatic, oncological or renal disease or other condition that in the opinion of the Investigators may affect subject safety or outcome measures.
27. Previous bariatric surgery for obesity including Roux-en-Y gastric bypass, gastric banding, sleeve gastrectomy.
28. Current pregnancy or breast-feeding in female volunteers (the Investigators would advise on using contraception for the duration of the visits).
29. Vegetarian, vegan, gluten or lactose-intolerant (as food pictures and test meals in the paradigms include meat, dairy and wheat products).
30. Volunteers who have donated, or intend to donate, blood within three months before the screening visit or following study visit completion.

Appendix 2: The Beck Depression Inventory

Please circle the appropriate answer

1. Sadness
0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all of the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism
0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my fortune is hopeless and will get only worse.

3. Past Failure
0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure
0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty most of the time.

6. Punishment Feelings
0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike
0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticisms
0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying
0 I don't cry any more than I used to.
1 I cry more than I used to.

2 I cry over every little thing.
3 I feel like crying, but I can't.

11. Agitation
0 I am no more restless or would up than usual.
1 I feel more restless or would up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless that I have to keep moving or doing something.

12. Loss of Interest
0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness
0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than usual.
3 I have trouble making any decision.

14. Worthlessness
0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy
0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Patterns
0 I have not experienced any change in my sleeping pattern.
1 I sleep somewhat more/less than usual.
2 I sleep a lot more/less than usual.
3 I sleep most of the day.
I wake up 1-2 hours early and can't get back to sleep.

17. Irritability
0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite
0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.

2a My appetite is much less than usual.
2b My appetite is much greater than usual.

3 I crave food all the time or I have no appetite at all.

19. Concentration Difficulty
0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue
0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.

2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex
0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

SCORE: _____

Appendix 3: Spielberger Trait Anxiety Inventory (STAI)

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	1 Almost never	**2** Sometimes	**3** Often	**4** Almost always
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am “calm, cool, and collected”	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over some-thing that really doesn’t matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thoughts run through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can’t put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Appendix 4: Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you felt that you were upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5. In the last month, how often have you felt that things were going your way?	0	1	2	3	4
6. In the last month, how often have you found that you could not cope with all the things you had to do?	0	1	2	3	4
7. In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
8. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9. In the last month, how often have you been angered because of things that were outside of your control?	0	1	2	3	4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Appendix 5: Fagerström Test for Nicotine Dependence

How soon after waking up do you smoke your first cigarette?

Within 5 minutes	(3 points)
5 – 30 minutes	(2 points)
31 – 60 minutes	(1 point)
After 60 minutes	(0 points)

Do you find it difficult to refrain from smoking in places where it is forbidden? e.g. Church, Library, etc.

Yes	(1 point)
No	(0 points)

Which cigarette would you hate to give up?

The first one in the morning	(1 point)
Any other one	(0 points)

How many cigarettes a day do you smoke?

10 or fewer	(0 points)
11 – 20	(1 point)
21 – 30	(2 points)
31 or more	(3 points)

Do you smoke more frequently in the morning?

Yes	(1 point)
No	(0 points)

Do you smoke even if you are sick in bed most of the day?

Yes	(1 point)
No	(0 points)

Appendix 6: Alcohol Use Disorders Identification Test

Questions	Scoring system					Your score
	0	1	2	3	4	
1. How often do you have a drink containing alcohol?	Never	Monthly or less	2 - 4 times per month	2 - 3 times per week	4+ times per week	
2. How many units of alcohol do you drink on a typical day when you are drinking?	1 -2	3 - 4	5 - 6	7 - 9	10+	
3. How often have you had 6 or more units if female, or 8 or more if male, on a single occasion in the last year?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
4. How often during the last year have you found that you were not able to stop drinking once you had started?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
5. How often during the last year have you failed to do what was normally expected from you because of your drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
6. How often during the last year have you needed an alcoholic drink in the morning to get yourself going after a heavy drinking session?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
7. How often during the last year have you had a feeling of guilt or remorse after drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
9. Have you or somebody else been injured as a result of your drinking?	No		Yes, but not in the last year		Yes, during the last year	
10. Has a relative or friend, doctor or other health worker been concerned about your drinking or suggested that you cut down?	No		Yes, but not in the last year		Yes, during the last year	

Appendix 7: Wechsler Test of Adult Reading

Say, **I will show you some words that I will ask you to pronounce.** Place the WTAR Word Card in front of the examinee. As you point to the card, say, **Beginning with the first word on the list, pronounce each word aloud. Start with this word** (point to item 1), **and go down this column, one after the other, without skipping any. When you finish this column, go to the next column** (point to the second column). **Pronounce each word even if you are unsure. Do you understand?** When you are sure that the examinee understands the task, say, **Ready? Begin.**

