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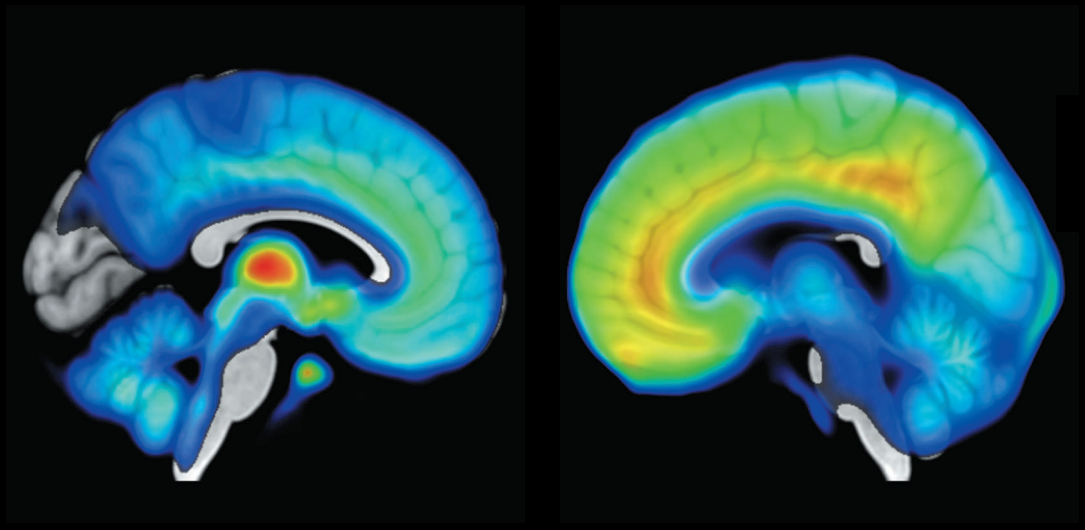

BRAIN OPIOID AND ENDOCANNABINOID SYSTEMS AS RISK FACTORS FOR OBESITY

Positron emission tomography studies of μ-opioid and CB_1 receptors with glucose uptake analysis

Tatu Kantonen

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ABSTRACT

The prevalence of obesity is increasing globally. Obesity is a major threat to public health since it predisposes individuals to multiple non-communicable diseases. Obesity is difficult to treat or prevent. The modern environment has been blamed for the obesity epidemic, due to the abundance of energy-dense and aggressively advertised foods. The brain is the most important organ controlling energy homeostasis and feeding. However, we do not know which brain pathways render some individuals susceptible to the obesity development in the current environment.

The aim of this thesis was to examine whether variation in the brain opioid and endocannabinoid pathways explains differences in the risk for obesity development. Two receptor systems associated with food intake and reward processing were investigated: μ-opioid receptors (MOR) and cannabinoid CB_1 receptors (CB_1R). MORs were measured with [^{11}C]carfentanil, and CB_1Rs with [^{18}F]FMPEP-d_2. In addition, brain glucose uptake (BGU) was quantified with [^{18}F]FDG. Healthy, non-obese humans were studied with positron emission tomography in four studies investigating I) the effects of demographic factors on MORs, II) the associations of obesity risk factors on MORs, CB_1Rs and BGU, III) the physical fitness and MOR function, and IV) how MORs and CB_1Rs associate with feeding behavior.

Age, sex and smoking influenced MOR availability, which may contribute to obesity development in specific populations. Familial obesity risk associated with increased BGU but low neuroreceptor availability, suggesting that vulnerability to obesity may be mediated by disruption of these interconnected pathways. Impulsive feeding was associated with reduced MOR availability, which may underlie excessive food intake and weight gain. Central capacity for releasing endogenous MOR ligands was dependent on aerobic fitness, suggesting that the MOR function may be critical in habitual exercise and weight maintenance. Obesity risk factors and circulating cannabinoids associated with reduced CB_1R availability, suggesting that an overactive cannabinoid system may facilitate weight gain. In conclusion, multiple neurochemical alterations previously associated with obesity are already present in a number of non-obese individuals, which may increase their risk for future obesity.

KEYWORDS: brain, opioid, cannabinoid, obesity, positron emission tomography

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
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TIIVISTELMÄ

Lihavuus yleistyy ympäri maailmaa. Lihavuus on yksi merkittävimmistä uhista väestön terveydelle, sillä painon kertyminen altistaa useille kansansairauksille. Lihavuutta on vaikea hoitaa tai ehkäistä. Nykyistä elinympäristöä on syytetty lihavuusepidemiasta, sillä ympäristö on täynnä energiatiiviitä ja voimakkaasti markkinoituja ruokatuotteita. Aivot ovat tärkein energiatasapainoa ja syömistä säätelevä elin. Emme kuitenkaan tiedä, mitkä muutokset aivojen toiminnassa saavat osan ihmisistä lihomaan tässä ympäristössä.

Väitöskirjan tavoitteena oli selvittää, selittävätkö aivojen opioidi- ja endokannabinoidijärjestelmän muutokset eroja ihmisten välisessä lihomisriskissä. Tutkimme kahta aivojen välittäjäainejärjestelmää, jotka säätelevät syömisen palkkiokokemuksia: μ-opioidireseptoreja (MOR) ja CB_1-kannabinoidireseptoreja (CB_1R). MOR-sitoutumista mitattiin [^{11}C]karfentaniililla, ja CB_1R-sitoutumista [^{18}F]FMPEP-d_2-merkkaineella. Aivojen glukoosinottoa mitattiin lisäksi [^{18}F]FDG-merkkiaineella. Tutkimme terveitä, ei-lihavia ihmisiä positroniemissiotomografialla neljässä tutkimuksessa, joissa selvitettiin: I) väestömuuttujien vaikutusta MOR-sitoutumiseen, II) lihavuuden riskitekijöiden vaikutusta MOR- ja CB_1R-sitoutumiseen sekä aivojen glukoosinottoon, III) fyysistä kuntoa ja MOR-toimintaa, ja IV) MOR- ja CB_1R-sitoitumisen yhteyttä syömiskäyttäytymiseen.

Ikä, sukupuoli ja tupakointi vaikuttivat MOR-sitoutumiseen, mikä voi selittää eroja lihavuuden kehittymisessä eri väestöryhmissä. Perheeseen liittyvä lihomisriski oli yhteydessä aivojen glukoosinottoon ja reseptorimääriin, ja näiden järjestelmien häiriintyminen saattaa altistaa lihavuudelle. Impulsiivinen syömiskäyttäytyminen liittyi alentuneeseen MOR-sitoutumiseen, mikä voi altistaa liialliselle syömiselle ja painon nousulle. Sisäsyntyisten opioidien vapauttamiskyky oli yhteydessä fyysiseen kuntoon, viitaten MOR-toiminnan merkitykseen liikuntaharrastuksen ylläpidossa ja painonhallinnassa. Lihavuuden riskitekijät ja verenkierron kannabinoidit liittyivät alentuneeseen CB_1R-sitoutumiseen, mikä viittaa siihen, että yliaktiivinen kannabinoidijärjestelmä voi altistaa lihomiselle. Osalla terveistä ihmisistä on siis havaittavissa useita aivokemiallisia muutoksia, jotka saattavat altistaa lihavuudelle.

AVAINSANAT: aivot, opioidi, kannabinoidi, lihavuus, positroniemissiotomografia

Table of Contents

Abbreviations

[^{11}C]carfentanil	^{11}C-labeled carfentanil
[^{18}F]FDG	^{18}F-labeled fluorodeoxyglucose
[^{18}F]FMPEP-d_2	^{18}F-labeled 3-fluoromethoxy-d_2
2-AG	2-arachidonoylglycerol
AEA	Anandamide, *N*-arachidonoylethanolamine
AGRP	Agouti-related protein
BED	Binge eating disorder
BGU	Brain glucose uptake
BMI	Body mass index
CB_1R	Cannabinoid receptor type 1
CB_2R	Cannabinoid receptor type 2
CNS	Central nervous system
CSF	Cerebrospinal fluid
CVD	Cardiovascular disease
D_2R	Dopamine D_2 receptor
DEBQ	Dutch Eating Behavior Questionnaire
DOR	δ-opioid receptor
ECS	Endocannabinoid system
FDA	Food and Drug Administration
FDR	False discovery rate
FUR	Fractional uptake rate
FWE	Family-wise error
GABA	γ-aminobutyric acid
GLP-1	Glucagon-like peptide-1
GPCR	G protein-coupled receptor
HR	High-risk
IDE	Insulin-degrading enzyme
KOR	κ-opioid receptor
LOR	Line of response
LR	Low-risk
MCH	Melanin-concentrating hormone

MOR	μ-opioid receptor
MRI	Magnetic resonance imaging
MVPA	Moderate to vigorous physical activity
NOR	Nociceptin-orphanin FQ receptor
OPRM1	Opioid receptor μ 1 gene
PET	Positron emission tomography
POMC	Pro-opiomelanocortin
PVN	Paraventricular nucleus
ROI	Region of interest
T2D	Type 2 diabetes
$TRPV_1$	Transient receptor potential vanilloid type 1 receptor
VO_{2peak}	Peak oxygen consumption
α-MSH	α-melanocyte-stimulating hormone

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Kantonen, T., Karjalainen, T., Isojärvi, J., Nuutila, P., Tuisku, J., Rinne, J., Hietala, J., Kaasinen, V., Kalliokoski, K., Scheinin, H., Hirvonen, J., Vehtari, A., Nummenmaa, L. Interindividual variability and lateralization of μ-opioid receptors in the human brain. *Neuroimage*, 2020; 217: 116922.

II Kantonen, T., Pekkarinen, L., Karjalainen, T., Bucci, M., Kalliokoski, K., Haaparanta-Solin, M., Aarnio, R., Dickens, A.M., von Eyken, A., Laitinen, K., Houttu, N., Kirjavainen, A.K., Helin, S., Hirvonen, J., Rönnemaa, T., Nuutila, P., Nummenmaa, L. Obesity risk is associated with altered cerebral glucose metabolism and decreased μ-opioid and CB_1 receptor availability. *International Journal of Obesity*, 2021: 1-8.

III Saanijoki, T., Kantonen, T., Pekkarinen, L., Kalliokoski, K., Hirvonen, J., Malén, T., Tuominen, L., Tuulari, J.J., Arponen, E., Nuutila, P., Nummenmaa, L. Aerobic fitness is associated with cerebral mu-opioid receptor activation in healthy humans. 2021. *Manuscript*.

IV Kantonen, T., Karjalainen, T., Pekkarinen, L., Isojärvi, J., Kalliokoski, K., Kaasinen, V., Hirvonen, J., Nuutila, P., Nummenmaa, L. Cerebral μ-opioid and CB_1 receptor systems have distinct roles in human feeding behavior. *Translational Psychiatry*, 2021; 11: 442.

1 Introduction

Obesity affects hundreds of millions of people and is strongly linked to numerous major non-communicable diseases. Worldwide, there were over 600 million obese adults in 2016, and the prevalence continues to rise around the globe (Abarca-Gómez et al., 2017; Malik et al., 2020). Effective means for treating and preventing obesity would have immense global health benefit (Nyberg et al., 2018). Today, the most effective strategy for treating severe or morbid obesity is bariatric surgery, which leads to a sustained weight loss of over 20% on average (Adams et al., 2017; Lingvay et al., 2021). However, invasive surgery carries multiple risks for acute complications and long-term adverse effects (e.g. dumping syndrome, nutrient deficiencies), and is not feasible for the routine management of common obesity (Lingvay et al., 2021).

The need for widely applicable and non-invasive treatments for obesity is thus evident. Intensive weight-loss programs lead on average to only ~ 3% reduction in body weight (Anderson et al., 2001), and only two in ten individuals are able to reach clinically meaningful and long-term weight loss with such lifestyle interventions (Lingvay et al., 2021). According to a recent meta-analysis, there exists three anti-obesity drug types that are clearly more effective in lowering body weight than lifestyle interventions alone (mean reductions in body weight are provided): Phentermine/topiramate (~ 8%), glucagon-like peptide-1 (GLP-1) receptor agonists (~ 6%), and naltrexone/bupropion (~ 4%) (Shi et al., 2021). Phentermine/topiramate is a combination of two obesity monotherapies. Pharmacodynamically phentermine is related to amphetamine, and promotes weight loss by activating the brain's norepinephrine pathways, while topiramate probably mediates its effects via multiple different central pathways. However, phentermine/topiramate has not been approved by the European Medicines Agency due to safety concerns, and side-effects including dry mouth and paresthesia are common (Lei et al., 2021; Smith et al., 2013). GLP-1 receptor agonists stimulate insulin secretion and are used in diabetes to improve glycemic control, but they also suppress food intake and thus promote weight loss (Larsen, 2008; Sharma et al., 2018). Naltrexone/bupropion targets the brain's opioid system to reduce appetite (Srivastava et al., 2018) (Chapter 2.4.2).

Despite multiple available treatment options, current pharmacological and behavioral treatments have proven unsuccessful in halting the continuously rising prevalence of obesity (Malik et al., 2020). At the core of the problem is the fact that once established increased body adiposity is extremely difficult to revert to healthy levels due to the homeostatic defense of the elevated body mass (Guyenet et al., 2012). We should thus be able to direct more resources to effective weight maintenance interventions for pre-obese risk individuals. Development of such interventions is dependent on a clear-cut characterization of the physiological processes that predispose a proportion of humans to excess adiposity. It is thus evident that we need more profound understanding of the individual risk factors for obesity development.

Obesity is usually defined as a body mass index (BMI) equal or over 30 kg/m^2 (i.e. weight divided by height squared), and overweight is the BMI range 25–29.9 kg/m^2 (Engin, 2017). These definitions are also used in this thesis. Compared to normal weight, overweight and obesity are associated with a loss of disease-free years, and morbidity increases with the severity of obesity (i.e. with higher BMI) (Nyberg et al., 2018). The known risk factors for the development of obesity include genetic (Locke et al., 2015), psychological (Gerlach et al., 2015), environmental (Hill et al., 2003) and socio-economic factors (Parsons et al., 1999). However, fairly little is known about how these factors are related to the function of the organ ultimately regulating our feeding patterns – the brain.

The brain coordinates energy intake based on internal hunger signals and external cues about food availability (Berthoud et al., 2017; Gadde et al., 2018). The hypothalamus is a central homeostatic hub in the brain, long known for its key role in energy balance regulation and its diverse connections across the body (Berthoud et al., 2017). Recent neuroscientific studies have established that the food intake regulation in the hypothalamus is intimately influenced by the decision-making and reward processes of the corticolimbic brain areas, including the amygdala, hippocampus, basal ganglia and frontal cortex (Berthoud et al., 2017). Central opioid and endocannabinoid systems operate in these brain networks and are essential mediators of food reward. Specifically, activation of μ-opioid and CB_1 receptors promotes food intake and the hedonic impact derived from feeding (Richard, 2015). Brain imaging studies have found alterations in these neuroreceptor systems in subjects with morbid obesity and eating disorders (Ceccarini et al., 2016; Karlsson et al., 2015; Majuri et al., 2017). Both μ-opioid and CB_1 receptor systems have been used as drug targets to treat obesity, but with only partial success (Bermudez-Silva et al., 2010; Srivastava et al., 2018). In addition to reduced neuroreceptor availability, insulin-dependent brain glucose uptake (BGU) is widely disrupted in morbid obesity (Tuulari et al., 2013). However, it is unclear whether these central alterations are already present in pre-obese individuals, and if these alterations might

predispose individuals to the development of obesity and dysfunctional feeding behavior. Interindividual differences in the function of μ-opioid and CB_1 receptor networks might increase the risk for obesity development in prone individuals, but thus far there has been no direct evidence to support this idea.

The aim of this thesis was to examine, whether variation in μ-opioid and CB_1 receptor systems is associated with the risk of developing obesity. The μ-opioid and CB_1 receptors were quantified with positron emission tomography (PET) imaging using ^{11}C-labeled carfentanil ([^{11}C]carfentanil) and ^{18}F-labeled 3-fluoromethoxy-*d*$_2$ ([^{18}F]FMPEP-*d*$_2$), respectively. To examine the interactions of neuroreceptor function and central insulin signaling, BGU was measured with ^{18}F-labeled fluorodeoxyglucose ([^{18}F]FDG). Obesity risk was assessed by a physician with access to individual medical history and biobehavioral metrics. The results show that alterations in μ-opioid and CB_1 receptor availability in addition to BGU changes are associated with specific obesity risk factors already in non-obese individuals. The findings underline the role of opioidergic and cannabinergic regulation of distinct feeding behavior traits. The data also show that μ-opioid receptor function is variable across specific populations, which may contribute to obesity risk via the opioidergic regulation of food intake (energy gain) and physical exercise habits (energy expenditure).

2 Review of the Literature

2.1 Obesity – prevalence and related health effects

In the past decades, obesity has emerged as one of the most prevalent public health concerns worldwide. While in 1975 it was estimated that 100 million adults were obese, in 2016, the global estimate had already reached 671 million (Abarca-Gómez et al., 2017). Regionally, obesity is most prevalent in high-income Western counties and among women in Central Asia, the Middle East and North Africa (Malik et al., 2020). Change in the global prevalence of obesity is summarized in **Figure 1**. In certain Pacific islands (including Nauru and Cook Islands), the prevalence of obesity is over 50 to 60 % ((Abarca-Gómez et al., 2017), country-specific data is available at http://ncdrisc.org/data-downloads-adiposity.html).

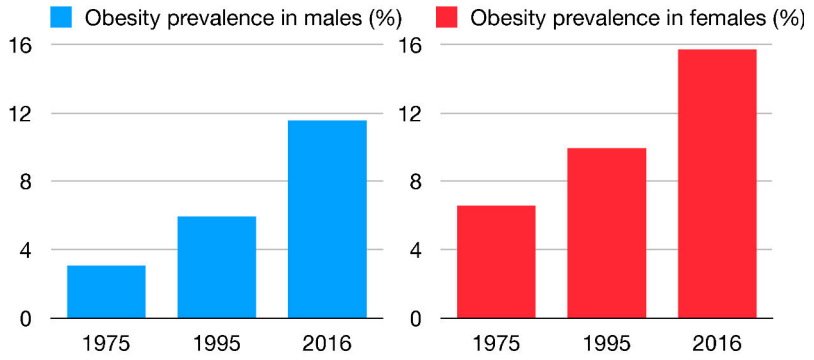

Figure 1. The global prevalence of obesity (BMI 30 kg/m^2 or more) in 1975, 1995 and 2016 separately for males (blue) and females (red). The data is from http://ncdrisc.org/data-downloads-adiposity.html (Abarca-Gómez et al., 2017).

Obesity and overweight account annually for approximately 4 million deaths, most of which are attributable to the accompanying cardiovascular disease (CVD) (Afshin et al., 2017). In addition to CVD, obesity predisposes individuals to type 2 diabetes (T2D) due to the pathological cascade of metabolic processes including systemic inflammation and insulin resistance (Heymsfield et al., 2017; Van Gaal et al., 2006). Adverse metabolic outcomes and major non-communicable diseases associate with

obesity irrespective of an individual's underlying cardiorespiratory fitness or physical activity level (Jukarainen et al., 2017; Nyberg et al., 2018). With regards to brain health, midlife obesity is associated with white and gray matter atrophy (Karlsson et al., 2013) and cognitive decline later in life (Kivipelto et al., 2005). Possibly due to the altered respiratory physiology and inflammatory responses, obesity is also a risk factor for severe forms of viral respiratory disease, including COVID-19, highlighting the importance of obesity management in the face of the most recent and future pandemics (Kalligeros et al., 2020; Zakka et al., 2021). Due to the burden of associated health issues, obese individuals have decreased health-related quality of life (Fontaine et al., 2001) while having substantially increased (~ 30%) individual medical care costs (Withrow et al., 2011). Finally, obesity is directly associated with increased oxidative metabolism but also to increased consumption of food and fossil fuels, and thus it has been estimated that obesity accounts for 1.6% of global greenhouse gas emissions (Magkos et al., 2020).

Treatment of obesity is difficult. The body's homeostatic mechanisms actively defend the increased fat mass, rendering obesity highly treatment resistant once developed (Guyenet et al., 2012). Although promising results are achieved with bariatric surgery, such a complex and invasive operation it is not suitable for routine obesity management (Lingvay et al., 2021). Currently available behavioral or pharmacological interventions have not been successful in reversing the global obesity pandemic (Heymsfield et al., 2017). Thus, effective preventive policies to influence the risk factors of obesity are recognized as a high-priority global health objective (Heymsfield et al., 2017; Malik et al., 2020).

2.2 Risk factors for common obesity

Obesity results from the simple math of positive energy balance where individual's long-term energy gain surpasses energy expenditure (Spiegelman et al., 2001). The modern environment has been called 'obesogenic' because it promotes overconsumption of energy-dense foods and sedentary lifestyle, efficiently resulting in a net positive energy balance (Chaput et al., 2011; Malik et al., 2013). Despite this, variation in body adiposity phenotype is remarkable, suggesting that there is considerable variation in the individual susceptibility for obesity in humans even in the current environment. Accordingly, studies have identified personality traits that protect against ('conscientiousness', 'self-control') and increase the risk for ('neuroticism', 'impulsivity', 'sensitivity to reward') obesity (Gerlach et al., 2015).

Obesity can be viewed as neuro-behavioral condition with major hereditary predisposition (Silventoinen et al., 2020). An individual's risk for obesity increases two to three times higher with a first-degree obese relative (Loos et al., 2003). Apart from rare monogenic obesity syndromes (e.g. leptin or pro-opiomelanocortin

(POMC) deficiency) (Farooqi et al., 2005), genetic risk for common obesity is mediated by multiple genes that are enriched in the central nervous system (CNS), the common genetic variation accounting for about of 20% of BMI variation (Locke et al., 2015). Hundreds of gene loci are associated with obesity in large-scale population studies, but incompletely understood environmental interactions mediate their effects, and one single polymorphism's effect to the actual BMI is weak (Pigeyre et al., 2016). However, normal-weight adolescents with obese parents show increased activation of brain reward pathways in response to palatable food (Stice et al., 2011), further suggesting that the predisposition to overeating is centrally mediated.

