Karlsson, Henry K.; Tuominen, Lauri; Tuulari, Jetro J.; Hirvonen, Jussi; Parkkola, Riitta; Helin, Semi; Salminen, Paulina; Nuutila, Pirjo; Nummenmaa, Lauri

Obesity Is Associated with Decreased µ-Opioid But Unaltered Dopamine D2 Receptor Availability in the Brain

Published in:
JOURNAL OF NEUROSCIENCE

DOI:
10.1523/JNEUROSCI.4744-14.2015

Published: 01/01/2015

Document Version
Publisher's PDF, also known as Version of record

Please cite the original version:
Obesity Is Associated with Decreased μ-Opioid But Unaltered Dopamine D₂ Receptor Availability in the Brain

Henry K. Karlsson,1 Lauri Tuominen,1,2 Jetro J. Tuulainen,1 Jussi Hirvonen,1,3 Riitta Parkkola,5 Semi Helin,1 Paulina Salminen,1 Pirjo Nuutila,1,2 and Lauri Nummenmaa1,6

Copyright © 2015 the authors 0270-6474/15/353959-07$15.00/0

Neurochemical pathways involved in pathological overeating and obesity are poorly understood. Although previous studies have shown increased μ-opioid receptor (MOR) and decreased dopamine D₂ receptor (D₂R) availability in addictive disorders, the role that these systems play in human obesity still remains unclear. We studied 13 morbidly obese women [mean body mass index (BMI), 42 kg/m²] and 14 nonobese age-matched women, and measured brain MOR and D₂R availability using PET with selective radioligands [11C]carfentanil and [11C]raclopride, respectively. We also used quantitative meta-analytic techniques to pool previous evidence on the effects of obesity on altered D₂R availability. Morbidly obese subjects had significantly lower MOR availability than control subjects in brain regions relevant for reward processing, including ventral striatum, insula, and thalamus. Moreover, in these areas, BMI correlated negatively with MOR availability. Striatal MOR availability was also negatively associated with self-reported food addiction and restrained eating patterns. There were no significant differences in D₂R availability between obese and nonobese subjects in any brain region. Meta-analysis confirmed that current evidence for altered D₂R availability in obesity is only modest. Obesity appears to have unique neurobiological underpinnings in the reward circuit, whereby it is more similar to opioid addiction than to other addictive disorders. The opioid system modulates motivation and reward processing, and low μ-opioid availability may promote overeating to compensate decreased hedonic responses in this system. Behavioral and pharmacological strategies for recovering opioidergic function might thus be critical to curb the obesity epidemic.

Key words: dopamine; obesity; opioids; positron emission tomography; receptors; reward

Introduction

Obesity is a great challenge to human health worldwide because it is associated with serious medical conditions such as type 2 diabetes, coronary heart disease, and stroke. Food reward is driven by functionally distinct neurochemical mechanisms promoting incentive motivation (“wanting”) and hedonic impact (“liking”) when food is consumed (Berridge, 2009). Accumulating evidence suggests that obesity is related to altered neurochemistry of the reward circuitry of the brain, making obese individuals prone to overeating (Berridge et al., 2010; Kenny, 2011; Volkow et al., 2013). The dopamine system supports incentive motivation, and dopaminergic reward system dysfunctions are associated with addictive disorders. In the striatum, alcohol and drug dependence are associated with lower dopamine D₂ receptor (D₂R) availability (Volkow et al., 1996, 2001; Martinez et al., 2012). Obese animals with unhealthy eating habits also show downregulation of D₂R (Johnson and Kenny, 2010). However, studies in obese human subjects have provided conflicting results, with some finding lower striatal D₂R availability (Wang et al., 2001; Volkow et al., 2008; de Weijer et al., 2011), and others unaltered striatal D₂R availability (Haltia et al., 2007, 2008; Steele et al., 2010).