	Item	Pronunciation	Score (0, 1)		Item	Pronunciation	Score (0, 1)
1.	again	ah-GEHN ah-GAIN *or* uh-GEHN or uh-GAIN		26.	conscientious	con-shee-EN-shss	
2.	address	ah-DRESS *or* uh-DRESS		27.	homily	HOM-ih-lay *or* HOM-ih-lee	
3.	cough	kawf *or* kof		28.	malady	MAL-uh-day *or* MAL-uh-dee	
4.	preview	PREE-vyue		29.	subtle	SUH-tl	
5.	although	awl-THO		30.	fecund	FE-cund *or* FEE-cund	
6.	most	mohst		31.	palatable	PAL-ah-tuh-bul *or* PAL-uh-tuh-bul	
7.	excitement	eck-SITE-munt *or* ik-SITE-munt		32.	menagerie	meh-NA-juh-ree	
8.	know	noh *or* no		33.	obfuscate	OB-fuh-skate	
9.	plumb	plum		34.	liaison	lee-AY-zon *or* lee-AY-zn	
10	decorate	DEK-oh-rate *or* DEK-uh-rate		35.	exigency	eks-IH-jen-say *or* eks-IH-jen-see	
11.	fierce	fee-us *or* feerss		36.	xenophobia	zen-oh-FO-bee-uh	
12.	knead	need		37.	ogre	OH-gur	
13.	aisle	iyle		38.	scurrilous	SKUR-ih-lus *or* SKUR-uh-lus	
14.	vengeance	VEN-jnss		39.	ethereal	ih-THEE-ree-ul *or* ih-THEER-ee-ul	
15.	prestigious	pre-STIJ-us *or* pre-STEEJ-us		40.	paradigm	PAH-rah-dime	
16.	wreathe	reeTH *or* REEEth		41.	perspicuity	per-spuh-KYEW-uh-tee	
17.	gnat	nat		42.	plethora	PLETH-oh-rah *or* PLETH-eh-rah	
18.	amphitheatre	AM-fih-thee-uh-ter		43.	lugubrious	loo-GOOB-ree-uss *or* loo-GOO-bree-uss	
19.	lieu	loo *or* l(y)oo		44.	treatise	TREE-tiz *or* TREET-iz	
20.	grotesque	gro-TESK		45.	dilettante	DILL-ih-tan-tay *or* DILL-uh-tahnt	
21.	iridescent	ihr-ih-DESS-unt *or* ihr-uh-DESS-unt		46.	vertiginous	ver-TIDJ-in-iss	
22.	ballet	BA-lay *or* ba-LAY *or* bal-ay		47.	ubiquitous	you-BIC-wuh-tiss *or* you-BIC-wuh-tus	
23.	equestrian	eh-KWESS-tree-un *or* ih- KWESS-tree-un		48.	hyperbole	hy-PER-bul-lay *or* hy-PUR-bul-lay	
24.	porpoise	PAW-pss *or* POR-poyz (Scots)		49.	insouciant	in-SOO-see-yunt	
25.	aesthetic	ess-THET-ik *or* ees-THET-ik		50.	hegemony	heh-GEM-o-nee *or* heh-JEM-o-nee *or* HEH-geh-mon-ee	
						WTAR Raw Score	
						WTAR Standard Score	

Appendix 8: Profile of mood states 2 (POMS-2)

Below is a list of words that describe feelings people have. Please read each one carefully. Then fill in ONE circle that best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY?

	Not at all	A little	Moderately	Quite a bit	Extremely		Not at all	A little	Moderately	Quite a bit	Extremely
1. Tense	O	O	O	O	O	20. Discouraged	O	O	O	O	O
2. Angry	O	O	O	O	O	21. Resentful	O	O	O	O	O
3. Worn out	O	O	O	O	O	22. Nervous	O	O	O	O	O
4. Unhappy	O	O	O	O	O	23. Miserable	O	O	O	O	O
5. Lively	O	O	O	O	O	24. Cheerful	O	O	O	O	O
6. Confused	O	O	O	O	O	25. Bitter	O	O	O	O	O
7. Peeved	O	O	O	O	O	26. Exhausted	O	O	O	O	O
8. Sad	O	O	O	O	O	27. Anxious	O	O	O	O	O
9. Active	O	O	O	O	O	28. Helpless	O	O	O	O	O
10. On edge	O	O	O	O	O	29. Weary	O	O	O	O	O
11. Grouchy	O	O	O	O	O	30. Bewildered	O	O	O	O	O
12. Blue	O	O	O	O	O	31. Furious	O	O	O	O	O
13. Energetic	O	O	O	O	O	32. Full of pep	O	O	O	O	O
14. Hopeless	O	O	O	O	O	33. Worthless	O	O	O	O	O
15. Uneasy	O	O	O	O	O	34. Forgetful	O	O	O	O	O
16. Restless	O	O	O	O	O	35. Vigorous	O	O	O	O	O
17. Unable to concentrate	O	O	O	O	O	36. Uncertain about things	O	O	O	O	O
18. Fatigued	O	O	O	O	O	37. Bushed	O	O	O	O	O
19. Annoyed	O	O	O	O	O						