Multiple lifestyle and environmental factors have been associated with obesity. In the modern environment, palatable energy-dense food products, large portion sizes and sedentary lifestyle promote obesity in populations (Hill et al., 2003). Low physical activity constitutes a well-known risk factor for weight gain, but also a history of low activity in childhood increases the risk for obesity later in life (Fogelholm et al., 2000; Yang et al., 2007). Other predisposing factors from childhood and youth have also been identified. Early-life BMI correlates strongly with midlife BMI (Juhola et al., 2011), and low socio-economic status in early life (Parsons et al., 1999) and particularly low family income (Juonala et al., 2011) predict obesity in adulthood. In addition, a history of parental obesity or T2D increases the risk for obesity (Anjana et al., 2009; Juonala et al., 2011; Parsons et al., 1999). Smoking cessation is also associated on average with 4.1kg weight gain (Tian et al., 2015), but for a minority of those who quit, the weight gain is substantial (Williamson et al., 1991). In addition, shift work and abnormal sleeping patterns associate with obesity (Antunes et al., 2010). Despite the identification of multiple obesity risk factors, it is still largely unknown how specific risk factors might influence the brain's homeostatic processes controlling the body's energy gain and expenditure.

2.3 Central regulation of energy homeostasis

The brain integrates internal homeostatic signals with external nutritional information to control appropriate feeding behavior (Berthoud et al., 2017). In humans, the five classical senses monitor external information, the visual system being predominant in the nutritional signal processing (Berthoud et al., 2017). Visual food cues strongly activate central reward networks, and these neural responses are increased in obese subjects (Devoto et al., 2018; Nummenmaa et al., 2012). A feeling of hunger also acutely increases these brain responses (LaBar et al., 2001). The frontal cortex, hippocampus, insula, amygdala and striatum are key brain areas for processing food related sensory information and subsequent cognitive processes

such as memory formation (Berthoud et al., 2017; Morris et al., 2001). Via these neural pathways, sensory representations of food are stored as "food memories" to guide future feeding behavior (Berthoud et al., 2017). Memory formation and eating behavior are directly modulated by the sensory input provided by the vagus nerve, which carries information from the gut to the brain (Clark et al., 1999; Fülling et al., 2019). The major brain interface processing external sensory and visceral information is schematically presented in **Figure 2**.

The hypothalamus is a major central hub for long-term energy balance regulation (Berthoud et al., 2017; Perez-Tilve, 2017). Within the hypothalamus, the arcuate nucleus contains agouti-related protein (AGRP) neurons and POMC neurons, which are crucial for basal feeding drive and sensations of hunger and satiety (Austin et al., 2008). Animal studies have shown that activation of the AGRP neurons rapidly induces food intake and also establishes sustained motivation to obtain food, while the inhibition has the opposite effect (Chen et al., 2016; Krashes et al., 2011). Neurons in lateral hypothalamus project to the arcuate nucleus to stimulate AGRP neurons and feeding (Horvath, 2005). Accordingly, the direct stimulation of the lateral hypothalamus manifolds the amount of food eaten (Delgado et al., 1952), while bilateral damage to these neurons leads to cessation of spontaneous eating (Anand et al., 1951). Activation cascades inside the hypothalamus are influenced by a diverse set of neurohumoral signals containing information about the nutritional state of the body. A prime example of this kind of messaging is leptin, which is produced in white adipose cells proportionally to the amount of fat tissue (Kelesidis et al., 2010). Circulating leptin activates its central receptors in the arcuate nucleus, which inhibits AGRP neurons and activates POMC neurons, resulting in net upregulation of anorexigenic pathways (Cowley et al., 2001; Horvath, 2005). In addition to leptin, hypothalamus is also sensitive to multiple other peripherally derived hormones. These include ghrelin and insulin, which function as peripheral signals of hunger and satiety, respectively (Austin et al., 2008; Guyenet et al., 2012).

Hypothalamic control of energy homeostasis is strongly influenced by the corticolimbic system and basal ganglia, which form the essential CNS network processing food reward via multiple co-operating neurotransmitter systems (Berthoud et al., 2017). Reward comprises of several partially overlapping cognitive processes including learning, motivation ("wanting") and affective "liking" (Berridge et al., 2003). Hedonic "liking" of foods and objective pleasure reactions are mediated by opioid receptor activation especially in nucleus accumbens, ventral pallidum and brainstem (hedonic hotspots) (Berridge et al., 2010; Berridge et al., 2003). This reward-sensitive brain circuitry has evolved in the past when calories were scarce in the environment. However, the circuit continues to generate pleasurable experiences upon consumption of energy-dense foods, promoting food intake (Berridge et al., 2010). Endocannabinoids also amplify feeding pleasure in the

hedonic hotspots (Berridge et al., 2010). These subcortical hubs are densely connected to prefrontal cortex and insula, which are key cortical areas for conscious incentive processing (Berridge et al., 2003; Berthoud et al., 2017). The hedonic hotspots also promote the incentive motivation to feed ("wanting" of food), which is supported by a wider opioidergic network extending to amygdala and striatum (Berridge et al., 2010). Pleasant foods and reward cues also activate the mesolimbic dopamine system, which further promotes "wanting" and the motivational drive to feed, also in the absence of apparent subjective pleasure (Berridge et al., 2010). The above-illustrated neurochemical pathways also mediate rewarding effects of addictive behaviors such as drug intake, further highlighting the potential reinforcing effects of feeding in vulnerable individuals (Volkow et al., 2013).

In summary, the brain has a paramount role in the control of feeding and subsequently in the management and development of body weight. It is thus reasonable to hypothesize that certain individual differences in the food intake regulating neural networks might predispose individuals to obesity in the modern environment. In the next sections, this idea is more closely examined with the emphasis on opioid and endocannabinoid systems, which are integral components of food reward and hedonics.

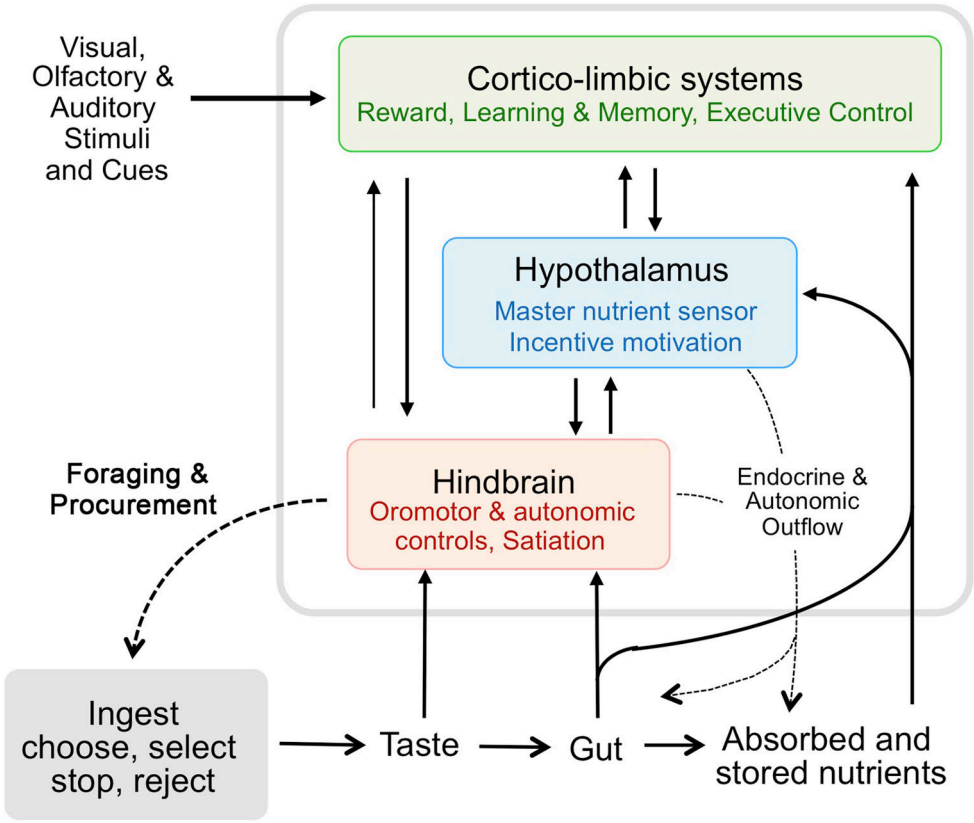

Figure 2. Central food-intake regulating brain areas and their relationships to peripheral signals. The figure is reprinted as originally published by Berthoud et al. under the Creative Commons CC-BY-NC-ND license (Berthoud et al., 2017) with permission.

2.4 Endogenous opioid system in the brain

Opium poppy (*Papaver somniferum*) was already cultivated by Sumerians at the end of third millennium BCE to produce opium, which was probably consumed in religious rituals for its euphoric effects (Brownstein, 1993). Morphine, the still widely used active analgesic component of opium, was successfully isolated in the beginning of 19th century (Brownstein, 1993; Serturner, 1805). Morphine relieves pain via opioid receptors, which were discovered in the brain only in 1973 (Pert et al., 1973; Simon et al., 1973; Terenius, 1973). This was followed by the discovery of multiple endogenous opiate molecules that physiologically target these receptors (Akil et al., 1998). The success story of morphine and its derivatives in the treatment of pain has however been contrasted by widespread problem of recreational misuse, highlighting the roles of the opioid system in addiction and reinforcement behaviors (Le Merrer et al., 2009). Importantly, subsequent research has found that central

opioid receptors and their endogenous ligands have a key role in the reward of feeding.

2.4.1 Opioid receptors and their ligands

Opioid receptors are highly conserved G protein-coupled receptors (GPCRs) with seven protein helices spanning across the cell membrane (Waldhoer et al., 2004). On the cell surface, opioid receptor agonists bind to the extracellular regions of these GPCRs, resulting in a helix movement and the initiation of an intracellular messaging cascade, which involves the inhibition of adenylyl cyclase and effects to multiple ion channel systems, including the inhibition of Ca^{2+} channels and stimulation of K^{+} channels (Huang et al., 2015; Waldhoer et al., 2004). After agonist binding, the receptor-ligand complex is rapidly *internalized* to the cell by endocytosis, and through complex sorting mechanism, the receptor is either returned to the cell surface or routed to degradation (*downregulation*) (Von Zastrow, 2010). Chronic opioid receptor agonist exposure may lead to downregulation (reduced density) of the corresponding receptors (Stafford et al., 2001). Conversely, chronic antagonist exposure results in *upregulation* of opioid receptors (Lesscher et al., 2003). The primary function of opioid receptor activation is inhibitory, resulting in decreased release of other neurotransmitters, but excitatory net effects are also prominent in certain parts of the CNS (Corbett et al., 2006). The opioid system is capable of influencing multiple emotional states and behaviors due to regional variation in receptor type density and their endogenous ligand expression (Corbett et al., 2006; Le Merrer et al., 2009).

There are three classical opioid receptors: the μ-opioid, δ-opioid and κ-opioid receptors (MOR, DOR and KOR, respectively). The nociceptin-orphanin FQ receptor (NOR) is also frequently classified as an opioid receptor, and additional less known receptors have been proposed as belonging to the opioid receptor family (Corbett et al., 2006; Cox, 2013; Stein, 2016). The classical opioid receptors and NOR were already expressed in jawed vertebrates around 450 million years ago (Dreborg et al., 2008). In addition to euphoria and respiratory depression, the general effects of MOR activation include analgesia and reward (also produced by DORs), while KORs mediate dysphoria (Stein, 2016; Waldhoer et al., 2004). CNS is a major site for expression of opioid receptors, although they are also found in various peripheral nervous, immune and ectodermal cells (Stein, 2016). Endogenous opioid molecules (endogenous opioid receptor ligands) have variable affinities for each opioid receptor type and are derived from distinct precursor proteins. β-endorphin is derived from POMC, and activates both MORs and DORs. Proenkephalin is cleaved to enkephalins, which have high affinities for DOR. In turn, dynorphins primarily activate KORs, and are derived from prodynorphin. (Corbett et al., 2006)

Endogenous opioid levels can be measured from peripheral blood samples. It is, however, unclear whether the peripheral concentrations are related to those in CNS, as at least the β-endorphin molecule is too large to cross the blood-brain barrier (Dietrich et al., 2004). In the brain, β-endorphin is produced mainly in the pituitary gland and the arcuate nucleus of the hypothalamus. From the arcuate nucleus, β-endorphin is delivered to more distal brain sites including basal ganglia, cingulate and frontal cortex via axonal transport and volume transmission in the cerebrospinal fluid (CSF) (Veening et al., 2012). β-endorphin can be degraded by several proteolytic enzymes, including the insulin-degrading enzyme (IDE) (Asvadi et al., 2014; Reed et al., 2008). In animal studies, multiple behavioral challenges (including physical exercise or feeding) have been associated with changes in the β-endorphin concentration of the CSF (Hoffmann et al., 1990; Veening et al., 2012; Yamamoto et al., 2000). Accordingly, the β-endorphin and its target receptors (MORs) are prominent regulators of food intake (Peciña et al., 2010; Veening et al., 2015).

2.4.2 Opioidergic regulation of feeding and weight maintenance

Opioid receptors are expressed widely in the central reinforcement pathways that process natural rewards derived from e.g. feeding and sexual behavior (Le Merrer et al., 2009). Central endogenous MOR agonists are released following feeding (Dum et al., 1983; Tuulari et al., 2017; Winterdahl et al., 2019), and MOR activation (agonism) in the hedonic hotspots, insula and orbitofrontal cortex amplifies pleasure derived from feeding (Castro et al., 2014, 2017). In humans, certain single nucleotide polymorphism (rs1799971) in the MOR coding gene (opioid receptor μ 1 gene, OPRM1) associates with lower central MOR availability (Weerts et al., 2013), but it is not known whether this variation directly affects the eating phenotype.

Multiple pharmacological studies have examined the effects of MOR ligands on feeding behavior. In rodents, injection of MOR agonist to nucleus accumbens or prefrontal cortex increases food intake, while the opioid antagonist naloxone has the opposite, inhibitory effect (Castro et al., 2014; Mena et al., 2011; Yeomans et al., 2002; Zhang et al., 1997). In humans, naloxone also reliably suppresses food intake and reduces the subjective pleasure derived from food (Gosnell et al., 2009; Yeomans et al., 2002). Another MOR antagonist naltrexone has been shown to reduce both liking and wanting behaviors associated with food rewards in healthy human subjects (Korb et al., 2020). The potential of exogenous MOR agonists to induce hyperphagia in humans has been less studied, although the results point towards increased feeding (Morley et al., 1985; Yeomans et al., 2002). Based on psychopharmacological studies, MOR agonists have been suggested to be especially

important in increasing the hedonic value of the most energy-dense food products (Eikemo et al., 2016).

In addition to the cortico-limbic reward enhancement, opioid peptides also modulate homeostatic feeding behavior via multiple hypothalamic sites, including paraventricular nucleus (PVN) and lateral hypothalamus (Le Merrer et al., 2009). The effects of opioids in PVN may be dependent on food preference and current metabolic needs (Naleid et al., 2007). However, MORs are likely to be important for basal feeding drive. MOR knockout mice have decreased feeding motivation (Papaleo et al., 2007), they exhibit less food-anticipatory behavior (Kas et al., 2004), and their food intake is decreased (Awad et al., 2020) compared to wild type controls. Interestingly, distinct eating behavior patterns facilitated in experimental setting may also induce dynamic changes in the central MOR system. In minipigs, repetitive sucrose solution intake reduces MOR availability in multiple brain regions already within 12 days, which potentially results from frequent endogenous opioid bursts downregulating MORs (Winterdahl et al., 2019).

Human PET studies with MOR-selective agonist radioligand [^{11}C]carfentanil have corroborated the role of endogenous opioid system in feeding physiology and pathology. In a PET challenge study by Tuulari et al., it was shown that feeding induces rapid and widespread release of endogenous opioids in the brain (Tuulari et al., 2017). Notably, this opioid release was independent of the subjective pleasure of feeding, suggesting that the essential part of opioidergic feeding regulation may operate via the unconscious reward system of the gut–brain axis (de Araujo et al., 2020). In morbidly obese subjects, the MOR availability is lowered in the central food reward processing regions including the orbitofrontal cortex, insula and frontal striatum (Karlsson et al., 2015). Compared with lean controls, obese subjects also exhibit disrupted opioid–dopamine crosstalk in the frontal striatum (Tuominen et al., 2015). In subjects with obesity, successful weight loss via both bariatric surgery and dieting normalizes central MOR availability (Burghardt et al., 2015; Karlsson et al., 2016). Interestingly, in surgical weight loss patients, the preoperative MOR availability in amygdala predicts postoperative weight in a two-year follow up after bariatric surgery, highlighting the role of the amygdala in weight maintenance after surgery (Karlsson et al., 2021). It has been hypothesized that the reduced MOR availability in obesity could be caused by the repeated overstimulation of MORs by feeding induced opioid release and following downregulation of these receptors, or that reduced MOR availability itself could form a predisposing factor to overeating and obesity (Karlsson et al., 2015). Based on the literature reviewed above, both mechanisms are likely to play a role.

In addition to obesity, the endogenous opioid system has been studied with PET in subjects with eating disorders. Patients with binge eating disorder (BED) have reduced MOR availability in multiple central regions, including nucleus accumbens

and prefrontal cortex (Majuri et al., 2017). Furthermore, the rs1799971 polymorphism of OPRM1 is over-represented in subjects with BED (Davis et al., 2009). In bulimia nervosa, insular MOR availability is lowered and it correlates inversely with recent fasting behavior (Bencherif et al., 2005). In a study by Galusca et al., patients with anorexia nervosa also had reduced availability of opioid receptors compared to controls, but it was not possible to differentiate which opioid receptor subtypes are affected in anorexia due to the unselective ligand ([^{11}C]diprenorphine) used in the study (Frost et al., 1990; Galusca et al., 2020). Unfortunately, thus far patients with anorexia nervosa have not been studied with MOR-specific PET imaging. Overall, previous studies suggest that alterations in the central opioidergic system and particularly in MORs may have an important role in the pathophysiology of obesity and eating disorders. While morbid obesity has been previously associated with reduced MOR availability, it remains unexplored whether the altered function of the MOR system forms a risk factor to the development of future obesity. It is also not known whether the MOR system mediates some specific feeding behavior traits that could be targeted with behavioral or pharmacological means to combat weight gain.

The central MOR system may also contribute to weight maintenance by mediating the affective responses of physical exercise. Rat studies have found that β-endorphin concentration increases in nucleus accumbens following treadmill running (Blake et al., 1984), and that both acute and chronic exercise modulate central MOR expression (de Oliveira et al., 2010). In humans, circulating β-endorphin levels are elevated by exercise (Goldfarb et al., 1997). Physical exercise also triggers central endogenous opioid release, which likely contributes to the following euphoric state colloquially known as "runner's high" (Boecker et al., 2008; Saanijoki et al., 2018b). Furthermore, increased MOR activation following moderate-intensity training predicts increased euphoria after exercise (Saanijoki et al., 2018b). Increased MOR agonist release by exercise also predicts higher hemodynamic brain responses to subsequent palatable food cues (Saanijoki et al., 2018a), suggesting an opioidergic link in homeostatic energy gain and expenditure mechanisms. However, whether the MOR function contributes to a long-term commitment to regular exercise is still unknown.

Currently the only anti-obesity drug approved by Food and Drug Administration (FDA) that primarily utilizes the brain's central opioid pathways is a combination of naltrexone/bupropion (trade name Contrave) (Srivastava et al., 2018). Buproprion stimulates hypothalamic POMC-producing neurons (Greenway et al., 2009), which release α-melanocyte-stimulating hormone (α-MSH) and β-endorphin. The inhibitory feedback of β-endorphin is blocked with opioid antagonist naltrexone, resulting in increased activity of α-MSH and activation of the anorexigenic pathways (Ornellas et al., 2011). It has been proposed that the drug also further modulates the

mesolimbic reward pathway and enhances the control of food intake and cravings (Greenway et al., 2010). Pharmacotherapy with naltrexone/bupropion induces approximately 4% weight loss, which is superior compared to intensive lifestyle intervetions alone (~ 3%), but the drug has common side-effects that limit its use (e.g. nausea, constipation, headache) (Shi et al., 2021; Srivastava et al., 2018).

In summary, the previous preclinical and human studies have established that central MOR system is critically involved in food intake regulation and weight maintenance. Furthermore, clinical studies have shown that the MOR system is altered in morbid obesity and eating disorders such as BED. However, little is known about variation in the MOR function among healthy humans, and importantly, whether interindividual differences in the central MOR system might explain why only some individuals are vulnerable to weight gain in the common obesogenic environment. Finally, the central MOR system does not operate alone. Instead, it forms a highly interconnected network with other neurotransmitter systems. The brain's cannabinoid system has been especially implicated in feeding regulation both independently and via its links with MORs.

2.5 Endocannabinoid system in the brain

Humans have used *Cannabis sativa* for medical and recreational purposes for several millennia, at least since 500 BCE, and the appetite stimulating properties of *Cannabis* have also been long recognized (Kirkham et al., 2001; Mechoulam et al., 2013; Ren et al., 2019). It was not until 1964 that Δ^9-tetrahydrocannabinol (THC), the principal compound responsible for the psychoactive effects of *Cannabis*, was isolated (Gaoni et al., 1964). Subsequent studies revealed that THC binds to specific cannabinoid receptors (Herkenham et al., 1990) in order to mediate its effects. After the discovery of the cannabinoid receptors, the existence of endogenous cannabinoids (endocannabinoids) was immediately hypothesized, and these were indeed discovered in 1992 in a porcine brain (Devane et al., 1992; Mechoulam et al., 2013). Since then, an extensive body of research has been carried out to investigate the role of cannabinoids and their receptors in energy homeostasis physiology and pathology.

2.5.1 Cannabinoid receptors and their ligands

The endocannabinoid system (ECS) is a widespread homeostatic regulatory system with receptors in both CNS and periphery (Cristino et al., 2014). The two major cannabinoid receptors identified are cannabinoid receptor type 1 (CB_1R) and cannabinoid receptor type 2 (CB_2R). Endocannabinoids also activate the transient receptor potential vanilloid type 1 receptor ($TRPV_1$), and potentially additional

cannabinoid receptors that are yet to be characterized (Brown, 2007; Morales et al., 2017). Phylogenetically, the evolution of cannabinoid receptors antedates the development of vertebrates (Anday et al., 2005).