Whereas the dopaminergic system is implicated in the desire for eating, the endogenous opioid system is involved in both incentive motivation and hedonic functions, also generating pleasurable sensations when palatable foods are consumed (Berridge et al., 2010). The μ-opioid receptors (MORs) function as a part of complex opioid system, and mediate the effects of endogenous opioids, such as β-endorphins and endomorphins, and various exogenous opioid agonists (Henriksen and Willoch, 2008). Alcohol dependence is associated with increased MOR availability in ventral striatum, possibly due the upregulation of MORs or a reduction in endogenous opioids (Heinz et al., 2005; Weerts et al., 2011). Moreover, cocaine dependence is linked to increased MOR availability in more extensive neural
areas, such as anterior cingulate and frontal cortex (Gorelick et al., 2005). However, long-term opiate drug use is associated with downregulation in MORs (Koch and Höll, 2008; Whistler, 2012).

Animal studies suggest that endogenous opioid system has an important role in the control of appetite. MOR agonists increase and opioid antagonists decrease food intake and hedonic pleasures caused by palatable foods (Gosnell and Levine, 2009). Opioid antagonists also prevent food seeking and binge-like eating (Giuliano et al., 2012; Cambridge et al., 2013). Moreover, stimulation of the MOR in the shell of nucleus accumbens increases the pleasure responses for foods and may also trigger eating behavior (Pecína and Berridge, 2005). The μ-opioid receptor gene OPRM1 also modulates the intake of fat and possibly the risk for gaining weight in humans (Haghighi et al., 2014). Accordingly, changes in MOR rather than D₂R availability can maintain excessive energy uptake due to altered hedonic processing of food. Here we determined the association between of obesity on the availability of D₂R and MOR using positron emission tomography (PET) in a cross-sectional design. We hypothesized that obesity would be associated with opioid and possibly dopamine neurotransmitter systems, which is reflected in decreased D₂R and MOR availabilities.

### Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of the Hospital District of South-Western Finland (SleevePET2, NCT01373892, http://www.clinicaltrials.gov). All participants signed ethics committee-approved informed consent forms before scans.

**Subjects.** We recruited 13 morbidly obese women [mean body mass index (BMI), 41.9 kg/m²; mean age, 39.1 years] for the study (Table 1). The BMI range was 37.1–49.3 kg/m². The obese subjects were compared with 14 healthy nonobese female subjects (mean BMI, 22.7 kg/m²; mean age, 44.9 years) that were age and height matched with the obese subjects (Table 1). Clinical screening included history, physical examination, anthropometric measurements, and laboratory tests. Exclusion criteria involved binge-eating disorders (BEDs); neurological or severe mental disorders; and any kind of opiate drug use, substance abuse, excessive alcohol consumption (>8 U/week), determined by clinical interview, medical history, and blood tests. Subjects also completed questionnaires that measured emotional and reward functioning [Beck Depression Inventory-II (BDI-II), State-Trait Anxiety Inventory (STAI), and behavioral inhibition system (BIS)/behavioral approach system (BAS) scales] as well as food craving and eating behavior [Trait and State Food Cravings Questionnaires (FCQ), Dutch Eating Behavior Questionnaire (DEBQ), Yale Food Addiction Scale (YFAS)]. None of the controls smoked tobacco, but five obese subjects were light smokers (smoking range, 3–15 cigarettes/d). None of the obese subjects had type 2 diabetes or used antidiabetic medications. Of the obese group, five subjects used oral medication for treatment of elevated blood pressure, three for treatment of hypothyroidism, and two for treatment of hypercholesterolemia. Use of antihypertensive and cholesterol-lowering drugs were discontinued before the experiments.