Appendix 9: Positive and Negative Affect Schedule

This scale consists of a number of words that describe different feelings and emotions.
Read each item and then mark [x] the appropriate answer in the space next to the word.
Indicate to what extent you have felt this way **on average during the past week**.
Use the following scale to record your answer:

No.	Feeling	very slightly or not at all	a little	moderate	quite a bit	extremely
1	interested					
2	distressed					
3	excited					
4	upset					
5	strong					
6	guilty					
7	scared					
8	hostile					
9	enthusiastic					
10	proud					
11	irritable					
12	alert					
13	ashamed					
14	inspired					
15	nervous					
16	determined					
17	attentive					
18	jittery					
19	active					
20	afraid					

Appendix 10: Spielberger State Anxiety Inventory (SSAI)

Read each statement and select the appropriate response to indicate how you feel *right now*, that is, *at this very moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	1 Not at all	**2** A little	**3** Somewhat	**4** Very Much So
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I feel tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel uncomfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I feel jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

Appendix 11: Dutch Eating Behaviour Questionnaire

Please place an **(✓)** in the box which applies best to each of the numbered statements. All of the results will be *strictly* confidential. Most of the questions directly relate to food or eating, although other types of questions have been included. Please answer each question carefully. Thank you.

1. If you have put on weight, do you eat less than you usually do?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

2. Do you try to eat less at mealtimes than you would like to eat?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

3. How often do you refuse food or drink offered because you are concerned about your weight?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

4. Do you watch exactly what you eat?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

5. Do you deliberately eat foods that are slimming?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

6. When you have eaten too much, do you eat less than usual the following days?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

7. Do you deliberately eat less in order not to become heavier?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

8. How often do you try not to eat between meals because you are watching your weight?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

9. How often in the evening do you try not to eat because you are watching your weight?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

10. Do you take into account your weight with what you eat?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

11. Do you have the desire to eat when you are irritated?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

12. Do you have a desire to eat when you have nothing to do?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

13. Do you have a desire to eat when you are depressed or discouraged?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

14. Do you have a desire to eat when you are feeling lonely?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

15. Do you have a desire to eat when somebody lets you down?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

16. Do you have a desire to eat when you are cross?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

17. Do you have a desire to eat when you are approaching something unpleasant to happen?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

18. Do you get the desire to eat when you are anxious, worried or tense?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

19. Do you have a desire to eat when things are going against you or when things have gone wrong?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

20. Do you have a desire to eat when you are frightened?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

21. Do you have a desire to eat when you are disappointed?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

22. Do you have a desire to eat when you are bore or restless?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

23. Do you have a desire to eat when you are emotionally upset?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often ❑ Not Relevant

24. If food tastes good to you, do you eat more than usual?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

25. If food smells and looks good do you eat more than usual?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

26. If you see or smell something delicious, do you have the desire to eat it?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

27. If you have something delicious to eat, do you eat it straight away?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

28. If you walk past the baker do you have the desire to buy something delicious?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

29. If you walk past a snackbar or a café, do you have the desire to buy something delicious?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

30. If you see others eating, do you also have the desire to eat?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

31. Can you resist eating delicious foods?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

32. Do you eat more than usual, when you see others eating?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

33. When preparing a meal are you inclined to eat something?

❑ Never ❑ Seldom ❑ Sometimes ❑ Often ❑ Very Often

Appendix 12: Three Factor Eating Questionnaire

Please circle the response that you feel best describes you

Part I	True	False
1. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	**T**	**F**
2. I usually eat too much at social occasions, like parties and picnics.	**T**	**F**
3. I am usually so hungry that I eat more than three times a day.	**T**	**F**
4. When I have eaten my quota of calories, I am usually good about not eating any more.	**T**	**F**
5. Dieting is so hard from me because I just get too hungry.	**T**	**F**
6. I deliberately take small helpings as a means of controlling my weight.	**T**	**F**
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	**T**	**F**
8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.	**T**	**F**
9. When I feel anxious, I find myself eating.	**T**	**F**
10. Life is too short to worry about dieting.	**T**	**F**
11. Since my weight goes up and down, I have gone on reducing diets more than once.	**T**	**F**
12. I often feel so hungry that I just have to eat something.	**T**	**F**
13. When I am with someone who is overeating, I usually overeat too.	**T**	**F**
14. I have a pretty good idea of the number of calories in common food.	**T**	**F**
15. Sometimes when I start eating, I just can't seem to stop.	**T**	**F**
16. It is not difficult for me to leave something on my plate.	**T**	**F**
17. At certain times of the day, I get hungry because I have gotten used to eating then.	**T**	**F**
18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.	**T**	**F**
19. Being with someone who is eating often makes me hungry enough to eat also.	**T**	**F**
20. When I feel blue, I often overeat.	**T**	**F**
21. I enjoy eating too much to spoil it by counting calories or watching my weight.	**T**	**F**
22. When I see a real delicacy, I often get so hungry that I have to eat right away.	**T**	**F**
23. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.	**T**	**F**
24. I get so hungry that my stomach often seems like a bottomless pit.	**T**	**F**
25. My weight has hardly changed at all in the last ten years.	**T**	**F**
26. I am always hungry, so it is hard for me to stop eating before I finish that food on my plate.	**T**	**F**
27. When I feel lonely, I console myself by eating.	**T**	**F**
28. I consciously hold back at meals in order not to gain weight.	**T**	**F**
29. I sometimes get very hungry late in the evening or at night.	**T**	**F**