CB_1Rs are among the most common receptors found in the brain, and are found in all cortical brain regions. The major sites of CB_1R expression are in the frontotemporal cortex, basal ganglia and midbrain, where they regulate the synaptic release of several other neurotransmitters (Glass et al., 1997; Mechoulam et al., 2013; Terry et al., 2010). Both sensory and motor brain regions have a high density of CB_1Rs (Mechoulam et al., 2013). In addition to CNS, CB_1Rs have also been found in multiple peripheral tissues but in a lower quantity (Mechoulam et al., 2013). In turn, CB_2Rs are almost exclusively found in peripheral immune tissues (Matias et al., 2006). As with opioid receptors, cannabinoid receptors also belong to the GPCR protein superfamily (Matsuda et al., 1990; Munro et al., 1993), activate similar intracellular messaging cascades (Howlett et al., 2002; Wenzel et al., 2018), and are also regulated by agonist-induced internalization and recycling processes (Hsieh et al., 1999).

Endocannabinoids are body's own lipid molecules that act as ligands to endocannabinoid receptors (Hillard, 2018; Mechoulam et al., 2013). The most studied endocannabinoids are anandamide (*N*-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) (Hillard, 2018) (**Figure 3**), which are synthesized from precursor lipids via multiple adjacent pathways (Hillard, 2015). Endocannabinoids are not stored in vesicles. Instead, they are produced on demand close to their central binding sites, where they act as retrograde messengers in nerve synapses (Mechoulam et al., 2013). After being released into the nerve synapse by postsynaptic neuron, endocannabinoids activate cannabinoid receptors in the presynaptic neuron, inducing a cell signaling cascade resulting in a reduced release of other neurotransmitters (e.g. glutamate, γ-aminobutyric acid (GABA)) (Kano et al., 2009). Endocannabinoids are then removed from the synapse and degraded by hydrolysis and oxidation (Kano et al., 2009). The central ECS has a major role in multiple physiological functions including stress recovery, cognition and reward processing (Mechoulam et al., 2013). Importantly, ECS is also a key regulator of energy homeostasis and food intake, potentially influencing an individual's risk for obesity.

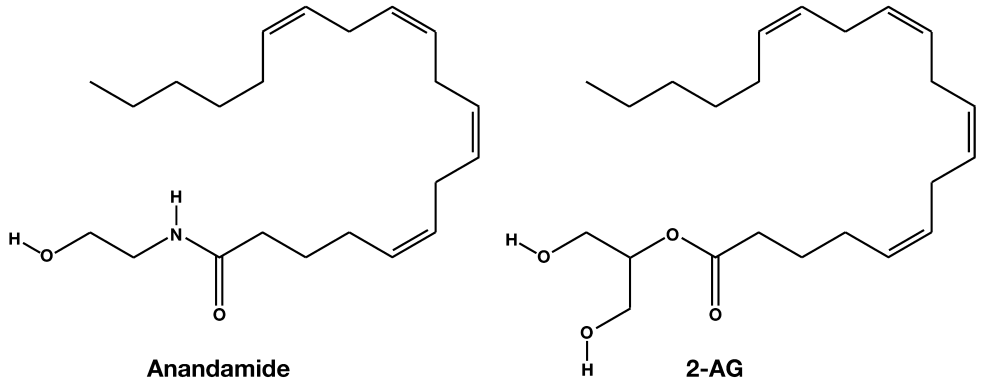

Figure 3. Structure of the two major endogenous cannabinoid molecules, anandamide and 2-AG.

2.5.2 Endocannabinoids and energy homeostasis

CB_1Rs are found in all energy balance regulating brain areas (Bermudez-Silva et al., 2010). In general, activation of central CB_1Rs (agonism) promotes food intake and energy storage as fat tissue (Cristino et al., 2014). It has been proposed that a "hyperactive" ECS could be an important pathological mechanism predisposing to obesity (Di Marzo et al., 2005). Indeed, rimonabant (SR141716A, trade name Acomplia), an inverse agonist of CB_1R, was successfully used to decrease food intake and BMI but also to improve the lipid profile in obese humans (Bermudez-Silva et al., 2010). Unfortunately, rimonabant had to be quickly withdrawn due to the psychiatric side effects, and therefore novel anti-obesity drugs utilizing ECS are under search (Bermudez-Silva et al., 2010; Cristino et al., 2014).

Cannabinoid molecules regulate food intake in multiple brain sites. CB_1R activation in the lateral hypothalamus stimulates melanin-concentrating hormone (MCH) activity, which in turn promotes feeding (Jo et al., 2005; Quarta et al., 2011). Other CB_1R-controlled hypothalamic sites of feeding regulation have been identified, including PVN, where cannabinoid agonists stimulate food intake (Verty et al., 2005). In addition to influencing homeostatic pathways in the hypothalamus, CB_1Rs also mediate hedonic feeding and motivation to eat through mesolimbic pathways (Di Marzo et al., 2005; Quarta et al., 2011). More specifically, CB_1R-agonists induce the release of dopamine in nucleus accumbens and interact with the opioid system (Di Marzo et al., 2005; Gardner, 2005), efficiently promoting food intake (ECS and opioid interactions described more closely in the next section). In rodents, limbic endocannabinoid concentration increases concurrently with the length of the fast, and a direct injection of 2-AG to the nucleus accumbens dose-dependently incudes feeding (Kirkham et al., 2002). Furthermore, CB_1R knockout in mice results to lean phenotype, resistance for diet-induced obesity (Ravinet Trillou

et al., 2004) and a devaluation of food reward (Sanchis-Segura et al., 2004). Obese rodents also have decreased CB_1R density in multiple brain areas, including the nucleus accumbens and neocortex (Harrold et al., 2002).

Human studies suggest that ECS is closely linked to eating and body mass. Brain PET imaging studies have found that increased BMI is associated with reduced central CB_1R availability (Ceccarini et al., 2016; Hirvonen et al., 2012), while anorexic patients have upregulated CB_1R availability (Gérard et al., 2011). Based on these cross-sectional studies it is, however, not known whether these alterations would serve as the cause or the effect of the corresponding BMI phenotype, or to what extent alterations in the brain's CB_1Rs are dependent on changes in the central endocannabinoid molecule concentrations. In obese humans, circulating AEA concentration is increased combined with decreased CB_1R gene expression in adipose tissue, which suggests that obesity is characterized by increased systemic levels of endocannabinoid molecules that downregulate endocannabinoid receptors via negative feedback loop (Engeli et al., 2005). Circulating endocannabinoid levels can be measured from peripheral blood samples, but it is still largely unknown how these peripheral endocannabinoid measures relate to the endocannabinoid levels in CNS, although there certainly is dense cross-talk between peripheral and central components of ECS (Cristino et al., 2014; Hillard, 2018). As a highly lipophilic molecule, AEA readily crosses the blood-brain barrier (Dietrich et al., 2004). Furthermore, *in vitro* evidence suggests that AEA is able to modulate the permeability of the blood-brain barrier, a putative mechanism for circulating AEA to influence CNS function (Hind et al., 2015). In addition, recently one small PET study reported an inverse association between certain circulating endocannabinoids (e.g. AEA) and the brain's CB_1R availability (Dickens et al., 2020), consistent with the systemic nature of the ECS function.

2.6 Anatomical and functional overlaps of central opioid and endocannabinoid pathways

Anatomically, the MOR and CB_1R systems overlap in the reward circuit (Cota et al., 2006). Distribution of MORs and CB_1Rs in the human brain *in vivo* is shown in **Figure 4**. Accumulating evidence suggests that these two receptor systems also interact in the regulation of feeding behavior. In rats, the MORs and CB_1R antagonists have a synergistic (supra-additive) effect on decreasing food intake (Rowland et al., 2001). Moreover, naloxone (MOR antagonist) administration blocks CB_1R agonist induced hyperphagia, and conversely rimonabant (CB_1R antagonist) blocks hyperphagia induced by a MOR agonist. This suggests that simultaneous activation of these neuroreceptor systems is crucial to a sustained feeding drive (Solinas et al., 2005). This functional cross-talk may be mediated via corresponding

state changes in endogenous neurotransmitter concentrations. Indeed, in rats, acute administration of MOR agonist increases AEA levels and CB_1R agonist increases β-endorphin levels in nucleus accumbens and other key feeding regulating brain sites (Solinas et al., 2004; Vigano et al., 2004). In addition, preclinical data suggest that co-localized MOR and CB_1R are able to form functional heterodimer in nucleus accumbens and to inhibit GABA release via this receptor complex (Schoffelmeer et al., 2006; Wenzel et al., 2018). However, whether this heterodimerization plays a significant role also *in vivo* neurotransmission remains to be clarified (Wenzel et al., 2018). Agonism of MORs and CB_1Rs also stimulates the mesolimbic dopaminergic signaling, further promoting the reinforcing effects of feeding (Cota et al., 2006; Richard, 2015). Overall, the central MOR and CB_1R systems work in synergistic manner to facilitate food intake and a positive net energy balance (Richard, 2015).

2.7 Other brain systems linked with obesity

In addition to opioids and endocannabinoids, multiple other brain systems have been proposed to be important in the pathophysiology of obesity, including the insulin system, the dopamine system, and the serotonin system.

2.7.1 Insulin and brain glucose metabolism

Insulin is a satiety hormone derived from the pancreas, and has a key role in regulating feeding behavior in addition to blood glucose levels (Davis et al., 2010). Most of the central insulin is actively transported from the periphery via the blood-brain barrier, while CNS might also have some synthesis of its own (Banks et al., 2012; Mehran et al., 2012; Rebelos et al., 2021). Insulin levels increase after feeding and during periods of positive energy balance (Austin et al., 2008; Benoit et al., 2002). In the hypothalamus, insulin activates its receptors to stimulate POMC neurons, which reduces food intake (Benoit et al., 2002). Appetite stimulating signals such as increased levels of β-endorphin partly attenuate the central effects of insulin (Shiraishi et al., 2008).

Peripheral insulin resistance is a well-known complication associated with obesity and a part of the metabolic cascade which ultimately leads to diabetes. PET studies have also examined the brain glucose metabolism in subjects with obesity and prediabetes. During a low-dose insulin infusion, overweight subjects with peripheral insulin resistance show diminished metabolic response in food intake regulating brain areas including the ventral striatum (Anthony et al., 2006). On the other hand, experimentally induced euglycemic hyperinsulinemia (DeFronzo et al., 1979) globally accelerates brain glucose metabolism in obese subjects with peripheral insulin resistance, but not in healthy controls (Hirvonen et al., 2011;

Tuulari et al., 2013). This cerebral metabolic alteration can be attenuated with bariatric surgery (Tuulari et al., 2013). The pathophysiology of obesity-associated alterations in brain glucose metabolism is currently poorly understood (Rebelos, 2020), since in healthy subjects, hyperinsulinemia has no effect on brain glucose metabolism or blood-brain barrier glucose transport (Hasselbalch et al., 1999). Indeed, in obesity, a putative mechanism for increased central glucose metabolism could be the dysfunction of the insulin transport in the blood-brain barrier, since the obesity-associated metabolic alterations are widespread throughout the brain (Rhea et al., 2018; Tuulari et al., 2013). However, it is not known whether alterations in the central insulin signaling might promote the development of obesity. Given the intimate mesolimbic links between insulin and opioid signaling regulating food reward (Davis et al., 2010), the cerebral insulin messaging is also (briefly) examined in this thesis as a potential risk factor for obesity.

2.7.2 Dopamine neurotransmission

Dopaminergic pathways are crucial for goal-directed behavior and incentive motivation, and the availability of dopamine D_2 receptors (D_2R) is decreased in multiple drug addictions (Martinez et al., 2012; Wise, 2004). Dopamine is also released in the striatum in response to feeding, and the release is proportional to the meal's pleasantness (Small et al., 2003). Paralleling the well-known role of D_2Rs in addictive disorders, a PET study by Wang et al. found that severely obese subjects (mean BMI 51.2 kg/m^2) had decreased D_2R availability in the striatum compared to normal weight subjects (Wang et al., 2001). However, later studies have reported conflicting results, and meta-analyses lend only modest support for the proposed link between brain D_2R availability and obesity (Benton et al., 2016; Karlsson et al., 2015). Thus, it has been proposed that the relationship between BMI and D_2R availability may be nonlinear, the decrease of receptors being evident only in severe forms of obesity (Van Galen et al., 2018). Decreased striatal dopamine synthesis capacity has been found in subjects with BED (Majuri et al., 2017). A recent meta-analysis found no association between overweight or obesity and central dopamine transporter protein availability (Pak et al., 2021). In summary, current evidence does not allow strong inferences to be made regarding the relationship between the brain dopamine function and obesity development (Malén et al., 2021; Van Galen et al., 2018).

2.7.3 Serotonin system

Most serotonergic projections relevant for energy homeostasis regulation arise from the raphe nuclei of the brainstem (Van Galen et al., 2021). There are over ten

serotonin receptors arranged in seven receptor families (Barnes et al., 2011). A bulk of PET studies investigating central serotonin system has reported increased availability of serotonin receptors in obese subjects (Van Galen et al., 2018). BMI has been found to associate with serotonin receptor 5-HT_{2A} throughout cerebral cortex (Erritzoe et al., 2009; Van Galen et al., 2018), and with 5-HT_4 in the reward circuit (Haahr et al., 2012). In addition, higher preoperative brain 5-HT_{2A} availability is associated with increased weight loss after bariatric surgery, in other words brain 5-HT_{2A} may predict who benefits most from surgical management of obesity (Haahr et al., 2015). Based on imaging studies and supporting preclinical data, the increased availability of serotonin receptors detected in obesity is usually considered as a marker of reduced amount of serotonin in the brain synapses (Van Galen et al., 2018; Van Galen et al., 2021). Accordingly, pharmacologically increasing the brain serotonergic signaling has been studied as a strategy to combat obesity (Oh et al., 2016). However, these attempts have been complicated by major side effects of the studied serotonergic anti-obesity drugs, and also promising serotonin receptor (5-HT_{2C}) agonist lorcaserin had to be withdrawn by FDA in 2020 due to associated cancer risk (Sharretts et al., 2020; Van Galen et al., 2021).

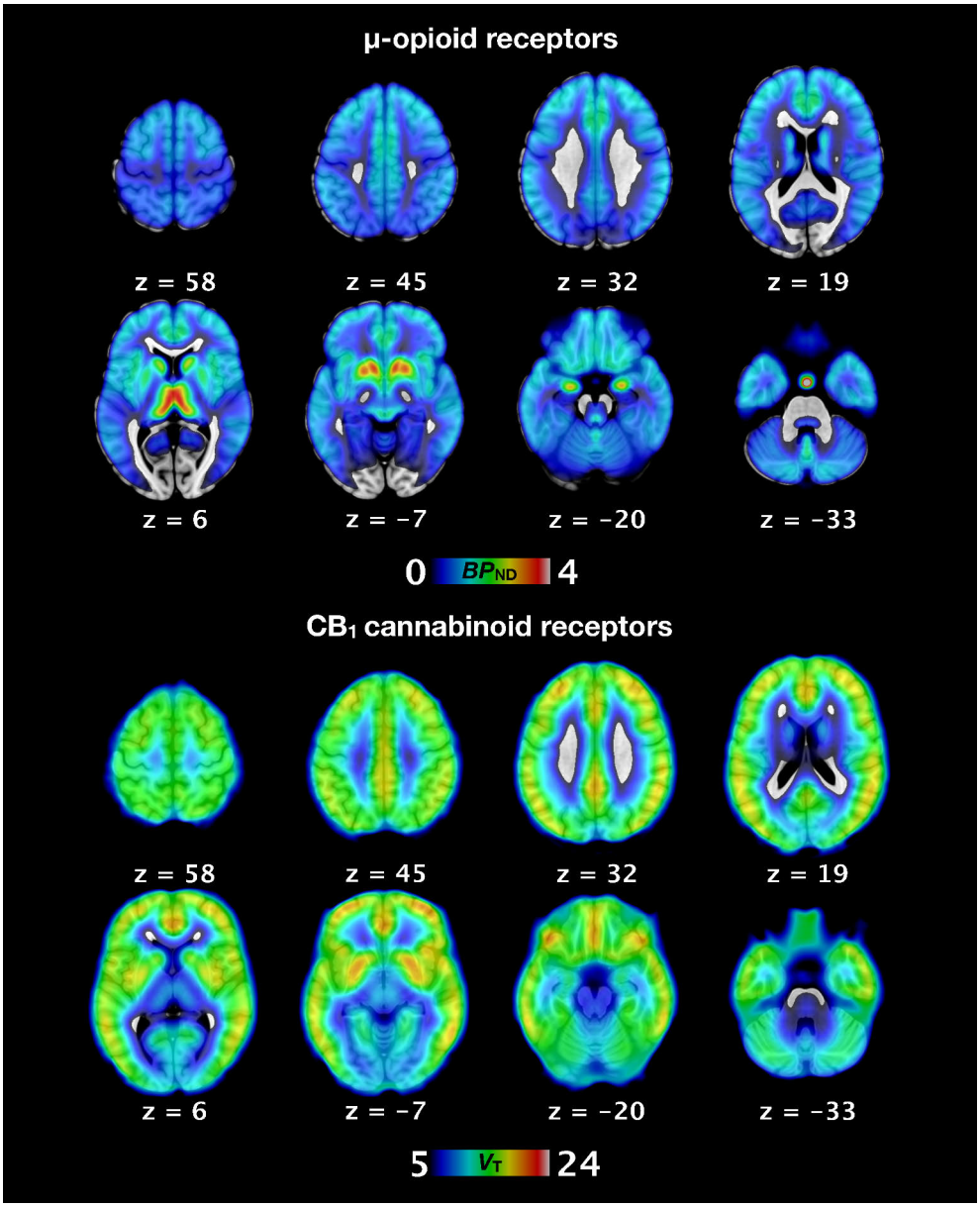

Figure 4. Mean distribution of central μ-opioid and CB_1 receptors. Image is based on 204 [^{11}C]carfentanil scans (132 males, 72 females) and 36 [^{18}F]FMPEP-d_2 scans (all males) from healthy subjects, compiled from the AIVO database (http://aivo.utu.fi) hosted by Turku PET Centre. μ-opioid receptor availability is expressed as [^{11}C]carfentanil nondisplaceable binging potential (BP_{ND}), and CB_1 receptor availability as [^{18}F]FMPEP-d_2 volume of distribution (V_T). z represents the axial coordinates in the MNI152 space.

2.8 Summary of the literature

Humans have used opium poppy and *Cannabis* for thousands of years to induce euphoria, pleasure, and analgesia. Advancements in 20th-century molecular biology have revealed the specific components of the brain networks responsible for these effects – opioid and endocannabinoid receptors, and their endogenous ligand molecules. An extensive body of preclinical and clinical studies has shown that these receptor systems (especially MORs and CB_1Rs) are prime mediators of food reward. As such, they influence our day-to-day feeding behavior, and consequently to our body composition and health.

The obesity pandemic is one of the most difficult challenges for modern societies. Despite intensive public efforts, the prevalence of obesity and related illness is increasing worldwide. Effective means to prevent obesity development are currently lacking. Excessive adiposity has adverse effects on multiple organ systems, including the cardiovascular and central nervous systems. Genetic, pharmacological, behavioral, and imaging studies have emphasized that brain function has a paramount role in determining individual's body mass. Accordingly, most of the past and current pharmacological strategies to combat obesity target the brain's energy regulation pathways.

Energy homeostasis is regulated by a multi-level brain network, where the basal feeding drive of hypothalamus is influenced by cortico-limbic MORs and CB_1Rs. These receptor systems are densely interconnected anatomically and functionally, both promoting food intake and anabolic energy balance. Neuroimaging studies in subjects with obesity and eating disorders have found alterations in their central MOR and CB_1R pathways. Although it is reasonable to expect that the evolutionarily old MOR or CB_1R systems might promote feeding in the modern obesogenic environment, it is nevertheless evident that not everyone gains excessive weight. Based on available (mostly cross-sectional) data it is not yet known whether the variation in the function of MOR or CB_1R could predispose a proportion of humans to the development of obesity.

3 Aims

The aim of this thesis was to investigate whether variation in the human brain's MOR and CB_1R system function explains variation in individual obesity risk. To this end, four studies were carried out, utilizing positron emission tomography, magnetic resonance imaging (MRI), biobehavioral metrics, and both clinical and historical datasets.

The specific study questions were:

I Is the central MOR availability associated with demographic factors, including age, BMI, sex, and smoking? (Study I)

II Are the brain's MOR and CB_1R availabilities related to classical obesity risk factors including overweight, low physical exercise, and parental risk factors? (Study II)

III Is physical fitness related to the central MOR availability and to the magnitude of endogenous opioid release following physical exercise? (Study III)

IV Do the brain's MOR and CB_1R availabilities associate with distinct eating behavior traits? (Study IV)

4 Materials and Methods

To answer the study questions presented in the Aims, four Studies were carried out:

I A large-scale PET study using a historical database of brain scans. This study analyzed the effects of age, BMI, sex, and smoking on central MOR availability in healthy humans.

II A clinical study in which young males were recruited based on obesity risk profiles and examined with three PET brain scans, to analyze the associations of obesity risk factors with central opioid, endocannabinoid and insulin function.

III A clinical study in which young males were studied with repeated PET brain scans and cardiorespiratory fitness tests, to examine the endogenous opioid release and MOR function.

IV A PET brain scan database study which assessed the effects of central MOR and CB_1R availability on eating behavior traits, quantified by a validated questionnaire.

All Studies were performed in accordance with the Declaration of Helsinki, and all volunteers signed written informed consent forms prior to participating. Studies II (NCT03106688) and III (NCT02615756) were clinical studies involving healthy male volunteers, who were recruited via newspaper advertisements, university-hosted email lists, bulletin boards and Internet discussion forums. Studies I and IV were retrospective register-based studies using pre-existing PET data. The clinical study protocols were approved by the Ethical Committee of the Hospital District of South-Western Finland before the data gathering, and the register study protocols were approved by the Turku University Hospital Clinical Research Services.

4.1 Subjects

The study samples are described in **Table 1**. In all the Studies, exclusion criteria included neurologic and psychiatric disorders, current use of medications that could

affect CNS, abuse of alcohol or illicit drugs, and any standard contraindication for PET or MRI imaging.