**Image acquisition and quantification of receptor availability.** We measured D₂ receptor availability with the antagonist [¹¹C]raclopride (Farde et al., 1986), and μ-opioid receptor availability with the high-affinity agonist [¹¹C]carfentanil (Frost et al., 1985) using PET on two separate visits. [¹¹C]Raclopride was synthesized using [¹¹C]methyl triflate, where cyclotron-produced [¹¹C]methane was halogenated by gas phase reaction into [¹¹C]methyl iodide (Larsen et al., 1997) and converted on-line into [¹¹C]methyl triflate (Jewett, 1992). The approach used was adapted from the published method (Langer et al., 1999) with the following modifications. The [¹¹C]methane was produced with a CC18/9 cyclotron (Efremov Institute, St. Petersburg, Russia) using 17 MeV protons for [¹¹C]N(p,a)¹¹C nuclear reaction in a N₂-H₂ gas target (10% H₂). [¹¹C]methyl triflate was bubbled into a solution containing acetone (200 μl), O-s-desmethyl precursor (0.4 mg, 1.2 μmol), and NaOH (2.8 μl, 0.5 m) at 0°C. At the HPLC purification step, the mobile-phase composition was adjusted into (32:68) acetonitrile/0.1 M H₃PO₄, and [¹¹C]raclopride peak was cut into a rotary evaporator already containing propylene glycol/ethanol (7:3, 0.4 ml). The evaporation residue was formulated in phosphate buffer (8 ml, 0.1 m) and sterile filtered. A analytical HPLC column (Kinetex XB-C18, Phenomenex; 2.6 μm, 3.0 mm × 50 mm), acetonitrile in 0.05 M H₃PO₄ (23:77) mobile phase, 1 ml/min flow rate, 3.5 min run time, and detectors in series for UV absorption (214 nm) and radioactivity were used for the determination of identity, radiochemical purity, and mass concentration. [¹¹C]Carfentanil was produced as previously described (Hirvonen et al., 2009), except the mobile phase was changed into CH₃OH/0.1 M NH₄HCO₃ (70:30).

Both radioligands had high radiochemical purity (>99%). Before scanning, a catheter was placed in the subject’s left antecubital vein for tracer administration. Head was strapped to the scanner table to prevent head movement. Subjects fasted for 2 h before scanning. A computed tomography (CT) scan was performed to serve as an attenuation map and a reference anatomical image of the brain. The clinical well being of subjects was monitored during the scanning.

We injected 251 ± 24 MBq of [¹¹C]raclopride and 251 ± 10 MBq of [¹¹C]carfentanil in separate scans on separate days. After injection, radioactivity in brain was measured with the GE Healthcare Discovery 690 PET/CT scanner for 51 min, using 13 time frames. MNI was performed with the SPM software (Wellcome Unit, London) with a default spatial resolution of 3.00 mm × 3.00 mm × 3.00 mm. Two voxel size. The subject-wise parametric BPᵥC/VNgoner was used to exclude structural abnormalities and to provide anatomical reference images for the PET scans. High-resolution anatomical images (1 mm × voxel size) were acquired using a T₁-weighted sequence (TR, 25 ms; TE, 4.6 ms; flip angle, 30°; scan time, 376 s).

All alignment and coregistration steps were performed using SPM8 software (www.fil.ion.ucl.ac.uk/spm/) running on Matlab R2012a (MathWorks). To correct for head motion, dynamic PET images were first realigned frame to frame. The individual T₁-weighted MR images were coregistered to the summation images calculated from the realigned frames. Regions of interest (ROIs) for reference regions were drawn manually on MR images using PMOD version 3.4 software (PMOD Technologies). Occipital cortex was used as the reference region for [¹¹C]carfentanil and cerebellum for [¹¹C]raclopride. Receptor availability was expressed in terms of BPᵥC/ND, which is the ratio of specific to nondisplaceable binding in brain. BPᵥC/ND was calculated applying the basis function method for each voxel using the simplified reference tissue model with reference tissue time activity curves as input data (Gunn et al., 1997). This outcome measure is not confounded by differences in peripheral distribution or radiotracer metabolism.