30. I eat anything I want, any time I want.	**T**	**F**
31. Without even thinking about it, I take a long time to eat.	**T**	**F**
32. I count calories as a means of controlling my weight.	**T**	**F**
33. I do not eat some foods because they make me fat.	**T**	**F**
34. I am always hungry enough to eat at any time.	**T**	**F**
35. I pay a great deal of attention to changes in my figure.	**T**	**F**
36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.	**T**	**F**

Part II				
37. How often are you dieting in a conscious effort to control your weight?	rarely	sometimes	usually	always
38. Would a weight fluctuation of 5 lbs (2¼ kg) affect the way you live your life?	not at all	slightly	moderately	very much
39. How often do you feel hungry?	only at mealtime	sometimes between meals	often between meals	almost always
40. Do your feelings of guilt about overeating help you to control your food intake?	never	rarely	often	always
41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?	easy	slightly difficult	moderately difficult	very difficult
42. How conscious are you of what you are eating?	not at all	slightly	moderately	extremely
43. How frequently do you avoid 'stocking up' on tempting foods?	almost never	seldom	usually	almost always
44. How likely are you to shop for low calorie foods?	unlikely	slightly unlikely	moderately unlikely	very likely
45. Do you eat sensibly in front of others and splurge alone?	never	rarely	often	always
46. How likely are you to consciously eat slowly in order to cut down on how much you eat?	unlikely	slightly unlikely	moderately unlikely	very likely
47. How frequently do you skip dessert because you are no longer hungry?	almost never	seldom	at least once a week	almost every day
48. How likely are you to consciously eat less than you want?	unlikely	slightly likely	moderately likely	very likely
49. Do you go on eating binges though you are not hungry?	Never	rarely	sometimes	at least once a week

50. On a scale of 0 to 5, where 0 means no restraint (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number, would you give yourself?	0 eat whatever you want, whenever you want it	1 usually eat whatever you want, whenever you want it	2 often eat whatever you want, whenever you want it	3 often limit food intake, but often 'give in'	4 usually limit food intake, rarely 'give in'	5 constantly limiting food intake, never 'giving in'
51. To what extent does this statement describe your eating behaviour? 'I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow'	not like me	little like me		pretty good description of me	describes me perfectly	

Appendix 13: Power of Food Scale (PFS)

Please indicate the extent to which you agree that the following items describe you. Use the following 1-5 scale for your responses.

1. don't agree at all
2. agree a little
3. agree somewhat
4. agree
5. strongly agree

1. I find myself thinking about food even when I'm not physically hungry.
2. When I'm in a situation where delicious foods are present, but I have to wait to eat them, it is very difficult for me to wait.
3. I get more pleasure from eating than I do from almost anything else.
4. I feel that food is to me like liquor is to an alcoholic.
5. If I see or smell a food I like, I get a powerful urge to have some.
6. When I'm around a fattening food I love, it's hard to stop myself from at least tasting it.
7. I often think about what foods I might eat later in the day.
8. It's scary to think of the power that food has over me.
9. When I taste a favourite food, I feel intense pleasure.
10. When I know a delicious food is available, I can't help myself from thinking about having some.
11. I love the taste of certain foods so much that I can't avoid eating them even if they're bad for me.
12. When I see delicious foods in advertisements or commercials, it makes me want to eat.
13. I feel like food controls me rather than the other way around.
14. Just before I taste a favourite food, I feel intense anticipation.
15. When I eat delicious food, I focus a lot on how good it tastes.
16. Sometimes, when I'm doing everyday activities, I get an urge to eat "out of the blue" (for no apparent reason).
17. I think I enjoy eating a lot more than most other people.
18. Hearing someone describe a great meal makes me really want to have something to eat.
19. It seems like I have food on my mind a lot.
20. It's very important to me that the foods I eat are as delicious as possible.
21. Before I eat a favourite food my mouth tends to flood with saliva.