In the clinical Studies II and III, smoking or use of nicotine products, and poor compliance with the study schedule were additional exclusion criteria. Male sex was an inclusion criterion for Studies II and III. An age of 20–35 years was an inclusion criterion for Study II, and an age of 18–65 was an inclusion criterion for Study III. In the database Studies I and IV, there were no age, sex or smoking criteria. A completed Dutch Eating Behavior Questionnaire (DEBQ) form was an inclusion criterion for the database Study IV.

In Study II, all subjects were recruited for either to the high-risk (HR) group or to the low-risk (LR) group based on common obesity risk factors (current BMI, physical exercise and parental risk factors). Inclusion criteria for the HR group were overweight without obesity (BMI of 25–30 kg/m^2), leisure time physical exercise < 4 h/week, maternal / paternal overweight or obesity or maternal / paternal T2D. Inclusion criteria for the LR group were normal weight (BMI of 18.5–24.9 kg/m^2), leisure time physical exercise > 4 h/week, and no maternal / paternal T2D.

There was partial overlap among the studied populations (**Figure 5**). Specifically, 40 subjects from Study II were also included also in the sample (n = 64) of Study III (one subject from Study II missing due to technical problems in the PET data processing). Furthermore, 49 subjects from Study III were included in the sample (n = 204) of Study I (15 subjects from Study III missing because the corresponding data were acquired after the completion of Study I). 39 subjects from the Study II were included in the sample (n = 92) of Study IV (two subjects from Study II missing due to the lack of DEBQ data). 69 subjects were included in both database Studies I and IV (all subjects in Study I did not have completed DEBQ form, and part of the data analyzed in Study IV were acquired after the completion of Study I).

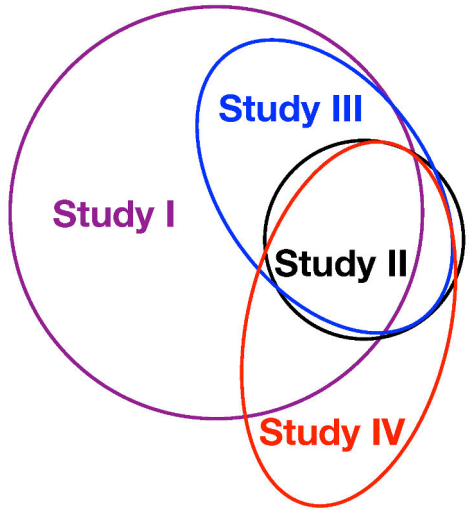

Figure 5. Visualization of the relationships between the study samples. Surface areas describe the sample size only approximately.

Table 1. Basic characteristics of the study samples in Studies I–IV. Numbers denote mean ± SD. Data are presented separately for males and females (m / f) and also for low-risk males and high-risk males (LR m / HR m) for the Study II.

Study		**n**	**Age** (years)	**BMI** (kg/m^2)	**Dose** (MBq) [^{11}C]carfentanil	**Dose** (MBq)* [^{18}F]FMPEP-d_2
I	m	132	27.8 ± 8.0	23.9 ± 2.7	297.2 ± 93.5	-
	/	/	/	/	/	
	f	72	40.7 ± 10.3	27.8 ± 8.1	341.1 ± 109.0	
II	LR m	22	23.0 ± 2.9	22.0 ± 1.9	244.5 ± 10.7	188.2 ± 11.0
	/	/	/	/	/	/
	HR m	19	27.1 ± 4.3	27.2 ± 1.9	252.6 ± 10.7	187.6 ± 14.8
III	m	64	25.4 ± 4.6	24.1 ± 2.8	248 ± 11.0	-
IV	m	70	27.4 ± 7.5	24.5 ± 2.8	277.0 ± 77.9	187.9 ± 12.8
	/	/	/	/	/	/
	f	22	47.7 ± 10.0	23.7 ± 3.1	352.3 ± 125.5	-

*The doses for [^{18}F]FMPEP-d_2. Study II: Doses for the LR (n = 20) and HR males (n = 16) that completed the [^{18}F]FMPEP-d_2 scan successfully. Study IV: Dose for 35 males scanned with [^{18}F]FMPEP-d_2.

4.2 Biobehavioral metrics

4.2.1 Obesity risk (Study II)

In Study II, obesity risk was quantified by three factors: current BMI, leisure time physical exercise and familial obesity risk (*Family Risk*, including a history of parental obesity and T2D). These factors that predict future obesity are based on previous longitudinal studies in a large Finnish cohort (the Cardiovascular Risk in Young Finns Study) and are therefore applicable to our sample of young Finnish males. BMI exhibits strong tracking from childhood to adulthood, and especially overweight males in early adulthood are at increased risk for obesity in middle age (Juhola et al., 2011). Abdominal obesity in adulthood is inversely related to leisure time physical activity, and adhering to regular physical activity from youth to

adulthood reduces weight gain and risk for obesity (Yang et al., 2007). Increased parental BMI is also a substantial risk factor for adult obesity (Juonala et al., 2011). Finally, parental history of T2D increases the individuals' risk for the development of metabolic syndrome, a state characterized by obesity, hypertension and insulin resistance (Mattsson et al., 2008). The presence of obesity risk factors was evaluated by licensed physician as a part of standardized medical history checkup. Based on the risk factors, all subjects were classified either to LR or HR group as described above. The familial obesity risk scoring is presented in **Table 2**. The obesity risk variables of the Study II sample are presented in **Table 3**.

Table 2. Familial obesity risk scoring in Study II. The principles of familial obesity risk (Family Risk) scoring, total score ranging from 0 to 4. Gestational diabetes (one subject) was scored as type 2 diabetes.

Parent overweight or obesity	**no**	**one parent**	**both parents**
	0	1	2
Parent type 2 diabetes	**no**	**one parent**	**both parents**
	0	1	2

Table 3. Obesity risk variables in the subjects of Study II.

	Low-risk males (n = 22)		**High-risk males** (n = 19)	
	mean	SD	mean	SD
BMI (kg/m^2)	22.0	1.9	27.2	1.9
Physical exercise (hours/week)	6.2	2.8	2.7	1.0
Family Risk score	0.1	0.3	1.4	0.9

4.2.2 Physical fitness (Study III)

In Study III, cardiorespiratory fitness was expressed as peak oxygen consumption (VO_{2peak}, mean±SD = 44.5±7.9 ml/kg/min), which was determined in a maximal exercise test at the Paavo Nurmi Centre (Turku, Finland). The maximal exercise test was performed with a cycle ergometer. Subjects started cycling with 40–50 W, and exertion was increased by 30 W every 2 minutes until volitional exhaustion.

Respiratory gas exchange and ventilation were measured (Jaeger Oxycon Pro, VIASYS Healthcare) and reported as the mean value per minute. VO_{2peak} is the subject's maximal 1-min mean value of oxygen consumption during the exercise test. In addition, subjects completed a questionnaire where they reported their weekly duration of moderate to vigorous physical activity (MVPA, mean±SD = 160±113.6 minutes/week).

4.2.3 Eating behavior (Study IV)

In Study IV, eating behavior was measured with the DEBQ (Van Strien et al., 1986a), which quantifies eating behavior traits that contribute to weight gain and maintenance (Van Strien et al., 2009; Van Strien et al., 2012b). The questionnaire is comprised of 33 items with Likert-type scoring in each item (response options ranging from 1−5, from "Never" to "Very often"). The DEBQ is constructed to measure three independent feeding behavior dimensions (Van Strien et al., 1995): *emotional eating* (tendency to overeat in response to emotional distress or negative emotions), *external eating* (tendency to overeat in response to external food cues) and *restrained eating* (tendency to eat less that desired) (Van Strien et al., 1986b; Van Strien et al., 2009; Van Strien et al., 2012a). The DEBQ subscales have good factorial validity, internal consistency and test-retest stability (Cebolla et al., 2014; Malesza et al., 2019; Van Strien et al., 1986a; Wardle, 1987). The DEBQ has been externally validated and found to successfully classify clinical feeding patterns in subjects with eating disorders or obesity, and also in healthy controls (Anschutz et al., 2009; Baños et al., 2014; Van Strien et al., 2012a; Wardle, 1987). In general, subjects with increased BMI and subjects with eating disorders such as BED score higher in the DEBQ across the three subscales (Baños et al., 2014; Joutsa et al., 2018; Svaldi et al., 2014; Van Strien et al., 2010). The DEBQ scores for Study IV subjects are presented in **Table 4**. The DEBQ form and scoring table are presented in Appendices.

Table 4. Dutch Eating Behavior Questionnaire scores in subjects of Study IV.

	[^{11}C]carfentanil scans			
	Males (n = 70)		Females (n = 22)	
	mean	SD	mean	SD
Total DEBQ score	67.0	12.8	73.4	12.8
Emotional eating score	21.0	6.8	22.1	5.7
External eating score	24.7	6.5	25.0	5.4
Restrained eating score	21.3	5.5	26.2	5.5

	[^{18}F]FMPEP-*d*$_2$ scans	
	Males (n = 35)	
	mean	SD
Total DEBQ score	68.6	14.5
Emotional eating score	20.7	7.4
External eating score	27.1	6.0
Restrained eating score	20.8	5.6

4.3 Radiochemistry

PET tracers are molecules with certain biological properties (*vehicles*), chemically combined (*labeled*) with positron-emitting unstable radionuclides. Knowledge of the molecular vehicle allows studies on specific biological processes in the human body, while the physical features of the radionuclide allow the measurement of PET signal ((Wadsak et al., 2010), see also Chapter 4.4.1). Radionuclides used in the PET tracer synthesis were produced in the cyclotrons (Lawrence et al., 1932) of Åbo Akademi University and Turku PET Centre. Inside a cyclotron, H^- ions are accelerated in a spiral path generated by magnetic and electric fields. Subsequently, accelerated ions are passed through a foil of graphite where they lose their electrons. The resulting protons are then bombarded onto the target material, producing radionuclides (Pichler et al., 2018). We used common radionuclides in the PET imaging with short half-lives, ^{11}C ($T_{1/2}$ = 20.4 min) and ^{18}F ($T_{1/2}$ = 110 min) (Townsend, 2004). These radionuclides were then chemically bound to specific ligands produce the desired PET tracers (Wadsak et al., 2010). In Studies I–IV, we used three different PET tracers, and their production details are summarized below.

^{11}C-labeled carfentanil ([^{11}C]carfentanil) was used to quantify MORs in Studies I–IV. The [^{11}C]carfentanil production before May 2017 has been described previously (Hirvonen et al., 2009; Karlsson et al., 2015). After May 2017 (Study II scans), [^{11}C]carfentanil was produced as follows: [^{11}C]carfentanil was synthesized using [^{11}C]methyl triflate, where cyclotron-produced [^{11}C]methane was halogenated by gas phase reaction into [^{11}C]methyl iodide (Larsen et al., 1997) and converted online into [^{11}C]methyl triflate (Jewett, 1992). The [^{11}C]methane was produced at the Accelerator Laboratory of Åbo Akademi University, using the ^{14}N(p,α)^{11}C nuclear reaction in a N_2-H_2 target gas (10 % H_2). [^{11}C]methyl triflate was bubbled into a solution containing acetone (200 µl), O-desmethyl precursor (0.3–0.4 mg, 0.79–1.05 µmol) and tetrabutylammonium hydroxide (aq) (4 µl, 0.2 M) at 0 °C. The reaction mixture was diluted and loaded into a solid phase extraction cartridge (C18 Sep-Pak® Light, Waters Corp., Milford, MA) and the cartridge was washed. Dilution and washing were done using 25% ethanol in a sterile water solution, 10 mL each step. The [^{11}C]carfentanil was extracted with ethanol from the cartridge, diluted with 0.1 M phosphate buffer solution into < 10 % ethanol level and finally sterile filtered (Millex GV, 0.22 µm polyvinylidene fluoride membrane, 33 mm, Merck Millipore). Analytical HPLC column (Phenomenex Luna® 5 µm C8(2) 100 Å, 4.6 × 100 mm), acetonitrile (32.5%) in 50 mM H_3PO_4 mobile phase, 1 ml/min flow rate, 7 min run time and detectors in series for UV absorption (210 nm) and radioactivity were used for determination of identity, radiochemical purity and mass concentration. Radiochemical purity of the produced [^{11}C]carfentanil was >98%.

^{18}F-labeled 3-fluoromethoxy-*d_2* ([^{18}F]FMPEP-*d_2*) was used to quantify CB_1Rs in Studies II and IV. [^{18}F]FMPEP-*d_2* was produced as described previously (Lahdenpohja et al., 2020). Radiochemical purity was >95%.

^{18}F-labeled fluorodeoxyglucose ([^{18}F]FDG) was used to quantify brain glucose uptake (BGU) in Study II. [^{18}F]FDG was produced using a FASTlab synthesis platform (GE Healthcare) according to a modified method of Hamacher et al. (Hamacher et al., 1986) and Lemaire et al. (Lemaire et al., 2002). Radiochemical purity was >98%.

4.4 Positron emission tomography (PET)

4.4.1 General principles of PET

PET enables imaging of *in vivo* biological processes in the molecular level. A PET tracer with the desired biochemical properties is produced as described in the previous chapter. The tracer is injected into the subject's vein, from where it enters into tissues via the bloodstream. The PET scanner records concentrations of tissue radioactivity as a function of time (Lammertsma, 2002). The radioactivity measured

by PET scanner emerges from the beta plus (β^+) decay (positron emission, (Anderson, 1933)) of the injected unstable radionuclides. The β^+ decay of a parent nuclide *X* to a daughter nuclide *Y* can be presented as

$$(1) \quad {}^{A}_{Z}X_N \rightarrow {}_{Z-1}{}^{A}Y_{N+1} + e^+ + \nu$$

where *A* denotes mass number, *Z* denotes atomic number, *N* is the number of neutrons. β^+ decay thus results in the formation of a daughter nuclide with one proton less and one neutron more than in a parent nuclide, and to an emission of a positron (e^+) and a neutrino (ν). The positron travels through tissue (~ 1mm range) losing kinetic energy, subsequently colliding with tissue electron (e^-), which results in annihilation

$$(2) \quad e^+ + e^- \rightarrow \gamma + \gamma$$

where two 511 keV photons (γ) are formed and emitted in opposite directions. PET imaging is based on the detection of these two photons (*coincidence detection*) in a small (~ 4–12 ns) temporal window. The detection is possible due to scintillation detectors that are positioned side by side in a circular shape. The path between two given detectors is called a line of response (LOR). During a PET scan, the scanner records the number of coincidence events in the LORs between detectors. Using the coincidence data from multiple LORs with variable angles, it is possible to reconstruct a three-dimensional PET image. (Mettler Jr et al., 2012; Townsend, 2004; Turkington, 2001)

The general operating principle of a PET scanner is schematically presented in **Figure 6**. The *spatial resolution* of PET images is influenced by multiple factors including the size of the photon detector elements, the positron travel range before annihilation and sampling errors, with theoretical limit estimated to be 1.83 mm for clinical studies (Moses, 2011). In practice, the PET/CT (GE Healthcare) scanner used in Studies I–IV has a spatial resolution of approximately 5 mm (Teräs et al., 2007). The *temporal resolution* of PET is generally considered relatively low (from minutes to hours, depending on the tracer used) when compared for example to functional MRI (~ seconds).

Tracer-specific kinetic modeling is needed to derive meaningful clinical information (e.g. about receptor availability) from the "raw" time-activity data measured with a PET scanner (Lammertsma, 2002). These models for [^{11}C]carfentanil, [^{18}F]FMPEP-d_2 and [^{18}F]FDG are described in Chapter 4.6.

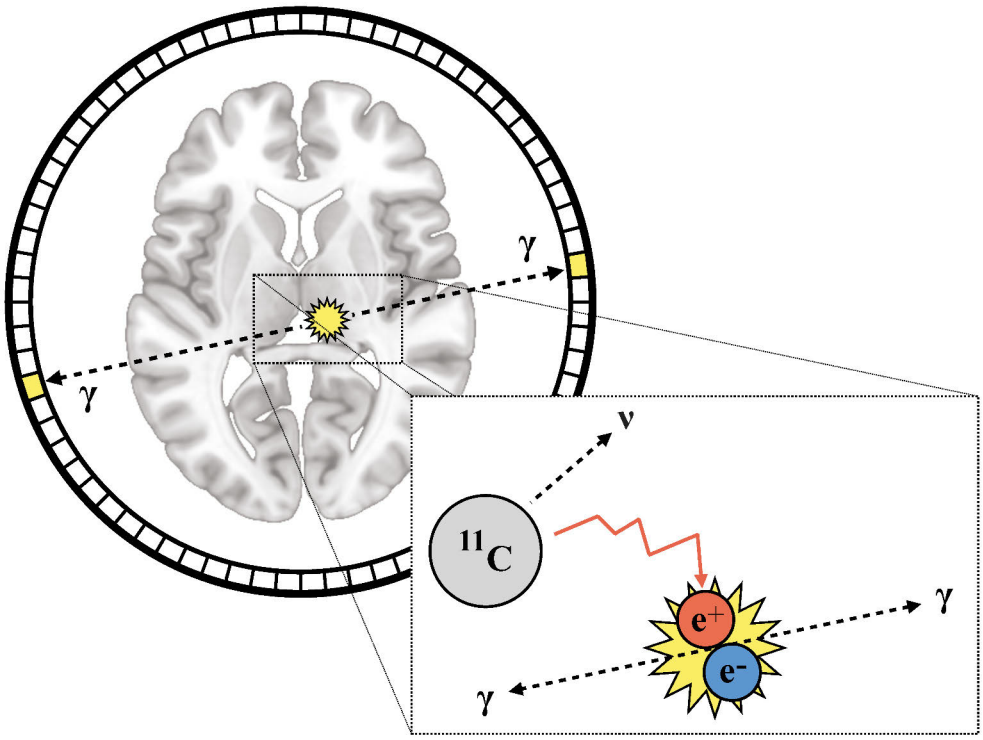

Figure 6. General principle of a PET scanner. In this schematic presentation, PET scanner scintillation detectors (positioned in circular shape) register a coincidence event based on two 511 keV photons (γ) arriving from thalamus. In the close-up image, unstable radionuclide ^{11}C decays to ^{11}B (omitted) and emits a neutrino (ν) and a positron (e^{+}). The positron travels a short distance losing kinetic energy, until it collides with a tissue electron (e^{-}). This results in annihilation of both particles and emission of two photons travelling in opposite directions. Based on these photons, PET scanner is able to localize and quantify tissue radioactivity as a function of time. An axial brain slice corresponds z = 7 in the MNI152 space.

4.4.2 Measurement of receptor availability in Studies I–IV

In Studies I–IV, MORs were studied by using [^{11}C]carfentanil, a high-affinity and specific agonist of MORs with high test-retest reliability and consistency (Frost et al., 1985; Hirvonen et al., 2009). GPCRs (including MORs and CB_1Rs) have at least two distinct affinity states depending e.g. on the presence of G protein (Warne et al., 2019). [^{11}C]carfentanil selectively binds to MORs with a high affinity state (Henriksen et al., 2008). The majority of [^{11}C]carfentanil (approximately 92–94%) binds to the cell surface MORs, while the binding is lesser in the intracellular space (Quelch et al., 2014). Endogenous opioid ligands released due to amphetamine (Quelch et al., 2014) and acetate administration (Ashok et al., 2021), social laughter (Manninen et al., 2017), physical exercise (Saanijoki et al., 2018b) and feeding (Tuulari et al., 2017) compete with [^{11}C]carfentanil in MOR binding. Thus, repeated

[^{11}C]carfentanil scans coupled with biological or psychological "challenges" can be used to measure opioid release in the brain. In all Studies, MOR availability was expressed as [^{11}C]carfentanil binding potential (BP_{ND}), which is detailed in Chapter 4.6.2. Regional MOR availabilities exhibit significant autocorrelation across most brain regions at an individual level (i.e. high BP_{ND} in a particular region predicts high BP_{ND} in other brain regions) (Tuominen et al., 2014).

In Studies II and IV, CB_1Rs were studied [^{18}F]FMPEP-d_2 (Donohue et al., 2008). The specific binding of [^{18}F]FMPEP-d_2 to CB_1Rs is approximately 90%, with low test-retest variability (Terry et al., 2010). As an inverse agonist of CB_1Rs, [^{18}F]FMPEP-d_2 may bind to both low and high affinity state receptors (Gullapalli et al., 2010; Hirvonen, 2015). To some degree, the [^{18}F]FMPEP-d_2 binding may be sensitive to the competitive binding of endogenous cannabinoids (Hirvonen et al., 2012; Takkinen et al., 2018). In Studies II and IV, CB_1R availability was expressed as [^{18}F]FMPEP-d_2 volume of distribution (V_T), which is detailed in Chapter 4.6.2.

In this thesis, receptor *availability* is used when referring to PET measures expressed as above ([^{11}C]carfentanil BP_{ND} and [^{18}F]FMPEP-d_2 V_T). In turn, receptor *density* is used when inferences of actual receptor count are made. To make such inferences of receptor density, usually evidence from other types of experiments is needed (e.g. *in vitro* autoradiography) in addition to single-scan PET studies. This is because changes in receptor availability measured with PET may potentially result from changes in receptor density, affinity or endogenous ligand competitive binding, or a combination of these factors (Henriksen et al., 2008).

4.4.3 PET data acquisition in Studies I–IV

Study I characterized the effects of demographic variables to the central MOR availability in healthy humans. The PET scans and associated demographic data were retrieved from the AIVO database (http://aivo.utu.fi). For Study I, all [^{11}C]carfentanil PET scans acquired between 2003 and 2018 which did not fulfill the exclusion criteria were identified, resulting in the study sample of 204 brain scans targeting MORs. Injected masses (μg, mean±SD) were 0.46±0.48 for males, and 0.47±0.50 for females. This sample consists of PET scans from 11 distinct research projects and five PET scanners. The PET scanners used were GE Advance (GE Healthcare, Wauwatosa, Wisconsin, USA), High Resolution Research Tool (HRRT, Siemens Medical Solutions, Erlangen, Germany), PET/CT (GE Discovery VCT PET/CT, GE Healthcare), GE Discovery (Discovery 690 PET/CT, GE Healthcare), and PET/MR (Ingenuity TF PET/MR, Philips Healthcare, Cleveland, Ohio, USA).

Study II examined whether central MOR or CB_1R availability associates with specific obesity risk factors. Subjects abstained from physical exercise the day before and fasted 6–12 hours before the PET scans, which were carried out on separate days.