Appendix 14: Yale Food Addiction Scale (YFAS)

This survey asks about your eating habits in the past year. People sometimes have difficulty controlling their intake of certain foods such as:

- Sweets like ice cream, chocolate, doughnuts, cookies, cake, candy, ice cream
- Starches like white bread, rolls, pasta, and rice
- Salty snacks like chips, pretzels, and crackers
- Fatty foods like steak, bacon, hamburgers, cheeseburgers, pizza, and French fries
- Sugary drinks like soda pop

When the following questions ask about "CERTAIN FOODS" please think of ANY food similar to those listed in the food group or ANY OTHER foods you have had a problem with in the past year. Use the following 1-5 scale for your responses.

1. Never
2. Once a month
3. 2-4 times a month
4. 2-3 times a week
5. 4 or more times a week or daily

IN THE PAST 12 MONTHS:

1. I find that when I start eating certain foods, I end up eating much more than planned
2. I find myself continuing to consume certain foods even though I am no longer hungry
3. I eat to the point where I feel physically ill
4. Not eating certain types of food or cutting down on certain types of food is something I worry about
5. I spend a lot of time feeling sluggish or fatigued from overeating
6. I find myself constantly eating certain foods throughout the day
7. I find that when certain foods are not available, I will to go out of my way to obtain them. For example, I will drive to the store to purchase certain foods even though I have other options available at home.
8. There have been times when I consumed certain foods so often or in such large quantities that I started to eat food instead of working, spending time with my family or friends, or engaging in other important activities or recreational activities I enjoy.
9. There have been times when I consumed certain foods so often or in such large quantities that I spent time dealing with negative feelings from overeating instead of working, spending time with my family or friends, or engaging in other important activities or recreational activities I enjoy
10. There have been times when I avoided professional or social situations where certain foods were available, because I was afraid I would overeat
11. There have been times when I avoided professional or social situations because I was not able to consume certain foods there
12. I have had withdrawal symptoms such as agitation, anxiety, or other physical symptoms when I cut down or stopped eating certain foods. (Please do NOT include withdrawal symptoms caused by cutting down on caffeinated beverages such as soda pop, coffee, tea, energy drinks, etc.)
13. I have consumed certain foods to prevent feelings of anxiety, agitation, or other physical symptoms that were developing. (Please do NOT include consumption of caffeinated beverages such as soda pop, coffee, tea, energy drinks, etc.)
14. I have found that I have elevated desire for or urges to consume certain foods when I cut down or stop eating them
15. My behaviour with respect to food and eating causes significant distress
16. I experience significant problems in my ability to function effectively (daily routine, job/school, social activities, family activities, health difficulties) because of food and eating

Please answer Yes or No for the following statements.

17. My food consumption has caused significant psychological problems such as depression, anxiety, self-loathing, or guilt
18. My food consumption has caused significant physical problems or made a physical problem worse
19. I kept consuming the same types of food or the same amount of food even though I was having emotional and/or physical problems
20. Over time, I have found that I need to eat more and more to get the feeling I want, such as reduced negative emotions or increased pleasure
21. I have found that eating the same amount of does not reduce my negative emotions or increase pleasurable feelings the way it used to
22. I want to cut down or stop eating certain kinds of food
23. I have tried to cut down or stop eating certain kinds of food
24. I have been successful at cutting down or not eating these kinds of food
25. How many times in the past year did you try to cut down or stop eating certain foods altogether?

1 or fewer times 2 times 3 times 4 times 5 or more times

Appendix 15: Binge Eating Scale (BES)

Below are groups of statements about behaviour, thoughts, and emotional states.
Please indicate which statement in each group best describes how you feel.

1.
   - ☐ I do not think about my weight or size when I'm around other people.
   - ☐ I worry about my appearance, but it does not make me unhappy.
   - ☐ I think about my appearance or weight and I feel disappointed in myself.
   - ☐ I frequently think about my weight and feel great shame and disgust.

2.
   - ☐ I have no difficulty eating slowly.
   - ☐ I may eat quickly, but I never feel too full.
   - ☐ Sometimes after I eat fast I feel too full.
   - ☐ Usually I swallow my food almost without chewing, then feel as if I ate too much.

3.
   - ☐ I can control my impulses towards food.
   - ☐ I think I have less control over food than the average person.
   - ☐ I feel totally unable to control my impulses toward food.
   - ☐ I feel totally unable to control my relationship with food and I try desperately to fight my impulses toward food.

4.
   - ☐ I do not have a habit of eating when I am bored.
   - ☐ Sometimes I eat when I am bored, but I can often distract myself and not think about food.
   - ☐ I often eat when I am bored, but I can sometimes distract myself and not think about food.
   - ☐ I have a habit of eating when I am bored and nothing can stop me.