[^{11}C]carfentanil and [^{18}F]FMPEP-d_2 PET images were acquired with PET/CT (GE Healthcare). On the [^{11}C]carfentanil day, the tracer was administered via an antecubital vein, and the brain's radioactivity was followed for 51 minutes. On the [^{18}F]FMPEP-d_2 day, two antecubital veins were cannulated. Blood samples for determining serum endocannabinoid levels were drawn and analyzed (Dickens et al., 2020). [^{18}F]FMPEP-d_2 was administered to another vein, and the brain's radioactivity was followed for 60 minutes. Arterialized blood samples for determining [^{18}F]FMPEP-d_2 metabolites and plasma radioactivity were drawn from another vein (Lahesmaa et al., 2018). In addition to MOR and CB_1R availabilities, the subjects' BGU was quantified in a separate study visit. This was accomplished by an additional PET scan with [^{18}F]FDG. The [^{18}F]FDG scans were performed during hyperinsulinemic euglycemic clamp, where blood glucose is held constant with a glucose infusion, and a steady rate insulin-infusion is also applied, enabling standardized measurement of tissue glucose uptake (DeFronzo et al., 1979; Tuulari et al., 2013). The [^{18}F]FDG was injected into an antecubital vein, and after 90-minute peripheral scan, the brain's radioactivity was followed for 10 minutes. Blood samples were obtained from an arterialized vein to measure plasma radioactivity. A GE Discovery (GE Healthcare) PET scanner was used in the [^{18}F]FDG scans. In Study II, altogether 41 subjects completed the [^{11}C]carfentanil scan, 38 subjects completed the [^{18}F]FDG scan (one subject discontinued due to cannula site pain and two due to scheduling problems) and 36 subjects completed the [^{18}F]FMPEP-d_2 scan successfully (the remaining scans were not possible to carry out due to scheduling problems and technical issues).

Study III examined whether physical fitness is associated with endogenous opioid release after exercise or with baseline MOR availability. The PET data were acquired with PET/MR (Philips Healthcare) (n = 24) and with PET/CT (GE Healthcare) (n = 40, subjects from the Study II). Subjects abstained from physical exercise the day before and fasted at least 2 hours before the PET scans. [^{11}C]carfentanil was administered via an antecubital vein, and the brain's radioactivity was followed for 51 minutes. On a separate day, a subset of participants (n = 23) also underwent an additional [^{11}C]carfentanil scan after a session of cycling exercise. The exercise session consisted of 60 minutes of moderate-intensity cycling (Tunturi E85, Tunturi Fitness, Almere, The Netherlands). The intensity level was determined individually based on preceding maximal exercise test (Saanijoki et al., 2018b).

Study IV determined whether variation in central MOR or CB_1R availability associates with feeding behavior traits measured with DEBQ. The PET data were retrieved from the AIVO database. For Study IV, all [^{11}C]carfentanil and [^{18}F]FMPEP-d_2 PET scans accompanied with completed DEBQ form which did not fulfill exclusion criteria were identified, resulting in 92 [^{11}C]carfentanil and 35

[^{18}F]FMPEP-d_2 PET scans. In Study IV, all subjects studied with [^{18}F]FMPEP-d_2 were also included in the [^{11}C]carfentanil sample (i.e. same subjects were scanned with two PET tracers). The Study IV sample consists of PET scans from five distinct research projects and three PET scanners. The PET scanners used were HRRT (Siemens Medical Solutions), PET/CT (GE Healthcare), and GE Discovery (GE Healthcare).

4.5 Magnetic resonance imaging (MRI)

4.5.1 General principles of MRI

MRI is an imaging method based on electromagnetic manipulation of tissue hydrogen atom nuclei and recording of the following MR signals. The complete presentation of MRI physics is outside of the scope of this thesis and is described in detail elsewhere (see for example (McRobbie et al., 2017)). Briefly, MRI utilizes hydrogen atom nuclei, which are abundant in most tissues as up to 75% of the human body consists of water. The hydrogen nuclei have magnetic moments with variable vector directions. In the MRI scanner, a strong external magnetic field (B_0) is applied to the nuclei, causing the sum vector of the magnetic moments of the nuclei (*net magnetization*) to align with B_0. This alignment of magnetic moments is then manipulated by radiofrequency pulses, generated by transmit coils. For example, with a 90° radiofrequency pulse, the net magnetization can be rotated perpendicular to the B_0. After the radiofrequency pulse, the magnetic moments start to re-align with B_0, emitting electromagnetic waves (MR signal) which can be registered with receiving coils. *T_1 relaxation* is the process where the hydrogen net magnetization vector returns to the alignment with the main magnetic field B_0, while *T_2 relaxation* is the process where the transverse magnetization decays. Durations for T_1 and T_2 are dependent of tissue specific factors, and T_2 is faster than T_1 in a given tissue. By modification of timing parameters used in radiofrequency pulse delivery and signal registration, it is possible to obtain differently *weighted* MR images. For example, T_1-weighted images offer an excellent contrast between different tissue types (e.g. between gray and white brain matter), while functional MRI is based on *T_2*-weighting* (depending on magnetic field inhomogeneities). (McRobbie et al., 2017)

4.5.2 MRI data acquisition in Studies I–IV

In all Studies, T_1-weighted MR images were used in preprocessing of PET images and to provide anatomical reference. In Study I, MRI data were acquired with Philips Ingenuity TF PET/MR 3T scanner (Philips Healthcare, Cleveland, Ohio, USA), Philips Gyroscan Intera CV Nova Dual 1.5T scanner (Philips Healthcare) and

Siemens Magnetom 1.5T scanner (Siemens Medical Solutions, Erlangen, Germany). In Study II and III, MRI data were acquired with Phillips Ingenuity TF PET/MR scanner. In Study IV, MRI data were acquired with Phillips Ingenuity TF PET/MR scanner and Philips Gyroscan Intera CV Nova Dual scanner.

4.6 Data processing and modeling

4.6.1 Magia pipeline

The "raw" dynamic PET images contain data of the radioactivity concentration as a function of time (time-activity curve) for each volumetric unit (voxel). This data is further modeled to yield biologically meaningful information. The PET images were preprocessed and modeled using Magia, an automated PET image processing pipeline running on MATLAB (The MathWorks, Inc., Natick, MA, USA). Development, validation and operation of Magia has been described in detail elsewhere (Karjalainen et al., 2020), and the open-source code is available at https://github.com/tkkarjal/magia. Compared to traditional manual processing methods, Magia reduces inter-operator variance, and is well suited for large-scale PET data analysis (e.g. in Study I) (Karjalainen et al., 2020).

The essential preprocessing steps in the Magia pipeline are:

i. Combining the PET image slices (2D) to volumes (3D),

ii. Motion correction to account for subject's head movement during the scan,

iii. Coregistration between the corresponding MR image to obtain the individual anatomical reference for further analyses,

iv. Generation of *reference regions* and *regions of interest* (ROIs) for kinetic modeling and analysis. These are produced with FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) from T_1-weighted MR images (i.e. anatomical labeling of each image voxel),

v. Generation of time-activity curves for reference regions and ROIs,

vi. *Tracer-specific kinetic modeling* in individual voxels and also in the aforementioned regions (detailed description in the next chapter),

vii. Normalization to the MNI 152 standard space to account for individual differences in brain size and shape and to allow population-level inference,

viii. Smoothing to increase signal-to-noise ratio. Neighboring data points are averaged with a Gaussian kernel, in our data the full width at half maximum was 8 mm for [^{18}F]FDG, and 6 mm for [^{11}C]carfentanil and [^{18}F]FMPEP-d_2.

4.6.2 Tracer-specific modeling (BP_{ND}, V_T, and BGU)

In Studies I–IV, MOR availability was expressed as [^{11}C]carfentanil binding potential, BP_{ND}, which is the ratio of *specifically bound* radioligand to that of *nondisplaceable* radioligand in tissue at equilibrium (Innis et al., 2007). Specific binding refers to the radioligand binding to its target receptor (e.g. [^{11}C]carfentanil to MOR), while nondisplaceable uptake refers to the combined concentration of radioligand binding to non-target molecules and free radioligand in tissue. BP_{ND} can be defined as

$$(3) \qquad BP_{ND} = \frac{V_T - V_{ND}}{V_{ND}} = \frac{V_T}{V_{ND}} - 1$$

where V_T is the total ligand concentration in tissue relative to the plasma radioligand concentration, and V_{ND} is the radioligand concentration in the nondisplaceable compartment relative to the concentration of ligand in the plasma (Innis et al., 2007). In [^{11}C]carfentanil studies it is not necessary to directly measure plasma radioactivity, since it is possible to use a reference tissue with no specific receptor binding (Endres et al., 2003). We used the occipital cortex as the reference region, since it contains only negligible amounts of MORs (Le Merrer et al., 2009; Pfeiffer et al., 1982), and the between-individual and within-individual variation of occipital cortex reference estimates is also negligible (Endres et al., 2003; Hirvonen et al., 2009). In the Magia pipeline, parametric BP_{ND} images are produced with basis function algorithms, which use the simplified reference tissue model of tissue radioactivity to estimate kinetic parameters, including BP_{ND} (Gunn et al., 1997; Lammertsma et al., 1996b). Essentially, this method compares the radioactivity derived from the time-activity curve of the target region (with specific binding) to the radioactivity of the reference region (with no specific binding) (Lammertsma et al., 1996a). Animal models suggest that time-activity curves or BP_{ND} measurements are not affected by changes in cerebral blood flow (Sander et al., 2019). The BP_{ND} measure is a unitless ratio (Innis et al., 2007).

In Study III, endogenous opioid release was measured with two [^{11}C]carfentanil PET scans conducted on separate days – one at baseline (rest) and one after physical exercise condition. Acute intra-individual changes in BP_{ND} are thought to represent the competitive receptor binding of endogenous neurotransmitters (Laruelle, 2000), i.e. released endogenous opioids that are competing with [^{11}C]carfentanil for the same binding sites. Accordingly, in Study III, the endogenous opioid release was quantified as a subtraction of the outcome measures, that is $\Delta BP_{ND} = BP_{ND(exercise)} - BP_{ND(baseline)}$.

In Studies II and IV, CB_1R availability was expressed as [^{18}F]FMPEP-d_2 volume of distribution, V_T (ml x cm^{-3}), which is the ratio of radioligand concentration in tissue to the plasma radioligand concentration at the equilibrium state (Innis et al., 2007). V_T was derived from graphical analysis as described by Logan (Logan, 2000). In this analysis, radioligand concentration-time curves for the target region and plasma are combined to form a single *Logan plot*, which achieves linearity (equilibrium) after a certain time, and from the slope of the line we can estimate V_T (Logan, 2000). [^{18}F]FMPEP-d_2 is metabolized in the plasma to radioactive metabolites (Terry et al., 2010), whose contribution to the detected plasma radioactivity was corrected (subtracted) as described elsewhere (Lahesmaa et al., 2018). In the model fitting, we used the image frames starting at 36 minutes and later after the injection, since the Logan plots became linear after 36 minutes. The equation for Logan plot and V_T measurement can be presented as

$$(4) \qquad \frac{\int_0^T C_{ROI}(t)dt}{C_{ROI}(T)} = V_T * \frac{\int_0^T C_p(t)dt}{C_{ROI}(T)} + Int$$

where $C_{ROI}(T)$ is the concentration of the radioligand in ROI at the time T, C_p is the radioligand concentration in the plasma, and *Int* is the intercept. For more detailed modeling presentation, please see: http://www.turkupetcentre.net/petanalysis/model_mtga.html#logan.

In Study II, brain glucose uptake (BGU) was quantified with [^{18}F]FDG. BGU is derived from the fractional uptake rate (FUR), which is the ratio of tissue radioactivity to the integral of plasma activity at a given time (Thie, 1995). To obtain the final FUR-values, the tissue radioactivity values were averaged over the time frames after 40 minutes from injection (late scan). Plasma activity was estimated by hybrid input functions. From 0 to 4.5 minutes the plasma activity was derived from the image (cardiac left ventricle), and then combined to activity measures derived from arterialized venous blood samples (Phelps et al., 1979). The FUR-estimates were converted to BGU (µmol/min/100g) with the equation

$$(5) \qquad BGU = 100 * \frac{glucose*FUR}{LC*density}$$

where *glucose* is the average glucose concentration in the blood from the time of the tracer injection until the end of the brain scan, *LC* is the lumped constant (irreversible uptake), and *density* is the gray matter tissue relative density in the brain. We used the values *LC* = 0.65 (Wu et al., 2003) and *density* = 1.04 (Snyder et al., 1975). At

least for muscle and adipose tissues, obesity does not influence the *LC* value (Peltoniemi et al., 2000; Virtanen et al., 2001).

4.7 Statistical analysis

In Study I, Bayesian hierarchical modeling was used to estimate the effects of age, BMI, sex and smoking to MOR availability ([^{11}C]carfentanil BP_{ND}). We specified seven linear models for the effects using the R package BRMS (Bürkner, 2017), which utilizes the sampling tools of RStan (https://mc-stan.org/users/interfaces/rstan). The first model included age, BMI, sex, smoking and sex-interactions for age and BMI. All other models were submodels of the first model. The PET scanner was used as a nuisance covariate to control for scanner-dependent variation. We used weakly informative priors in all models. BP_{ND} values were log-transformed, since an assumption of linear additivity works poorly when the dependent variable (BP_{ND}) is limited to positive values (Gelman et al., 2006), and posterior predictive checking showed that the log-transformation improves the model fit (Gabry et al., 2019; Gelman et al., 1995). The predictive performance of the specified models was compared with Bayesian 10-fold cross-validation (Vehtari et al., 2002) utilizing the R package LOO (https://CRAN.R-project.org/package=loo). This included creating subsamples where 10% of the subjects were randomly removed, and assessing the predictive accuracy of the models in these subsamples. This procedure was repeated ten times, and results were combined to select the model with best predictive accuracy. According to the cross-validation, the model without BMI was the best predictor of MOR availability. The reported posterior distributions at ROI-level and cluster level were obtained by fitting this model to the data. In Studies I–IV, *a priori* ROIs were examined. In Study I, ROIs were chosen based on previous reports of regions with MORs in moderate to high density (Nummenmaa et al., 2018b; Tuominen et al., 2014). In Studies II–IV, ROIs with moderate to high density of target receptors and potential roles in obesity risk and food intake regulation were studied (Bencherif et al., 2005; Berridge et al., 2003; Carta et al., 2019; Hirvonen et al., 2012; Karlsson et al., 2015; Majuri et al., 2017; Owens et al., 2017; Tuominen et al., 2014; Tuulari et al., 2013). A summary of the analyzed ROIs in Studies I–IV is presented in **Table 5**. In Study I, we used FreeSurfer to generate 15 ROIs, and estimated the effects of age, sex and smoking in these. Because voxel-wise analysis would be computationally prohibitive, the full-volume analysis was performed by clustering the atlas-derived (the AAL template, (Tzourio-Mazoyer et al., 2002)) anatomical ROIs into smaller volume units based on [^{11}C]carfentanil BP_{ND} map of the sample. This procedure defines clear anatomical boundaries for small (here 320) volumetric units. The

complete scripts used in Study I and their detailed description is available in https://github.com/tkkarjal/morvariability.

In Study II, [^{11}C]carfentanil BP_{ND}, [^{18}F]FMPEP-d_2 V_T, and BGU measured with [^{18}F]FDG were compared between the LR and HR groups using two-sample t-test. We analyzed full-volume data with nonparametric approach using SnPM13 (http://nisox.org/Software/SnPM13/). $p < 0.05$ was used as the cluster-defining threshold, and we reported clusters large enough to be statistically significant with family-wise error (FWE) $p < 0.05$. Additionally, the effects of serum endocannabinoids to the [^{18}F]FMPEP-d_2 V_T were examined in separate full-volume models. Since eight endocannabinoid compound models were specified, endocannabinoid results were confirmed using Bonferroni-corrected p value as the cluster-defining threshold ($0.05/8 = 0.00625$). We also analyzed the individual effects of distinct risk factors (BMI, physical exercise and Family Risk) to the outcome measures in 21 FreeSurfer-parcellated ROIs involved in the food reward processing via opioid, endocannabinoid and insulin messaging (Hirvonen et al., 2012; Tuominen et al., 2014; Tuulari et al., 2013) (**Table 5**). The ROI-level effects were estimated using the Bayesian approach similar to that used in Study I. We used weakly informative, regularizing priors with a zero-mean for the estimated regression coefficients in order reduce overfitting. The hierarchical (multilevel) Bayesian model used produces estimates that are partially pooled towards each other, consequently accounting for multiple comparisons (Gelman et al., 2012). Age was controlled for in all full-volume and ROI models.

In Study III, the effects of VO_{2peak} and MVPA to [^{11}C]carfentanil BP_{ND} change following the cycling exercise and to baseline BP_{ND} were assessed. These were modeled with SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) using linear regression model. BMI and PET scanner were entered as covariates to the models. Age was additionally controlled for in a complementary analysis. The statistical threshold was set at $p < 0.05$, false discovery rate (FDR) corrected at cluster level. Additionally, ten *a priori* ROIs with high density of MORs (**Table 5**) were generated using the AAL (Tzourio-Mazoyer et al., 2002) and SPM Anatomy (Eickhoff et al., 2005) toolboxes. The mean BP_{ND} were extracted from the ROIs, and the effects of VO_{2peak} and MVPA to the mean BP_{ND} values were analyzed with R statistical software (https://cran.r-project.org) using least squares regression and Pearson correlations.

In Study IV, Bayesian hierarchical modeling (as described in Study I and II) was utilized to analyze the effects of the DEBQ subscale scores (Emotional eating, External eating, Restrained eating) and Total DEBQ scores on [^{11}C]carfentanil BP_{ND} and [^{18}F]FMPEP-d_2 V_T. Effects were quantified in ten ROIs with high density of MORs (Tuominen et al., 2014) and CB_1Rs (Terry et al., 2010) (**Table 5**). ROIs were parcellated using FreeSurfer. SnPM13 was used to create corresponding full-volume models. $p < 0.01$ was used as the cluster-defining threshold, and we reported clusters

statistically significant with FWE $p < 0.05$. Age was controlled for in all full-volume and ROI models, and we also controlled for PET scanner in [^{11}C]carfentanil models. Sex, BMI and smoking were controlled for in a complementary [^{11}C]carfentanil analysis, and [^{18}F]FMPEP-d_2 models were also replicated with BMI as an additional covariate to rule out the confounding effects of these variables.

Table 5. The analyzed regions of interest in Studies I–IV.

	I	II	III	IV
AMYGDALA	x	x	x	x
CAUDATE	x	x	x	x
CEREBELLUM		x		x
DORSAL ANTERIOR CINGULATE	x	x		x
HIPPOCAMPUS		x	x	
INFERIOR TEMPORAL GYRUS	x	x		
INSULA	x	x	x	x
MEDULLA		x		
MIDBRAIN		x		
MIDDLE CINGULATE			x	
MIDDLE TEMPORAL GYRUS	x	x		x
NUCLEUS ACCUMBENS	x	x	x	x
ORBITOFRONTAL CORTEX	x	x	x	x
PARS OPERCULARIS	x	x		
POSTERIOR CINGULATE	x	x	x	
PONS		x		
PUTAMEN	x	x		x
ROSTRAL ANTERIOR CINGULATE	x	x	x	
SUPERIOR FRONTAL GYRUS	x	x		
SUPERIOR TEMPORAL GYRUS		x		
TEMPORAL POLE	x	x		
THALAMUS	x	x	x	x

5 Results

5.1 Study I: Sex, age and smoking have region-specific influence on central μ-opioid receptor availability

Study I analyzed the effects of age, sex, smoking and BMI on brain MOR availability. In Study I, the model including age, sex, age–sex interaction and smoking, but without BMI, was the best predictor of MOR availability. Thus, in this mostly normal weight sample, BMI was not a relevant predictor of MOR availability. Age had a regionally specific effect on MOR availability. In the thalamus, nucleus accumbens and amydgala, BP_{ND} *decreased* with age, while in other brain regions BP_{ND} *increased* with age. The proportional changes of BP_{ND} resulting from one SD (10.8 years) increase in age ranged from a 3–16% increase in the frontotemporal areas to a 2–8% decrease in subcortical areas. In females, the decrease of BP_{ND} in nucleus accumbens was more prominent, and the age-dependent increases in BP_{ND} were weaker than in males. Overall, 20-year-old females had a higher MOR availability than 20-year old males, but this sex difference reversed after the middle age, and 50- to 60-year-old males had had higher MOR availability compared to females of the same age in most brain regions. The sex-dependent effects of age on MOR availability are summarized in **Figure 7**.

Smoking had a global negative effect on central MOR availability. Compared to nonsmokers, smokers had reduced BP_{ND} (–8% to –14%). These reductions were most evident in subcortical regions including caudate, putamen and amygdala, while cortical associations were prominent in insula and fronto-cingulate cortex (**Figure 8**).

The anatomical receptor availability map of MORs and also the beta maps (model coefficients) for the effects of age and smoking can be found at https://neurovault.org/collections/GCELSAIA/.