5.
   - ☐ Usually when I eat it is because I am hungry.
   - ☐ Sometimes I eat on impulse without really being hungry.
   - ☐ I often eat to satisfy hunger even when I know I've already eaten enough. On these occasions I can't even enjoy what I eat.
   - ☐ Although I have not physically hungry, I feel the need to put something in my mouth and I feel satisfied or only when I can fill my mouth (for example with a piece of bread).

6.
   - ☐ After eating too much:
   - ☐ I do not feel guilty or regretful at all.
   - ☐ I sometimes feel guilty or regretful.
   - ☐ I almost always feel a strong sense of guilt or regret.

7.

- ☐ When I'm on a diet, I never completely lose control of food, even in times when I eat too much.
- ☐ When I eat a forbidden food on a diet, I think I've failed and eat even more.
- ☐ When I'm on a diet and I eat too much, I think I've failed and eat even more.
- ☐ I am always either binge eating or fasting.

8.

- ☐ It is rare that I eat so much that I felt uncomfortably full.
- ☐ About once a month I eat so much that I felt uncomfortably full.
- ☐ There are regular periods during the month when I eat large amounts of food at meals or between meals.
- ☐ I eat so much that usually, after eating, I feel pretty bad and I have nausea.

9.

- ☐ The amount of calories that I consume is fairly constant over time.
- ☐ Sometimes after I eat too much, I try to consume few calories to make up for the previous meal.
- ☐ I have a habit of eating too much at night. Usually I'm not hungry in the morning and at night I eat too much.
- ☐ I have periods of about a week in which I imposed starvation diets, following periods of when I ate too much. My life is made of binges and fasts.

10.

- ☐ I can usually stop eating when I decide I've had enough.
- ☐ Sometimes I feel an urge to eat that I cannot control.
- ☐ I often feel impulses to eat so strong that I cannot win, but sometimes I can control myself.
- ☐ I feel totally unable to control my impulses to eat.

11.

- ☐ I have no problems stopping eating when I am full.
- ☐ I can usually stop eating when I feel full, but sometimes I eat so much it feels unpleasant.
- ☐ It is hard for me to stop eating once I start, I usually end up feeling too full.
- ☐ It is a real problem for me to stop eating and sometimes I vomit because I feel so full.

12.

- ☐ I eat the same around friends and family as I do when I am alone.
- ☐ Sometimes I do not eat what I want around others because I am aware of my problems with food.
- ☐ I often eat little around other people because I feel embarrassed.
- ☐ I'm so ashamed of overeating, I only eat at times when no one sees me. I eat in secret.

13.

- ☐ I eat three meals a day and occasionally a snack.
- ☐ I eat three meals a day and I usually snack as well.
- ☐ I eat many meals or skip meals regularly.
- ☐ There are times when I seem to eat continuously without regular meals.

14.

- ☐ I don’t think about impulses to eat very much.
- ☐ Sometimes my mind is occupied with thoughts of how to control the urge to eat.
- ☐ I often spend much time thinking about what I ate or how not to eat.
- ☐ My mind is busy most of the time with thoughts about eating.
- ☐ I seem to be constantly fighting not to eat.

15.

- ☐ I don’t think about food any more than most people.
- ☐ I have strong desires for food, but only for short periods.
- ☐ There are some days when I think of nothing but food.
- ☐ Most of my days is filled with thoughts of food. I feel like I live to eat.

16.

- ☐ I usually know if I am hungry or not. I know what portion sizes are appropriate.
- ☐ Sometimes I do not know if I am physically hungry or not. In these moments, I can hardly understand how much food is appropriate.
- ☐ Even if I knew how many calories I should eat, I would not have a clear idea of what is, for me, a normal amount of food.

Appendix 16: Barratt Impulsiveness Scale

Please read the following statements and circle the number that you think most accurately describes you.

1= Rarely/Never
2= Occasionally
3= Often
4= Almost always/Always

1. I plan tasks carefully	**1**	**2**	**3**	**4**
2. I do things without thinking	**1**	**2**	**3**	**4**
3. I make up my mind quickly	**1**	**2**	**3**	**4**
4. I am happy-go-lucky	**1**	**2**	**3**	**4**
5. I don't 'pay attention'	**1**	**2**	**3**	**4**
6. I have 'racing' thoughts	**1**	**2**	**3**	**4**
7. I plan trips well ahead of time	**1**	**2**	**3**	**4**
8. I am self-controlled	**1**	**2**	**3**	**4**
9. I concentrate easily	**1**	**2**	**3**	**4**
10. I save regularly	**1**	**2**	**3**	**4**
11. I 'squirm' at plays or lectures	**1**	**2**	**3**	**4**
12. I am a careful thinker	**1**	**2**	**3**	**4**
13. I plan for job security	**1**	**2**	**3**	**4**
14. I say things without thinking	**1**	**2**	**3**	**4**
15. I like to think about complex problems	**1**	**2**	**3**	**4**
16. I change jobs	**1**	**2**	**3**	**4**
17. I act on impulse	**1**	**2**	**3**	**4**
18. I get easily bored when solving thought problems	**1**	**2**	**3**	**4**
19. I act on the spur of the moment	**1**	**2**	**3**	**4**
20. I am a steady thinker	**1**	**2**	**3**	**4**
21. I change residences	**1**	**2**	**3**	**4**
22. I buy things on impulse	**1**	**2**	**3**	**4**
23. I can only think about one problem at a time	**1**	**2**	**3**	**4**
24. I change hobbies	**1**	**2**	**3**	**4**
25. I spend or charge more than I earn	**1**	**2**	**3**	**4**
26. I often have extraneous thoughts when thinking	**1**	**2**	**3**	**4**
27. I am more interested in the present than the future	**1**	**2**	**3**	**4**
28. I am restless at the theatre or lectures	**1**	**2**	**3**	**4**
29. I like puzzles	**1**	**2**	**3**	**4**
30. I am future orientated	**1**	**2**	**3**	**4**

Appendix 17: Urgency, Premeditation, Perseverance, Sensation seeking, and Positive urgency (UPPS-P) Impulsive Behaviour Scale

Below are a number of statements that describe ways in which people act and think. For each statement, please indicate how much you agree or disagree with the statement. Use the following 1-4 scale for your responses.

1. Agree Strongly
2. Agree Somewhat
3. Disagree Somewhat
4. Disagree Strongly

---

1. I have a reserved and cautious attitude toward life
2. I have trouble controlling my impulses
3. I generally seek new and exciting experiences and sensations
4. I generally like to see things through to the end
5. When I am very happy, I can't seem to stop myself from doing things that can have bad consequences
6. My thinking is usually careful and purposeful
7. I have trouble resisting my cravings (for food, cigarettes, etc)
8. I'll try anything once
9. I tend to give up easily
10. When I am in a great mood, I tend to get into situations that could cause me problems
11. I am not one of those people who blurt out things without thinking
12. I often get involved in things I later wish I could get out of
13. I like sports and games in which you have to choose your next move very quickly
14. Unfinished tasks really bother me
15. When I am very happy, I tend to do things that may cause problems in my life
16. I like to stop and think things over before I do them
17. When I feel bad, I will often do things I later regret in order to make myself feel better now 18. I would enjoy water skiing
19. Once I get going on something, I hate to stop
20. I tend to lose control when I am in a great mood
21. I don't like to start a project until I know exactly how to proceed
22. Sometimes when I feel bad, I can't seem to stop what I'm doing even though it is making me feel worse
23. I quite enjoy taking risks
24. I concentrate easily
25. When I am really ecstatic, I tend to get out of control
26. I would enjoy parachute jumping
27. I finish what I start
28. I tend to value and follow a rational, "sensible" approach to things
29. When I am upset I often act without thinking
30. Others would say I make bad choices when I am extremely happy about something
31. I welcome new and exciting experiences and sensations, even if they are a little frightening and unconventional
32. I am able to pace myself so as to get things done on time
33. I usually make up my mind through careful reasoning
34. When I feel rejected, I will often say things that I later regret
35. Others are shocked or worried about the things I do when I am feeling very excited
36. I would like to learn to fly an airplane

37. I am a person who always gets the job done
38. I am a cautious person
39. It is hard for me to resist acting on my feelings
40. When I get really happy about something, I tend to do things that can have bad consequences
41. I sometimes like doing things that are a bit frightening
42. I almost always finish projects that I start
43. Before I get into a new situation I like to find out what to expect from it
44. I often make matters worse because I act without thinking when I am upset
45. When overjoyed, I feel like I can't stop myself from going overboard
46. I would enjoy the sensation of skiing very fast down a high mountain slope
47. Sometimes there are so many little things to be done that I just ignore them all
48. I usually think carefully before doing anything
49. When I am really excited, I tend not to think of the consequences of my actions
50. In the heat of an argument, I will often say things that I later regret
51. I would like to go scuba diving
52. I tend to act without thinking when I am really excited
53. I always keep my feelings under control
54. When I am really happy, I often find myself in situations that I normally wouldn't be comfortable with
55. Before making up my mind, I consider all the advantages and disadvantages
56. I would enjoy fast driving
57. When I am very happy, I feel like it is ok to give in to cravings or overindulge
58. Sometimes I do impulsive things that I later regret
59. I am surprised at the things I do wile in a great mood

Appendix 18: Behavioural activation / behavioural inhibition scale (BAS/BIS)