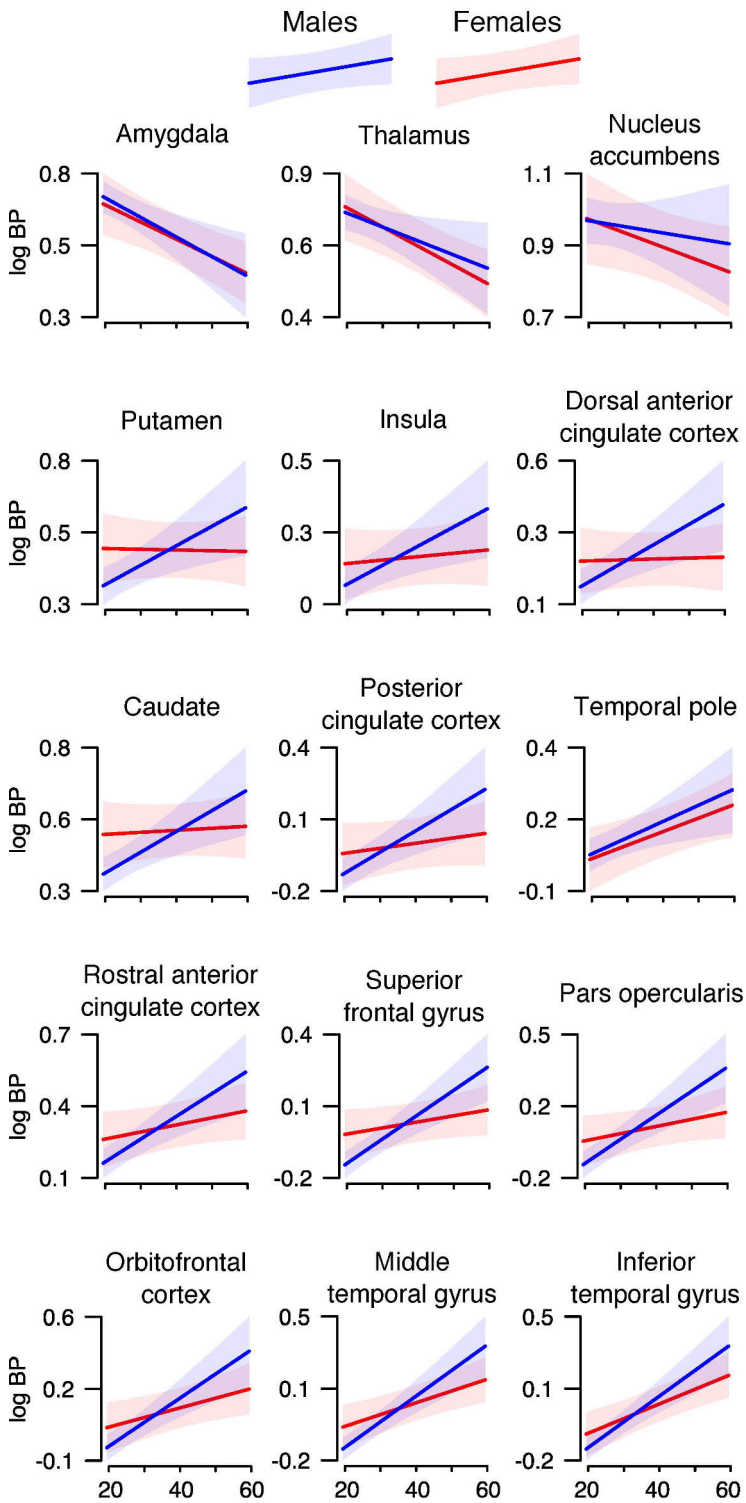

Figure 7. Relationship between age and central μ-opioid receptor availability. Effects of age (x-axis) to mean log [^{11}C]carfentanil binding potential BP_{ND} (y-axis) with 95% highest density posterior intervals for all examined regions of interest separately for males (blue) and females (red). Reproduced from the original publication I.

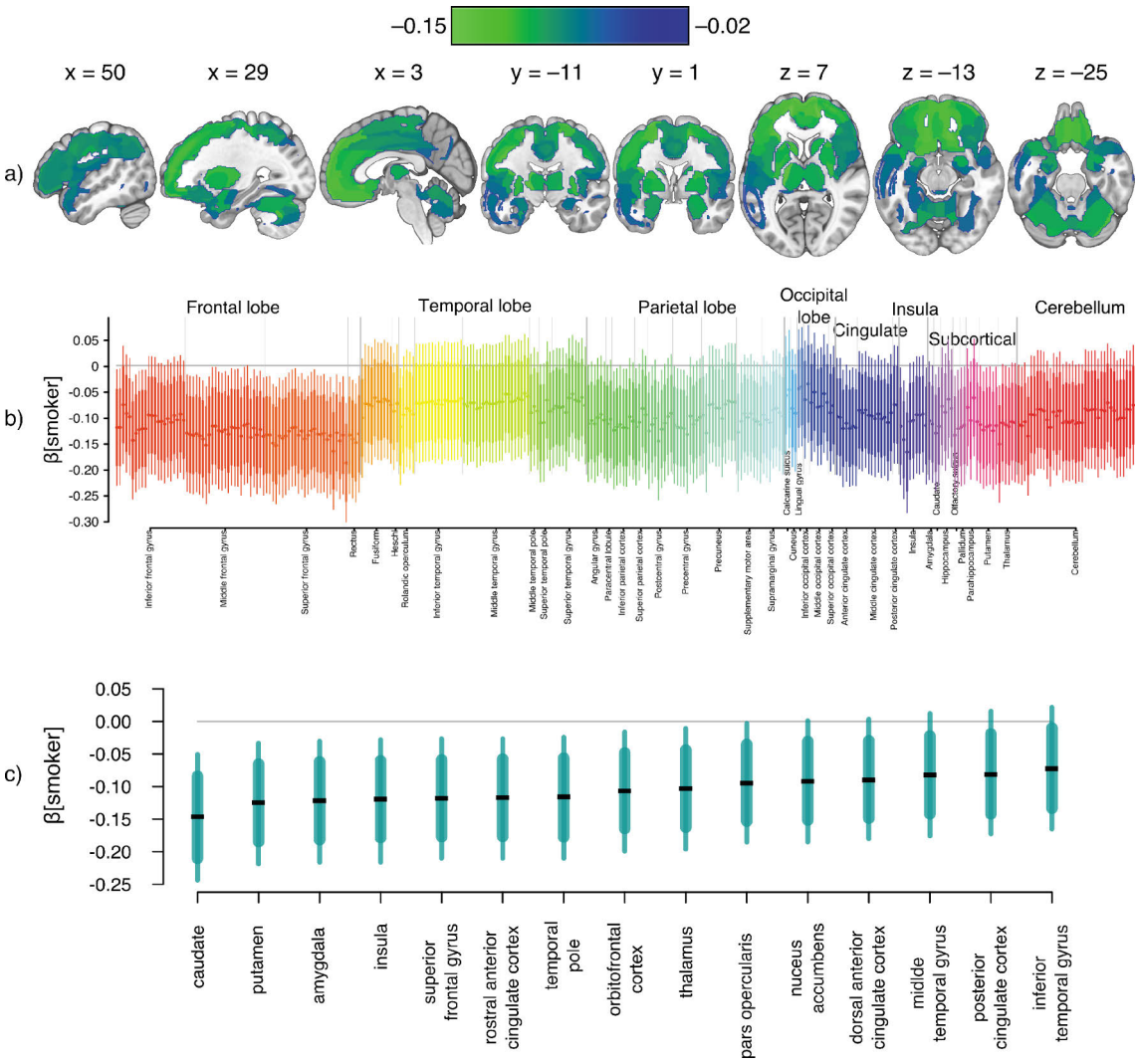

Figure 8. Effects of smoking on central μ-opioid receptor availability. a) Brain regions where smoking associated with decreased [^{11}C]carfentanil binding potential BP_{ND}. Shown are clusters whose 80% posterior interval excluded zero and where absolute value of the regression coefficient was at least 0.02. The coordinates are in the MNI152 space. b) Posterior distributions for the regression coefficients of smoking in all anatomical clusters. The filled circles represent posterior means, the thick lines 80% posterior intervals, and the thin lines 95 % posterior intervals. c) Posterior distributions for the regression coefficients of smoking in pre-specified regions of interest. The filled circles represent posterior means, the thick lines 80% posterior intervals, and the thin lines 95 % posterior intervals. Reproduced from the original publication I.

5.2 Study II: Familial obesity risk associates with altered brain glucose uptake and decreased availability of μ-opioid and CB_1 receptors

Study II investigated whether obesity risk is associated with brain glucose uptake (BGU) and central availability of MORs and CB_1Rs. Mean distribution of MORs, CB_1Rs and BGU was consistent with previous literature (Laurikainen et al., 2019; Nummenmaa et al., 2018b; Rebelos et al., 2019) (**Figure 9**). Subjects with high obesity risk had globally increased insulin-stimulated BGU compared to low-risk subjects, while group differences in neuroreceptor (MOR / CB_1R) availability did not reach statistical significance. Of the three distinct obesity risk factors, the familial obesity risk (including parental obesity / T2D) had the strongest association with the PET outcome measures. Increased familial obesity risk strongly associated with increased BGU, and also with the decreased availability of MORs and CB_1Rs (**Figure 9**). Increased family-mediated obesity risk had a global association to increased BGU, while the decrease of MORs was most prominent in the striatum and insula (**Figure 10**). BMI had a weaker effect on BGU and also on CB_1R availability (**Figure 9**). Of the eight studied circulating endocannabinoids studied, only AEA had significant associations with central CB_1R availability. Increase in serum AEA associated with a decrease of CB_1Rs in the frontal striatum (**Figure 11**).

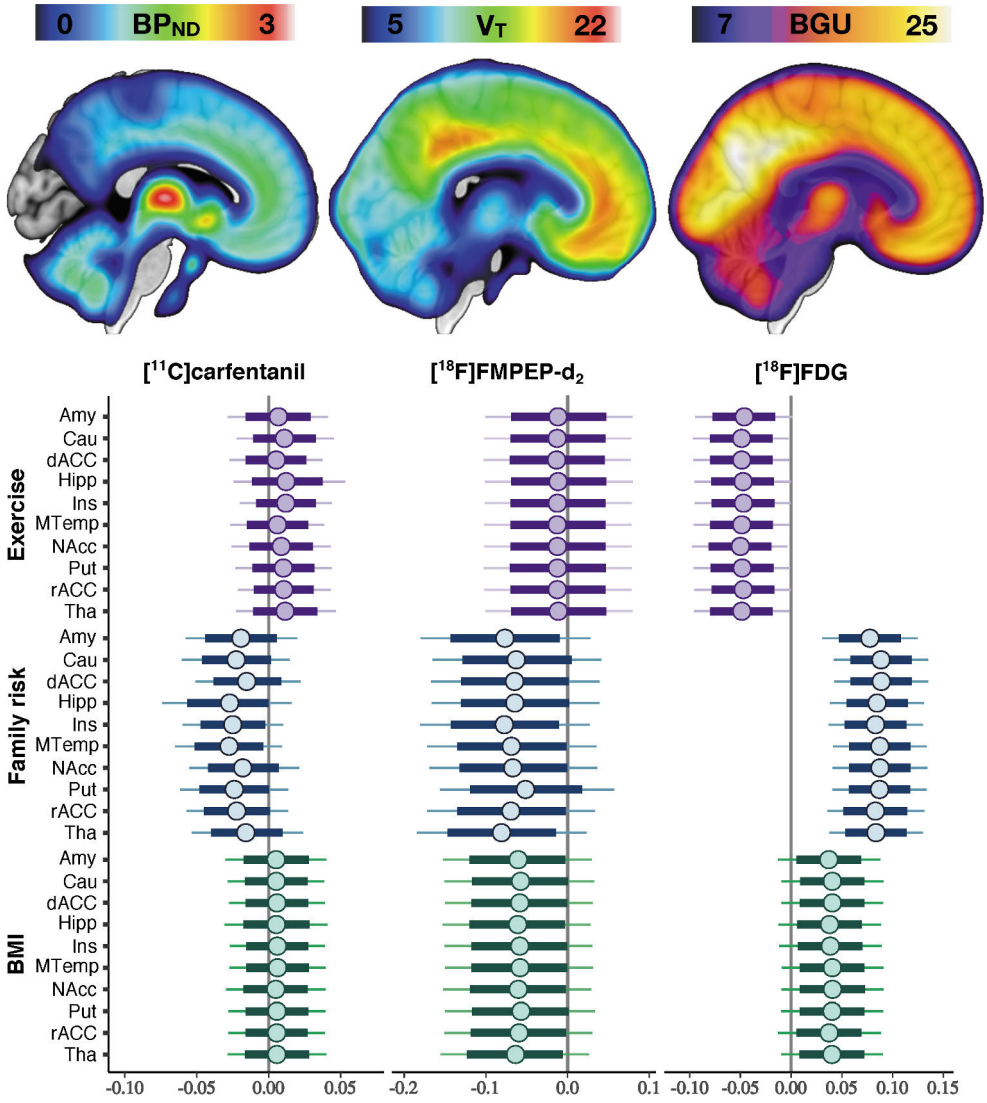

Figure 9. Neuroreceptor availability, brain glucose uptake (BGU) and obesity risk. Upper row shows the mean distribution of μ-opioid receptors ([^{11}C]carfentanil BP_{ND}, n = 41), CB_1 receptors ([^{18}F]FMPEP-d_2 V_T, n = 36) and BGU quantified by [^{18}F]FDG (n = 38) for the Study II sample. Brain images are in sagittal plane (x = -5) in the MNI152 space. Lower image shows the effects of distinct obesity risk factors (BMI, Family risk score and exercise) on the log-transformed and age-corrected BP_{ND}, V_T and BGU in 10 representative regions of interest. The effects are expressed as posterior distributions of the regression coefficients. The colored circles denote posterior means, the thick horizontal bars 80% posterior intervals, and the thin horizontal bars 95% posterior intervals. Abbreviations stand for Amy = amygdala, Cau = caudate, dACC = dorsal anterior cingulate cortex, Hipp = hippocampus, Ins = insula, MTemp = middle temporal gyrus, NAcc = nucleus accumbens, Put = putamen, rACC = rostral anterior cingulate cortex, Tha = thalamus. Modified from the original publication II.

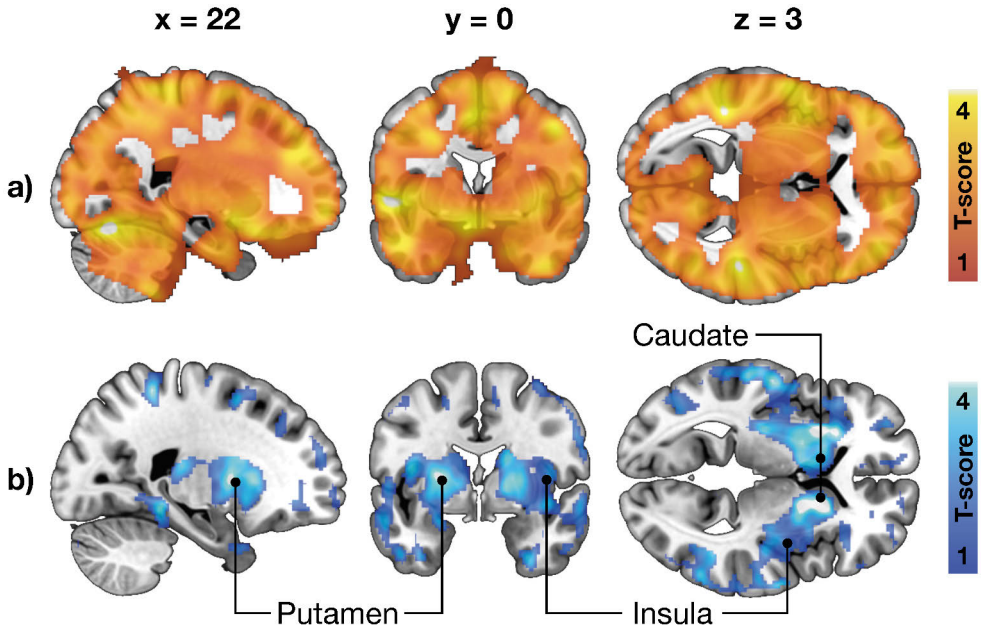

Figure 10. Familial obesity risk, brain glucose uptake (BGU) and μ-opioid receptor (MOR) availability. a) Brain areas where increased Family Risk score associated with increased BGU in the 38 subjects studied with [^{18}F]FDG. b) Brain areas where increased Family Risk score associated with decreased MOR availability in the 41 subjects studied with [^{11}C]carfentanil. The data are thresholded at $p < 0.05$, FWE corrected at cluster level. Other risk factors (BMI, exercise) and age act as covariates in the linear regression model. The coordinates are in the MNI152 space. Modified from the original publication II.

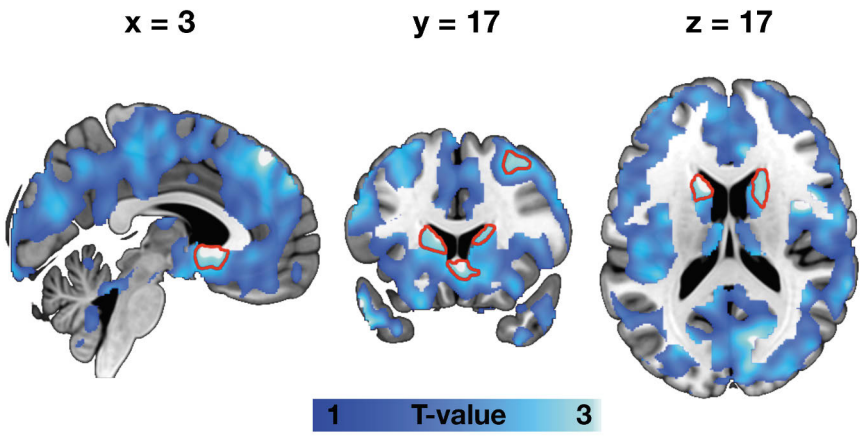

Figure 11. Circulating anandamide (AEA) and brain CB_1 receptors. Regions where increased AEA associated with lowered CB_1 receptors. The data are thresholded at $p < 0.05$, FWE corrected at cluster level, age corrected. Areas denoted with red were significant with additional Bonferroni cluster-correction. Modified from the original publication II.

5.3 Study III: Physical fitness predicts endogenous opioid release in the brain

Study III investigated whether physical fitness is associated with brain MOR availability and endogenous opioid releasing capacity. Higher cardiorespiratory fitness (VO_{2peak}) was associated with increased endogenous opioid release ([^{11}C]carfentanil ΔBP_{ND}) in multiple brain regions, most notably in the striatum, left hippocampus and frontotemporal cortices (**Figure 12**). VO_{2peak} also correlated with mood improvement after exercise, as measured with Positive Affect and Negative Affect Schedule ($r = 0.59$, $p < 0.01$). Physical exercise habits (weekly moderate to vigorous physical activity, MVPA, minutes/week) predicted endogenous opioid release – higher MVPA was associated with increased opioid release in striatum, insula and frontotemporal cortices (**Figure 12**). BP_{ND} variabilities in response to exercise were much higher (for example 350% higher in the thalamus and 60% higher in the anterior cingulate cortex) than the standard variability between two [^{11}C]carfentanil scans with no behavioral challenge (Hirvonen et al., 2009). VO_{2peak} was also negatively associated with baseline BP_{ND} in most regions with high MOR density, but this association did not survive *a priori* statistical threshold when age was controlled for.

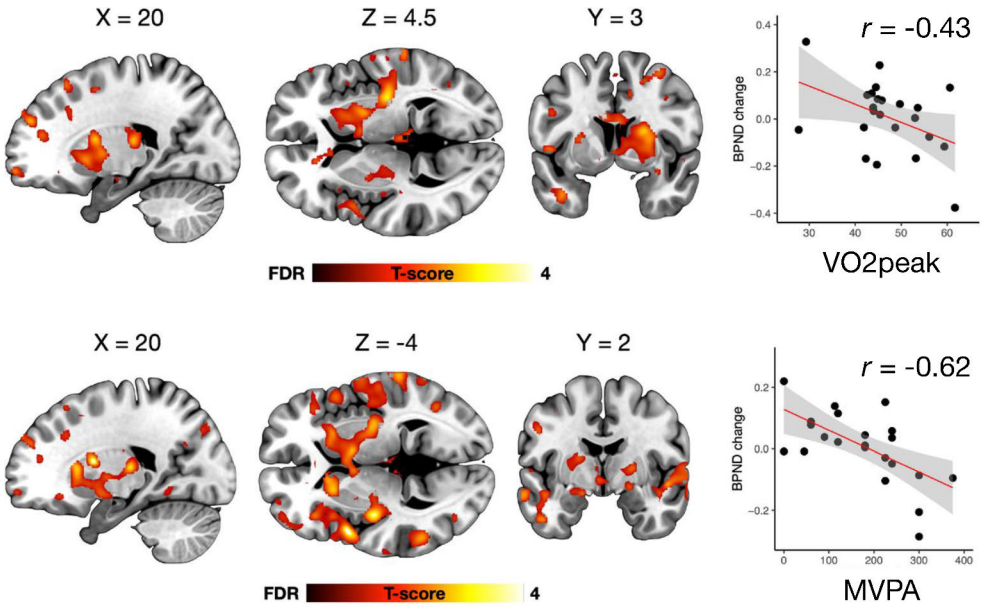

Figure 12. Associations between physical fitness and exercise habits on endogenous opioid release in the brain. Upper row: Increased cardiorespiratory fitness (VO_{2peak}, ml/kg/min) was associated with a larger decrease in [^{11}C]carfentanil BP_{ND} in multiple brain regions. The scatterplot shows the corresponding association in the putamen (least squares regression line in red with 95% CI). Lower row: Increase in regular physical activity (MVPA, minutes/week) was also associated with a larger decrease in [^{11}C]carfentanil BP_{ND}. The scatterplot shows the corresponding association in right fusiform gyrus. These data are thresholded at $p < 0.05$, FDR-corrected. The coordinates are in the MNI152 space. Modified from the original publication III.

5.4 Study IV: Decreased availability of μ-opioid receptors associates with external eating scores, while CB_1 receptor availability is associated with multiple eating behavior traits

Study IV analyzed the association of eating behavior traits with central MOR and CB_1R availabilities. Eating behavior was quantified with the DEDQ. For [^{11}C]carfentanil we observed that lower central MOR availability was associated with higher External eating scores in all ten examined ROIs. With other DEBQ subscales and Total DEBQ score, the 80% confidence intervals overlapped with zero. Full volume analysis identified the strongest associations between MOR availability and External eating score in the right frontotemporal cortex, anterior insula, and cerebellum (**Figure 13**). Results were essentially the same when controlling for sex, BMI and smoking. There were, however, no significant associations in the female subsample, possibly due to the limited sample size and

reduced statistical power. Results in the male-only subsample were similar to those with the whole sample.

In the [^{18}F]FMPEP-d_2 models, decreased central CB_1R availability associated with higher Total DEBQ score in all ROIs. The directions of the associations between distinct DEBQ subscales and CB_1R availability were all negative, but the 95% confidence intervals overlapped with zero. In the full volume analysis, lower CB_1R availability associated with higher Total DEBQ score in multiple brain regions bilaterally, the most prominent associations being in the frontotemporal cortex, parahippocampus, ventral striatum, cerebellum, insula, and anterior cingulate (**Figure 14**). Analyses yielded similar results when controlling for BMI.

The full-volume receptor availability maps for MORs and CB_1Rs, and statistically significant full-volume DEBQ association maps can be accessed via https://neurovault.org/collections/RZFLYXTL/.

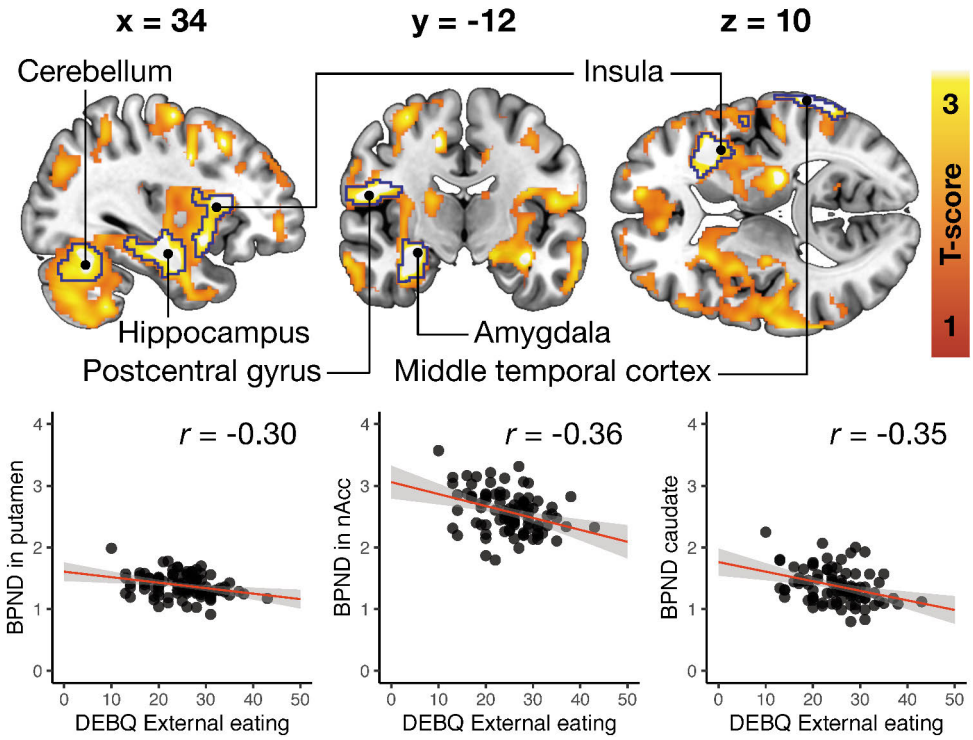

Figure 13. Association between external eating and μ-opioid receptor availability in the full sample (70 males and 22 females) of Study IV. Upper row: The blue outline denotes brain regions where lower [^{11}C]carfentanil binding potential (BP_{ND}) associated with higher External eating score, cluster forming threshold $p < 0.01$, FWE corrected, controlled for age and PET scanner. Additional associations significant with more lenient cluster-defining threshold ($p < 0.05$, FWE corrected) are shown in the red–yellow T-score scale. The coordinates are in the MNI152 space. Lower row: The scatterplot visualization shows the associations of External eating score and [^{11}C]carfentanil BP_{ND} in the putamen, nucleus accumbens (nAcc) and caudate (least squares regression line in red with 95% CI). Modified from the original publication IV.

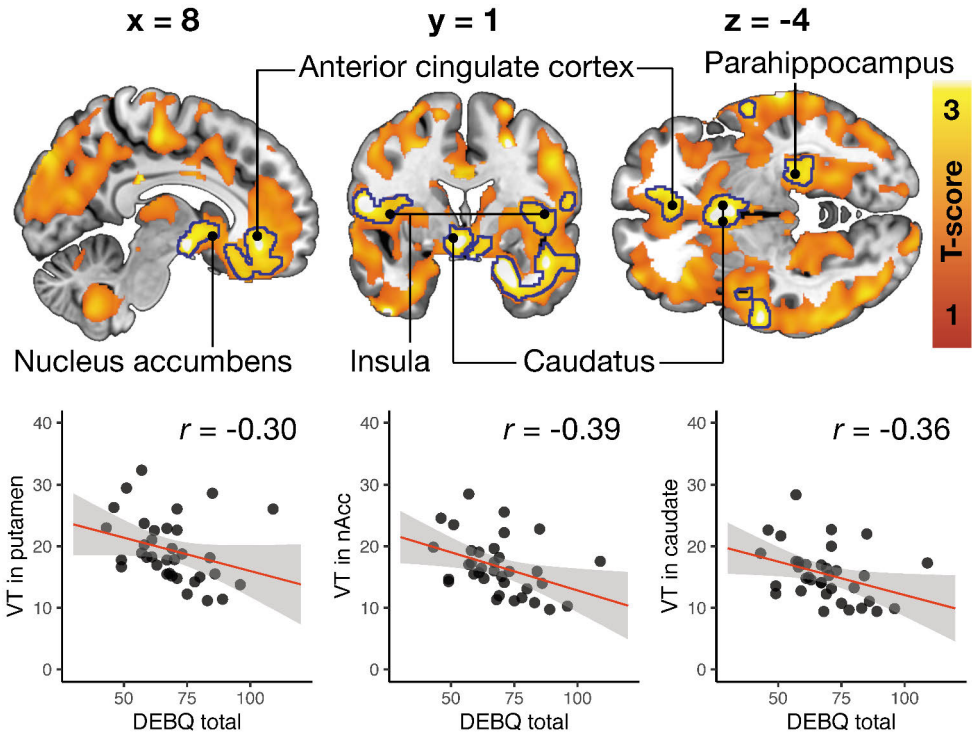

Figure 14. Association between the total Dutch Eating Behavior Questionnaire (DEBQ) score with CB_1 receptor availability in the 35 males of Study IV. Upper row: The blue outline denotes brain regions where lower [^{18}F]FMPEP-d_2 volume of distribution (V_T) associated with higher Total DEBQ score, cluster forming threshold $p < 0.01$, FWE corrected, controlled for age. Additional associations significant with more lenient cluster-defining threshold ($p < 0.05$, FWE corrected) are shown in the red–yellow T-score scale. The coordinates are in the MNI152 space. Lower row: The scatterplot visualization shows the associations of Total DEBQ score and [^{18}F]FMPEP-d_2 V_T in the putamen, nucleus accumbens (nAcc) and caudate (least squares regression line in red with 95% CI). Modified from the original publication IV.

6 Discussion

6.1 Variation in μ-opioid receptor availability – implications for obesity risk

Study I showed that age and sex have regionally specific effects on central MOR availability, while smoking was associated with widespread reductions in MOR availability. Globally, obesity is more prevalent in women across all age groups (Chooi et al., 2019). This sex difference is especially prominent after 50 years of age, when the obesity prevalence in females rises to 20% while remaining around 10% in men (Chooi et al., 2019). It has been suggested that the difference in obesity prevalence might result, at least partly, from sex differences in the brain systems processing reward and appetite (Kroll et al., 2020). For example, in women, obesity is associated more strongly with the volume of putamen and dorsolateral prefrontal cortex than in men (Horstmann et al., 2011; Kroll et al., 2020). Functional MRI studies have observed sex-dependent differences in central taste processing, but results have been mixed (Kroll et al., 2020). Although many neuroimaging studies on obesity have included only males or females (Kroll et al., 2020), decreased MOR availability in feeding regulating brain structures has been documented in both obese females (Karlsson et al., 2015) and males (Burghardt et al., 2015).

In Study I, we found that central MOR availability measured with *in vivo* PET is dependent on sex and age in healthy humans. This is in line with previous small post-mortem studies reporting increased cortical MOR density in the elderly (Gabilondo et al., 1995; Gross-Isseroff et al., 1990), while null findings have also been reported (Zalsman et al., 2005), potentially due to the limited sample size. One previous PET study on MOR sex differences reported that females in reproductive age have higher MOR availability compared to males in the same age (Zubieta et al., 1999). In our well-powered Study I, males older than 40 years had higher MOR availability than similar-age females in most brain regions, also in striatal areas including the caudate and putamen. This confirms that the sex difference in MOR availability reverses with aging. Notably, while the MORs in nucleus accumbens decreased with age in both sexes, this decrease was more profound in females versus males. Our study cannot reveal the biological mechanisms for this, but changes in hormonal milieu are one plausible driver to these sex-dependent alterations. Circulating estrogen has been

shown to directly affect central MOR expression. In female rats, subcutaneous estrogen injection elevates hypothalamic MOR mRNA levels (Quiñones-Jenab et al., 1997). Also in humans, females with a high-estrogen state in the menstrual cycle have increased MOR availability in nucleus accumbens compared to the low-estrogen state (Smith et al., 2006). Endogenous opioids might also modulate brain MOR expression differently depending on sex hormones (Gupta et al., 2021). It is also possible that central endogenous opioid levels change in different pace in aging males and females, which might lead to the detected differences in MOR availability.

Menopause is defined as the cessation of menses for at least 12 months, and is characterized by permanent decrease in female estrogen levels (Karvonen-Gutierrez et al., 2016). Menopause typically occurs around 50 years of age, and is preceded by 5–10 years of hormonal changes (Karvonen-Gutierrez et al., 2016; Van der Schouw et al., 1996). The menopausal transition period is also associated with weight gain (Al-Safi et al., 2015). Given the robust difference in obesity prevalence between males and females after the age of 50 (Chooi et al., 2019), it is possible that the menopause and associated estrogen decrease promotes weight gain in obesity-susceptible females. As estrogen directly modulates the expression of MORs in central areas involved in food reward processing (Quiñones-Jenab et al., 1997; Smith et al., 2006), the permanent low-estrogen state might result in lowered MOR density (**Figure 7**). Subsequently, lowered MOR density in food reward regulating brain areas might promote excessive and external food intake (Study IV), and potentially also weight gain in prone individuals (Karlsson et al., 2015).

In Study I, BMI was not associated with MOR availability when age, sex and smoking were included in the model. At first, this might seem like a contradictory finding, given the above-reviewed linkage between MORs, feeding and obesity. However, it must be noted that widespread decrease in central MORs has been previously found in subjects with morbid obesity (Karlsson et al., 2015), while our subjects had mostly normal weight. Bariatric surgery recovers downregulated MORs in morbidly obese subjects even when they are still clearly obese after the intervention (Karlsson et al., 2016). In another PET study by Majuri et al., BMI (22.8–42.1 kg/m^2) was not associated with MOR availability although the patients with BED had reduced MOR availability (Majuri et al., 2017). Accordingly, it has been suggested that the elevated BMI itself is not a prerequisite for MOR downregulation (or vice versa) – rather, it seems that the decreased central MOR availability is a common pathognomonic manifestation of dysregulated feeding behavior involving excessive food intake (Joutsa et al., 2018), and the downregulated MORs may only be directly linked with BMI only in most extreme cases. Absence of the association between BMI and MOR availability in Study I, with mostly normal-weight subjects without eating disorders, supports this view. Furthermore, in Study IV we directly investigated the association between MORs and specific

feeding habits and found that cue-induced external eating was associated with the decrease of MORs. This is consistent with the MOR system's role in promoting excessive, hedonic-driven feeding behaviors.

Smoking was associated with globally lowered MOR availability in Study I. This is consistent with previous studies (Scott et al., 2007; Weerts et al., 2014), while conflicting results have also been reported (Ray et al., 2011). Nicotine induces the release of endogenous opioids in CNS, and subsequent activation of MORs contribute to the highly rewarding and addictive properties of smoking (Davenport et al., 1990; Walters et al., 2005). Although the results from animal models have been mixed (Berrendero et al., 2010; Wewers et al., 1999), chronic tolerance-producing nicotine treatment has been found to decrease the density of striatal MORs in mice (Galeote et al., 2006). Accordingly, the reduced MOR availability in smokers may reflect reduced density of central MORs, potentially from the repeated endogenous opioid stimulation by smoking and following compensatory receptor downregulation.

Smoking cessation is associated with psychological withdrawal symptoms including anxiety, restlessness, discomfort, and craving for cigarettes (Shiffman et al., 1976). These aversive symptoms and anhedonia may result from MOR-dependent reward deficit, following the cessation of smoking and abolition of repetitive endogenous opioid bursts (Der-Avakian et al., 2012). Smoking cessation is also associated with increased caloric intake, weight gain, and in some individuals, the onset of obesity and T2D (Bush et al., 2016; Tian et al., 2015; Williamson et al., 1991). Indeed, it has been proposed that highly addictive substances such as tobacco and drugs *compete* with palatable food to form behavioral patterns using the same central reward pathways (Volkow et al., 2013). Thus, smoking cessation may predispose to compensatory reward seeking, and a potent way to re-establish the central endogenous opioid stimulation is consumption of food (Tuulari et al., 2017).

6.2 Central insulin messaging and neuroreceptor function link with familial obesity risk

In Study II, higher familial obesity risk was associated with increased insulin-stimulated brain glucose uptake, but also to the decreased availability of MORs and CB_1Rs, albeit with a lesser magnitude. Overall, obesity risk was most consistently and independently linked with the brain glucose uptake in Study II. It is possible that altered insulin messaging is one of the early central abnormalities in obesity pathogenesis, possibly accompanied by alterations in MOR and CB_1R systems in later phases of obesity development. Indeed, accumulating evidence suggests that certain alterations in brain insulin signaling might develop already *in utero* as a result of parental obesity and diabetes (Kullmann et al., 2020). Previous studies suggest

that alterations in brain glucose uptake in obesity are insulin-dependent, and we interpret our results similarly due to the same experimental setting, PET imaging during a hyperinsulinemic clamp (Rebelos et al., 2021).

Insulin functions as an important homeostatic signal, informing the CNS about satiety and peripheral energy stores (Sallam et al., 2021). Insulin regulates feeding behavior directly via hypothalamic neurons, and also by interacting with mesolimbic opioid and dopamine reward pathways (Davis et al., 2010). Favorite-food cues activate these reward pathways more strongly in obese compared to lean subjects, and these brain responses are dependent on plasma insulin levels (Jastreboff et al., 2013). Study IV further highlights that such responses and cue-induced feeding behavior are also affected by MOR function. Locally, in the hedonic hotspots of nucleus accumbens, insulin may influence palatable food intake by inducing the release of endogenous opioids and subsequent MOR activation (Fetterly et al., 2021). However, insulin is usually considered to be a hormone with a net anorexigenic effect, while MOR-agonists (especially β-endorphin) are orexigenic (Gerozissis, 2004). Based on Study II, familial obesity risk factors are associated with reciprocal alterations in both of these central feeding regulating signals (**Figure 10**).

A potential mechanism linking changes in insulin-dependent brain glucose uptake and opioid signaling may be altered function of the insulin-degrading enzyme, IDE. This enzyme has been found in most tissues including the liver and brain, and is ubiquitous also in the sense that it is present in the cell cytoplasm, membranes and can be secreted in the extracellular space and bloodstream (Pivovarova et al., 2016; Sofer et al., 2021; Valera Mora et al., 2003). In addition to insulin, IDE cleaves multiple other peptides including β-amyloid and β-endorphin (Valera Mora et al., 2003). Based on human and animal data, IDE is a critical homeostatic mechanism regulating basal insulin and other peptide levels, and decreased function of IDE is a metabolic alteration associated with hyperinsulinemia, T2D and obesity (Merino et al., 2020; Pivovarova et al., 2016; Valera Mora et al., 2003; Wei et al., 2014). In addition, genetic variation in the IDE gene is associated with T2D and altered glucose levels (Karamohamed et al., 2003; Rudovich et al., 2009). Due to its ability to degrade β-amyloid, IDE has been proposed as the possible explanation for the strong association between neurodegenerative diseases such as Alzheimer's and T2D (Pivovarova et al., 2016).

Previous neuroimaging studies have found that in morbidly obese humans, insulin-dependent brain glucose uptake is increased (Rebelos et al., 2021; Tuulari et al., 2013) while central MOR availability is decreased (Burghardt et al., 2015; Karlsson et al., 2015). Our Study II shows that similar alterations are already present in young non-obese adults who nevertheless have risk factors for developing obesity. In theory, reduced activity of IDE could result in both altered central insulin function and increased endogenous β-endorphin levels. While the central β-endorphin

concentration in obese humans is unknown, in obese rodents the basal β-endorphin levels are markedly increased (Khawaja et al., 1989). Since β-endorphin competes with [^{11}C]carfentanil (Henriksen et al., 2008), reduced MOR availability in human obesity (and obesity risk) probably also reflects elevated brain β-endorphin levels. Increased β-endorphin could result in reduced IDE function, or the increased amount of other IDE substrates (e.g. insulin) (Wacławczyk et al., 2021). Accordingly, an insulin injection to a rat brain directly decreases the rate of β-endorphin degradation by substrate competition (Kavushansky et al., 2013). As a potent endogenous MOR-agonist, β-endorphin promotes palatable food intake and may contribute to weight gain (Mendez et al., 2015; Nogueiras et al., 2012).

In summary, our results and previous data suggest that there may exist a link between central insulin and opioid signaling, which might be important in obesity development. Next, it would be necessary to examine if the function of IDE is the prime alteration resulting in other neurochemical phenomena detected with PET. To our knowledge, there are no data demonstrating that IDE could degrade endogenous cannabinoids, although this seems unlikely given the low molecular resemblance between endocannabinoids (AEA, 2-AG) compared to insulin or β-endorphin. Conversely, at least peripheral CB_1R activation has been shown to downregulate IDE and to promote hyperinsulinemia (Liu et al., 2012).

Central interactions between insulin and ECS have been recently extensively reviewed (Sallam et al., 2021). In obesity, reduced insulin sensitivity in the mesolimbic brain, accompanied by increased endocannabinoid drive promoting food intake irrespective of metabolic needs is a consistent finding (Anthony et al., 2006; Sallam et al., 2021). Multiple studies have found that circulating endocannabinoid levels are increased in obesity (Mazier et al., 2015). They also correlate with visceral fat mass and insulin resistance (Mazier et al., 2015), and are associated with reduced CB_1R gene expression (Engeli et al., 2005). In line with a previous small PET study (Dickens et al., 2020), we found in Study II that increased circulating AEA is associated with decreased CB_1R availability in the ventral striatum. In addition, increased family-mediated obesity risk and overweight were associated with lower CB_1R availability. These results are consistent with the view that an overactive ECS is critical in the development of obesity, and is already present in non-obese subjects with risk factors for obesity. Low CB_1R availability most likely represents reduced receptor density, based on autoradiography studies in obese rats (Harrold et al., 2002). Reduced CB_1R density may result from compensatory downregulation to increased circulating AEA entering to the CNS, or from increased formation of AEA in the CNS (Berger et al., 2001; Di Marzo et al., 2000).

6.3 Physical exercise and μ-opioid receptor neurotransmission

Study III showed that the physical activity level is associated with central opioid releasing capacity. Regular physical activity and exercise training are essential for weight maintenance (Swift et al., 2018), and obese humans are markedly less physically active than their normal-weight counterparts (Davis et al., 2006). Cardiovascular health improvement is already evident with low levels of moderate intensity activity (> 150 minutes/week), while high levels of physical activity (> 225–420 minutes/week) may lead to weight loss (Swift et al., 2018). Positive affective responses during exercise predict adherence to future physical exercise (Rhodes et al., 2015; Williams et al., 2008). Accordingly, the ability to derive subjective pleasure from exercise is crucial for long-term commitment to a physically active lifestyle (Ekkekakis, 2017). As the central MOR system mediates pleasure from natural rewards (Nummenmaa et al., 2018b), brain opioid neurotransmission could also potentially underlie the affective responses of regular exercise.

In Study III, we found that both a higher level of regular physical activity (MVPA) and better cardiorespiratory fitness (VO_{2peak}) associated with increased release of endogenous MOR agonists (e.g. β-endorphin) during acute aerobic exercise. Such exercise-induced central opioid release correlates with increased subjective euphoria after exercise (Saanijoki et al., 2018b). In line with this, VO_{2peak} also correlated with mood improvement after exercise in our sample. These data suggest that the magnitude of endogenous opioid release is integral to the amount of pleasure derived from exercise. It is possible that increased hedonic valuation of exercise mediates motivation and long-term commitment to physical activity (Van Steenbergen et al., 2019). In other words, inter-individual differences in the opioid-releasing capacity might explain inter-individual differences in affective responses and regularity of exercise. Furthermore, Study III and previous PET studies (Saanijoki et al., 2018b) have established that exercise pleasure is mediated via reward pathways that partially overlap with those associated e.g. with smoking (Study I) and pathological eating (Study IV). This further highlights why physical exercise may be highly rewarding and self-reinforcing, sometimes even escalating to an unhealthy addiction (Landolfi, 2013).

6.4 The roles of μ-opioid and CB_1 receptors in distinct feeding patterns

In Study IV, we found that lower central MOR availability was associated with a higher score in the External eating subscale of DEBQ. External eating score is based on the externality theory of food intake, which describes behavior characterized by

feeding in response to external food-related stimuli, regardless of the internal state of satiety (Schachter, 1968; Van Strien et al., 1986a). The DEBQ subscale of External eating quantifies this behavioral trait by ten questions such as "If you walk past a snackbar or a café, do you have the desire to buy something delicious?" and "If food tastes good to you, do you eat more than usual?" (Van Strien et al., 1986a). It has been suggested that individuals with obesity and subjects who are trying to lose weight would be especially prone to these kind of sensory stimuli (food advertisements, display windows) promoting food intake (Herman et al., 2008).

A higher score in External eating associates with increased energy-dense food craving (Burton et al., 2007) and palatable food intake triggered by external cues, such as television commercials (Van Strien et al., 2012a). Accordingly, externally-oriented eating behavior may contribute to weight gain, at least in a 2-year follow-up (Van Strien et al., 2012b). Individuals with higher external eating traits have a higher BMI and are also less physically active (Van Strien et al., 2010), which may reflect a preference to rather derive MOR-mediated reward from food (Tuulari et al., 2017) than from exercise (Study III, (Saanijoki et al., 2018b)).

In Study II, a history of parental obesity and T2D was associated with decreased MOR availability in young males. Study IV builds on this by showing that on a behavioral level, this central opioidergic phenotype is associated with increased cue-triggered feeding. This may be one of the brain mechanisms by which the risk for obesity is passed from parents to their offspring (Silventoinen et al., 2020). This view is supported by previous research, which has connected low MOR availability in the same brain areas to increased hemodynamic responses to visual food cues (Nummenmaa et al., 2018a) and to morbid obesity (Karlsson et al., 2015). These data may explain why individuals with low MOR availability are prone to external overeating in the obesogenic environment full of appetitive cues (Berthoud, 2012).