Each item of this questionnaire is a statement that a person may either agree with or disagree with. For each item, indicate how much you agree or disagree with what the item says by putting a circle around the appropriate number. Please respond to all the items; do not leave any blank. Choose only one response to each statement. Please be as accurate and honest as you can be. Respond to each item as if it were the only item. That is, don't worry about being "consistent" in your responses. Choose from the following four response options:

---

1 very true for me
2 somewhat true for me
3 somewhat false for me
4 very false for me

1. A person's family is the most important thing in life.
2. Even if something bad is about to happen to me, I rarely experience fear or nervousness
3. I go out of my way to get things I want.
4. When I'm doing well at something I love to keep at it.
5. I'm always willing to try something new if I think it will be fun.
6. How I dress is important to me.
7. When I get something I want, I feel excited and energized.
8. Criticism or scolding hurts me quite a bit.
9. When I want something I usually go all-out to get it.
10. I will often do things for no other reason than that they might be fun.
11. It's hard for me to find the time to do things such as get a haircut.
12. If I see a chance to get something I want I move on it right away.
13. I feel pretty worried or upset when I think or know somebody is angry with me.
14. When I see an opportunity for something I like I get excited right away.
15. I often act on the spur of the moment.
16. If I think something unpleasant is going to happen I usually get pretty "worked up."
17. I often wonder why people act the way they do.
18. When good things happen to me, it affects me strongly.
19. I feel worried when I think I have done poorly at something important.
20. I crave excitement and new sensations.
21. When I go after something I use a "no holds barred" approach.
22. I have very few fears compared to my friends.
23. It would excite me to win a contest.
24. I worry about making mistakes.

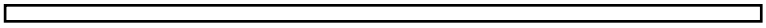
Appendix 19: Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria for Alcohol Use Disorder

In the past year, have you:

1. Had times when you ended up drinking more, or longer, than you intended?
2. More than once wanted to cut down of stop drinking, or tried to, but couldn't?
3. Spent a lot of time drinking? Or being sick or getting over aftereffects?
4. Wanted a drink so badly you couldn't think of anything else?
5. Found that drinking – or being sick from drinking - often interfered with taking care of your home or family? Or caused job troubles? Or school problems?
6. Continued to drink even though it was causing trouble with your family or friends?
7. Given up or cut back on activities that were important or interesting to you, or gave you pleasure, in order to drink?
8. More than once gotten into situations while or after drinking that increased your chances of getting hurt (such as driving, swimming, using machinery, walking in a dangerous area, or having unsafe sex)?
9. Continued to drink even though it was making you feel depressed or anxious or adding to another health problem? Or after having had a memory blackout?
10. Had to drink much more than you once did to get the effect you want? OR found that your usual number of drinks had much less effect than before?
11. Found that when the effects of alcohol were wearing off, you had withdrawal symptoms, such as trouble sleeping, shakiness, restlessness, nausea, sweating, a racing heart, or a seizure? Or sensed things that were not there?

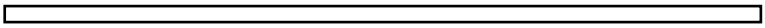
Appendix 20: Visual Analogue Scale (VAS)

HOW HUNGRY DO YOU FEEL RIGHT NOW?



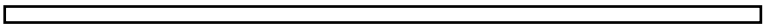
NOT AT ALL EXTREMELY

HOW SICK DO YOU FEEL RIGHT NOW?



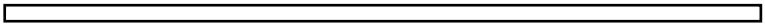
NOT AT ALL EXTREMELY

HOW PLEASANT WOULD IT BE TO EAT RIGHT NOW?



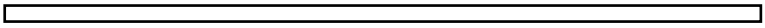
NOT AT ALL EXTREMELY

HOW ANXIOUS DO YOU FEEL RIGHT NOW?



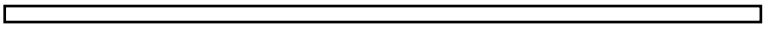
NOT AT ALL EXTREMELY

HOW MUCH DO YOU THINK YOU COULD EAT RIGHT NOW?



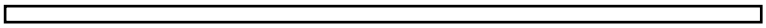
NOTHING A LARGE AMOUNT

HOW FULL DO YOU FEEL RIGHT NOW?



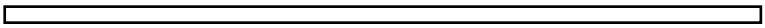
NOT AT ALL EXTREMELY

HOW STRESSED DO YOU FEEL RIGHT NOW?



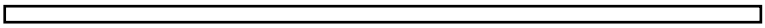
NOT AT ALL EXTREMELY

HOW SLEEPY DO YOU FEEL RIGHT NOW?



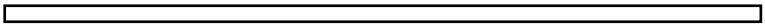
NOT AT ALL EXTREMELY

ALL I WANT TO DO IS TO EAT RIGHT NOW.



NOT AT ALL EXTREMELY

NOTHING WOULD BE BETTER THAN EATING RIGHT NOW.



NOT AT ALL EXTREMELY

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