PET methodology does not allow disentangling whether this central obesity risk phenotype with low MOR availability results primarily from reduced number of receptors or increased competitive binding of endogenous β-endorphin (Henriksen et al., 2008). It has been shown that repetitive endogenous bursts of endogenous opioids from feeding cause MOR downregulation (Tuulari et al., 2017; Winterdahl et al., 2019), suggesting that reduced MORs result from excessive food intake. However, another mutually non-exclusive mechanism is that the receptor number (or function) is initially determined by genetic factors (Weerts et al., 2013), thus individuals with fewer MORs would need more repetitive stimulation (food intake) to obtain the sufficient reward response (reward-deficit theory, (Stice et al., 2011)). Longitudinal studies with genetic assays are needed to definitely resolve this question. Regardless of the underlying cellular mechanism, decreased MOR availability may be critical in the maintenance of unhealthy, obesity-promoting

feeding patterns (Karlsson et al., 2015). A proposed model for disrupted MOR function leading to obesity is illustrated in **Figure 15**.

In contrast with MORs, central CB_1R availability did not clearly associate with any specific eating behavior trait measured with DEBQ. Rather, low CB_1R availability in multiple brain regions was associated with increased total score from DEBQ. Our analyses suggested a negative association with all DEBQ subscales, but these failed to reach statistical significance possibly due to the relatively low sample size (n = 35). Based on these data, the CB_1R system may play a role in multiple feeding behavior traits. In addition to feeding, CB_1R function is critical in other essential behaviors such as emotion regulation and stress-coping (Di Marzo, 2008; Lutz et al., 2015). Against this background, it easy to see why psychiatric side-effects are highly prevalent with direct CB_1R blockade, as witnessed with the promising but later withdrawn anti-obesity drug rimonabant (Bermudez-Silva et al., 2010): Only blocking CB_1R system is not a "clean" enough strategy to reduce the excessive food intake promoting obesity, or at least this can only be achieved with major adverse effects. Thus, one feasible approach could be to combine drugs targeting both the MOR and CB_1R systems, in order to reduce the dose of CB_1R blocker and potentially also the unwanted effects. Furthermore, in Study II, subjects with lowered central CB_1R availability had increased family-mediated obesity risk in addition to increased serum AEA levels. Instead of direct CB_1R blockade, one pharmacological strategy to combat obesity development might also be to reduce AEA levels. Accordingly, endocannabinoid biosynthesis inhibitors have been developed, and at least a 2-AG synthesis blocker has been successfully used to reduce palatable food intake in mice (Bisogno et al., 2009). CB_1R blockers that do not readily cross the blood-brain barrier have also been designed (Chorvat et al., 2012). Preclinical models suggest that these peripherally-restricted CB_1R blockers might promote weight loss by improving systemic leptin messaging and by activating lipolysis of fat tissue (Quarta et al., 2020).

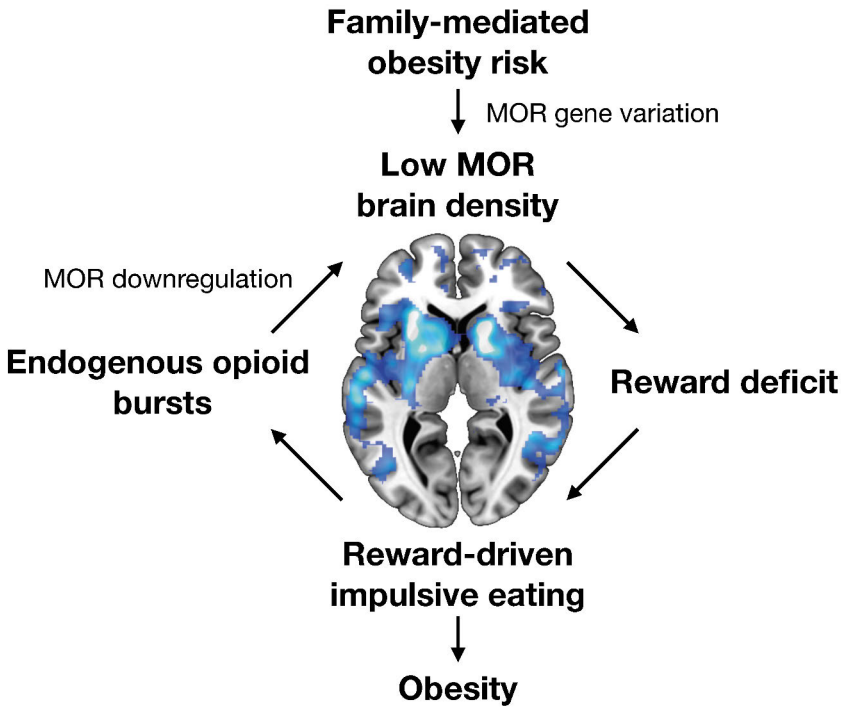

Figure 15. A proposed model of the brain μ-opioid receptor (MOR) function and obesity development. In the model, risk for obesity is passed from parent to offspring via MOR gene, leading to low MOR density. This leads to disruption in central reward response transmission, promoting compensatory externally-oriented feeding behavior. Excessive palatable food intake leads to repetitive endogenous opioid bursts in the brain and ultimately, to obesity. Increased endogenous opioid tone may further downregulate central MORs, as hypothesized previously (Karlsson et al., 2016). Brain areas where increased Family Risk score associated with decreased MOR availability in Study II are marked with blue. An axial brain slice corresponds z = 4 in the MNI152 space.

6.5 Limitations and future directions

Study I showed that sex influences [^{11}C]carfentanil binding, and also the phase of the menstrual cycle may affect MOR availability (Smith et al., 2006). Thus, we recruited only males in Studies II and III to keep the confounding factors at a minimum. In Study IV there were no females studied with [^{18}F]FMPEP-d_2, and the [^{11}C]carfentanil female sample was small. Since both [^{18}F]FMPEP-d_2 and [^{11}C]carfentanil binding are affected by sex (Study I, (Laurikainen et al., 2019; Zubieta et al., 1999)), and central food regulation may be different between males and females (Kroll et al., 2020), inferences based on Studies II–IV might not be fully generalizable to females. Preferably, these studies should be replicated in a female sample, since obesity is more prevalent in females (**Figure 1**). Furthermore, all smokers in Study I were females, potentially limiting the generalization of the

relationship between MORs and smoking for males. Only subjects that had completed the DEBQ were included in Study IV, which may limit the generalization of Study IV findings for example to subjects who are not able or willing to complete the DEBQ.

As discussed earlier in the Materials and Methods section and elsewhere (Henriksen et al., 2008), a single *in vivo* PET scan cannot reveal the distinct cellular mechanisms responsible for the detected changes in tracer binding: these may pertain to e.g. changes in receptor density, affinity or endogenous ligand binding. To further specify these cellular mechanisms, an interdisciplinary approach and additional research methods are warranted. For example, it is possible to separate a change in density from a change in affinity with post-mortem autoradiography (Gross-Isseroff et al., 1990; Tempel et al., 1987), and endogenous ligand concentrations can be measured with *in vitro* radioimmunoassays (Khawaja et al., 1989). Future studies might also examine whether the injected cold mass proportional to obese body weight influences the radiotracer binding or behavioral challenges, although prominent mass effects are thought to pertain only to small animal imaging (Kung et al., 2005). Our database Studies analyzed PET scans from multiple historical study projects, and different PET scanners used in these projects might produce minor variation in binding potential estimates (Nummenmaa et al., 2020). To account for this, in addition to PET scanner cross-calibration, all historical PET images were reprocessed with similar analysis pipeline (Karjalainen et al., 2020), and the PET scanners were entered as nuisance covariates to all statistical models. For [^{18}F]FDG and [^{18}F]FMPEP-d_2 data, arterialized venous blood samples were used to generate input functions, according to study protocols relevant to our subject characteristics (Lahesmaa et al., 2018; Phelps et al., 1979). However, arterial blood sampling remains as the gold standard, especially in pharmacokinetic PET studies.

We found many associations in striatal MOR and CB_1R systems, for example with regards to the DEBQ scores and the Family risk variable. Another limitation of these studies is that it is not possible to differentiate whether these changes co-occur due to some common driver, or would an alteration in one system precede and potentially cause the detected changes in the other. Since MOR and CB_1R systems exhibit intimate anatomical and functional interplay in the brain's reward pathways as discussed in Chapter 2.6, it may be challenging to entirely separate their effects in clinical studies. Animal studies with selective MOR or CB_1R gene knockout might provide additional information about their individual roles in feeding regulation (Alshaarawy et al., 2019; Kas et al., 2004).

Studies II and IV found that central neuroreceptor availability associates with obesity risk factors and certain eating behavior traits. However, these studies are cross-sectional by design, and thus follow-up studies are needed to resolve whether these alterations independently promote future weight gain. Study II was a part of

PROSPECT (Clinicaltrials.gov, NCT03106688), an ongoing research project, which quantified BGU and MOR and CB_1R availabilities in non-obese healthy young males. The next phase of PROSPECT is to measure the change of BMI and physical condition of these males in the following five years. Results from PROSPECT and other longitudinal studies are needed to definitively establish causal relationship between brain function and obesity development.

Furthermore, the Family Risk variable used in Study II does not directly differentiate between environmental and genetic risk factors. The genetic alterations potentially affecting the PET outcome measures in Study II might pertain to the MOR gene (Weerts et al., 2013) or the IDE gene (Pivovarova et al., 2016), and these should be explicitly determined in future studies. Another limitation of the current BGU analyses is that the blood-brain barrier glucose transport was not directly measured (Hasselbalch et al., 1999), which may be warranted to further elucidate the pathophysiological mechanisms behind familial obesity risk factors and increased BGU. In addition to the potential role of the IDE in increased BGU, it is also possible that systemic ketone bodies might play a role. Ketone bodies are produced during fasting, and also brain uses them as an energy source when there is scarcity of glucose (Bouteldja et al., 2014). [^{18}F]FDG-PET studies in hyperketonemic state have yielded somewhat mixed results, but they point towards that brain glucose metabolism decreases when blood ketone levels increase (Bouteldja et al., 2014; Zhang et al., 2013). It is thus possible that when compared to their low-risk controls, the subjects with familial obesity risk factors are not as efficient or metabolically accustomed to utilizing ketones as a cerebral fuel, which would explain at least some of their increased BGU. A limiting factor of the current Studies is that ketone bodies were not measured from the blood samples, which should be carried out in future studies of BGU.

Because we used a cross-sectional design, Study III cannot resolve whether increased activation of the MOR system could cause increased levels of regular exercise (or vice versa). Feasible strategies for future studies involve testing i) whether pharmacological or behavioral intervention that activates central MORs also increases the physical activity level and ii) does brain opioid-releasing capacity explain differences in the ability to form a regular exercise habit (Kaushal et al., 2015). Chronic exercise reduces the neural responses to appetitive food cues in the insula and visual cortex, which may be an additional mechanism for lasting weight maintenance (Cornier et al., 2012). In line with this, Study III suggests that habitual exercise may modulate the function of central MOR system, which has direct implications on feeding behavior (Study IV) and obesity risk (Study II). Accordingly, VO_{2peak} was associated with basal MOR availability in Study III, but this effect did not survive correction for age. Given the strong influence of age on

MOR availability (Study I) and on VO_{2peak} (Jackson et al., 1995), this analysis should preferably be replicated in a larger sample with less age variation.

Finally, BED is a common condition in obese subjects, with a prevalence of 16–30% among weight control program participants (de Zwaan, 2001; Kessler et al., 2013). BED is characterized by frequent episodes of eating large amounts of food without compensatory behaviors (de Zwaan, 2001; Razzoli et al., 2017). In individuals with BED, DEBQ External eating scores are particularly high (Joutsa et al., 2018; Svaldi et al., 2014), and BED is also associated with large reductions in central MOR availability (Majuri et al., 2017). Previous studies have suggested that the anti-obesity medication naltrexone/bupropion may be especially effective for patients with BED (Srivastava et al., 2018). Study IV showed that also in healthy subjects, trait-like patterns in eating behavior including external eating vary across individuals, and that the externally-oriented feeding is consistently associated with reduced central MORs. In current clinical practice, the treatment efficacy (weight loss) accomplished with opioid-blocking naltrexone/bupropion is variable and poorly predictable, and side effects are common (Greenway et al., 2010; Srivastava et al., 2018). Evidence from Study IV and former studies suggest that it might be beneficial to use DEBQ as a screening tool to identify subjects with high external eating, who might benefit most from the naltrexone/bupropion treatment. This DEBQ-guided treatment tailoring is in itself a testable hypothesis, which potentially can be formally examined in future studies.

7 Conclusions

Surface receptors transmit neurochemical signals from the outside of the cell to the inside. MOR and CB_1R systems work in the brain's interface between external signals and internal motivational states regulating human behavior, including feeding. Today, these phylogenetically ancient receptors systems interact with the modern obesogenic environment, promoting food intake in excess of metabolic needs. In this thesis, I have examined the function of MOR and CB_1R systems in relation with individual's risk for obesity development. I conclude my thesis with the following remarks:

I Common demographic factors including age, sex, and smoking are associated with central MOR availability. Aging affects brain's MOR function in a sex-specific manner, which may contribute to sex differences in obesity prevalence later in life. Smoking and feeding influence the same MOR-dependent reward pathways in the brain, which may explain the increased feeding and weight gain after smoking cessation.

II Familial obesity risk factors are associated with globally increased insulin-stimulated brain glucose uptake and with reduced availability of MORs and CB_1Rs in food intake regulating pathways. Thus, multiple neurochemical alterations previously associated with obesity are already present in non-obese individuals with obesity risk factors. These neural changes may lead to altered central integration of satiety and reward signals, predisposing to obesity development.

III The magnitude of endogenous opioid release during exercise is associated with the physical fitness level. The capacity to release central opioids and derive pleasure from physical exercise may be important to form an exercise habit, which helps in weight maintenance.

IV The brain's MOR and CB_1R availabilities associate with distinct eating behavior traits. Low MOR availability is associated with increased externally triggered eating, while CB_1Rs associate with multiple eating behavior traits. Specific alteration in the MOR system function is thus associated with impulsive eating behavior, which may increase the risk for obesity development in an environment with appetitive cues.

Appendices

Appendix I: The DEBQ form (Study IV)

DEBQ-kysely

Dutch Eating Behaviour Questionnaire (Strien, Frijters, Bergers, & Defares, 1986).
1 = Ei koskaan, 2 = Harvoin, 3 = Joskus, 4 = Usein, 5 = Hyvin usein.

1.	Kun olet lihonut, niin syötkö vähemmän kuin tavallisesti?	1	2	3	4	5
2.	Yritätkö syödä ruokaillessasi vähemmän kuin mielesi tekisi?	1	2	3	4	5
3.	Miten usein kieltäydyt sinulle tarjotusta ruuasta tai juomasta koska olet huolestunut painostasi?	1	2	3	4	5
4.	Valvotko huolellisesti mitä syöt?	1	2	3	4	5
5.	Yritätkö syödä laihduttavia ruokia?	1	2	3	4	5
6.	Kun olet syönyt liikaa, syötkö seuraavana päivänä vähemmän kuin tavallisesti?	1	2	3	4	5
7.	Yritätkö syödä vähemmän välttääksesi lihomista?	1	2	3	4	5
8.	Kuinka usein pyrit välttämään ylimääräisiä välipaloja sen takia, että tarkkailet painoasi?	1	2	3	4	5
9.	Miten usein yrität olla syömättä iltaisin sen takia, että tarkkailet painoasi?	1	2	3	4	5
10.	Vaikuttaako painosi siihen mitä syöt?	1	2	3	4	5
11.	Tekeekö mielesi syödä, kun tunnet itsesi ärtyneeksi?	1	2	3	4	5
12.	Tekeekö mielesi syödä, kun sinulla ei ole mitään tekemistä?	1	2	3	4	5
13.	Tekeekö mielesi syödä, kun olet masentunut tai lannistunut?	1	2	3	4	5
14.	Tekeekö mielesi syödä, kun tunnet itsesi yksinäiseksi?	1	2	3	4	5
15.	Tekeekö mielesi syödä, kun joku pettää luottamuksesi?	1	2	3	4	5
16.	Tekeekö mielesi syödä, kun olet kiukkuinen?	1	2	3	4	5
17.	Tekeekö mielesi syödä, kun tiedät, että pian tapahtuu jotain ikävää?	1	2	3	4	5
18.	Tekeekö mielesi syödä, kun tunnet itsesi rauhattomaksi, huolestuneeksi tai jännittyneeksi?	1	2	3	4	5

19.	Tekeekö mielesi syödä syödä, kun koet vastoinkäymisiä tai asiat ovat menneet pieleen?	1	2	3	4	5
20.	Tekeekö mielesi syödä, kun olet pelästynyt?	1	2	3	4	5
21.	Tekeekö mielesi syödä, kun olet pettynyt?	1	2	3	4	5
22.	Tekeekö mielesi syödä, kun olet järkyttynyt?	1	2	3	4	5
23.	Tekeekö mielesi syödä, kun olet tylsistynyt tai levoton?	1	2	3	4	5
24.	Mikäli ruoka maistuu mielestäsi hyvältä, syötkö enemmän kuin tavallisesti?	1	2	3	4	5
25.	Mikäli ruoka tuoksuu ja näyttää herkulliselta, syötkö enemmän kuin tavallisesti?	1	2	3	4	5
26.	Jos näet tai haistat jotain herkullista niin tekeekö mielesi syödä kyseistä ruokaa?	1	2	3	4	5
27.	Mikäli sinulla on jotain herkullista syötävää, syötkö sen välittömästi?	1	2	3	4	5
28.	Tekeekö mielesi ostaa jotain herkullista kävellessäsi leipomon ohi?	1	2	3	4	5
29.	Tekeekö mielesi ostaa jotain herkullista kävellessäsi kahvilan ohitse?	1	2	3	4	5
30.	Tekeekö mielesi syödä kun näet muiden ihmisten ruokailevan?	1	2	3	4	5
31.	Kykenetkö vastustamaan herkullisten ruokien syömistä?	1	2	3	4	5
32.	Syötkö enemmän kuin tavallisesti kun näet muiden ihmisten ruokailevan?	1	2	3	4	5
33.	Onko sinulla tapana syödä samanaikaisesti valmistaessasi ruokaa?	1	2	3	4	5

Appendix II: The DEBQ scoring table (Study IV)

The scoring for the DEBQ subscales: emotional eating (E), external eating (X), and restrained eating (R). Only one question is an inverse item, which is marked in red.

Skaala	Kysymys suomeksi	Ei koskaan	Harvoin	Joskus	Usein	Hyvin usein	Käännä	
E	Tekeekö mielesi syödä kun koet vastoinkäymisiä tai asiat ovat menneet pieleen?	1	2	3	4	5	Ei	X = DEBQ_External_eating
R	Valvotko huolellisesti mitä syöt?	1	2	3	4	5	Ei	E = DEBQ_Emotional_eating
R	Yritätkö syödä vähemmän välttääksesi lihomista?	1	2	3	4	5	Ei	R = DEBQ_Restraint_Eating
E	Tekeekö mielesi syödä kun olet pelästynyt?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä kun sinulla ei ole mitään tekemistä, ?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä kun tunnet itsesi rauhattomaksi, huolestuneeksi tai jännittyneeksi?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä, kun olet järkyttynyt?	1	2	3	4	5	Ei	
R	Kun olet lihonut niin syötkö vähemmän kuin tavallisesti?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä kun joku pettää luottamuksesi?	1	2	3	4	5	Ei	
R	Kun olet syönyt liikaa, syötkö seuraavana päivänä vähemmän kuin tavallisesti?	1	2	3	4	5	Ei	
X	Mikäli ruoka maistuu mielestäsi hyvältä, syötkö enemmän kuin tavallisesti?	1	2	3	4	5	Ei	
X	Syötkö enemmän kuin tavallisesti kun näet muiden ihmisten ruokailevan?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä kun tiedät, että pian tapahtuu jotain ikävää?	1	2	3	4	5	Ei	
R	Miten usein yrität olla syömättä itaisin sen takia, että tarkkailet painoasi?	1	2	3	4	5	Ei	
R	Miten usein kieläydyt sinulle tarjotusta ruuasta tai juomasta koska olet huolestunut painostasi?	1	2	3	4	5	Ei	
X	Mikäli sinulla on jotain herkullista syötävää, syötkö sen välittömästi?	1	2	3	4	5	Ei	
X	Tekeekö mielesi ostaa jotain herkullista kävellessäsi kahvilan ohitse?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä kun tunnet itsesi yksinäiseksi?	1	2	3	4	5	Ei	
R	Yritätkö syödä ruokaillessasi vähemmän kuin mielesi teksisi?	1	2	3	4	5	Ei	
X	Mikäli ruoka tuoksuu ja näyttää herkulliselta, syötkö enemmän kuin tavallisesti?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä kun olet kiukkuinen?	1	2	3	4	5	Ei	
X	Tekeekö mielesi syödä kun näet muiden ihmisten ruokailevan?	1	2	3	4	5	Ei	
X	Onko sinulla tapana syödä samanaikaisesti valmistaessasi ruokaa?	1	2	3	4	5	Ei	
X	Tekeekö mielesi ostaa jotain herkullista kävellessäsi leipomon ohi?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä kun olet masentunut tai lannistunut?	1	2	3	4	5	Ei	
R	Kuinka usein pyrit välttämään ylimääräisiä välipaloja sen takia, että tarkkailet painoasi?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä kun tunnet itsesi ärtyneeksi?	1	2	3	4	5	Ei	
E	Tekeekö mielesi syödä kun olet pettynyt?	1	2	3	4	5	Ei	
X	Jos näet tai haistat jotain herkullista niin tekeekö mielesi syödä kyseistä ruokaa?	1	2	3	4	5	Ei	
R	Vaikuttaako painosi siihen mitä syöt?	1	2	3	4	5	Ei	
X	Kykenetkö vastustamaan herkullisten ruokien syömistä?	1	2	3	4	5	KÄÄNNÄ!	1
E	Tekeekö mielesi syödä kun olet tylsistynyt tai levoton?	1	2	3	4	5	Ei	
R	Yritätkö syödä laihduttavia ruokia?	1	2	3	4	5	Ei	

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