The space using the T₁-weighted MR images, and smoothed with a Gaussian kernel of 8 mm FWHM. Subsequently, between-groups, voxel-
elwise differences in D2R and MOR BPND were compared using independent samples t tests in SPM8. The statistical threshold was set at \( p < 0.05 \), false discovery rate (FDR) corrected at the cluster level. In a complementary approach, anatomic regions of interest were generated in ventral striatum, dorsal caudate nucleus, putamen, insula, amygdala, thalamus, orbitofrontal cortex, anterior cingulate cortex, medial cingulate cortex, and posterior cingulate cortex using the AAL (Tzourio-Mazoyer et al., 2002) and Anatomy (Eickhoff et al., 2005) toolboxes. These data were analyzed with a 2 (group) \( \times 10 \) (ROI) mixed ANOVA. Associations among receptor availabilities (i.e., BPND values in each ROI), BMI, and questionnaire scores were assessed using Pearson correlations.

Finally, to weight the existing evidence on striatal D2R availability in obesity, we conducted a meta-analysis on human PET studies targeting obesity using \([11C]raclopride\). The meta-analysis includes peer-reviewed studies written in English and published through the end of April 2014. Several search methods were used. The Web of Science, PubMed, and Scopus databases were searched to retrieve documents containing the terms “dopamine,” “obesity,” “PET,” “raclopride,” and “receptor,” in article title, abstract, or keywords. Articles referred to in articles found by the preceding method were examined. Studies were accepted for the meta-analysis if they met the following criteria: (1) they had compared D2R availability in obese versus normal-weight subjects using PET; and (2) they used \([11C]raclopride\) as a radiotracer. Effect sizes were estimated using the \( r \) statistic based on means and variances and the number of participants, or, alternatively, the \( F \) or \( t \) test values and degrees of freedom (Rosenthal, 1984; Rosenthal and DiMatteo, 2001). Effect sizes were consistently computed in such a way that positive values reflect lowered D2R availability in obese individuals. Subsequently, weighted effect sizes were computed and subjected to meta-analysis using unbiased estimates of correlation coefficients and a restricted maximum likelihood estimator, yielding mean and 95% confidence intervals (CIs) for the effect sizes. This model assumes that effect sizes are contingent on study parameters, thus allowing for an estimation of both within- and between-studies variances. Altogether with the present data, the meta-analysis included data from 105 subjects stemming from five independent studies.

**Results**

Full-volume analysis revealed that morbidly obese patients had significantly lower \([11C]carfentanil\) BPND values (\( p < 0.05 \), FDR corrected in the SPM analysis), versus control subjects, throughout the reward circuit, including the ventral striatum, dorsal caudate, thalamus, insula, orbitofrontal cortex, and anterior cingulate cortex (Figs. 1, 2, 3). However, there were no significant differences in \([11C]raclopride\) BPND values in any brain region. Furthermore, there were no regions with higher \([11C]carfentanil\) or \([11C]raclopride\) BPND values in obese versus normal-weight individuals. These effects were corroborated in the ROI analysis. For \([11C]carfentanil\), the ANOVA revealed that BMI data show a main effect of group (obese vs. lean) with no interaction with ROI. *\( p < 0.05 \) in complementary contrast test.
0.89), yet no interaction between subject group and ROI was observed \((F = 2.16, p > 0.05)\). For \([11C]\)raclopride, there was a main effect of ROI \((F_{1,255} = 246.92, p < 0.001, \eta^2_p = 0.92)\), but there was neither a difference between groups \((F = 1.04, p > 0.05)\) nor an interaction between group and ROI \((F = 0.52, p > 0.05)\).

Across the whole sample, BMI correlated negatively with \([11C]\)raclopride \(BP_{ND}\) in ventral striatum, dorsal caudate, putamen, insula, amygdala, thalamus, as well as orbitofrontal cortex \((r_s = -0.42; p < 0.03; \text{Fig. 4})\). No significant correlations between BMI and \([11C]\)carfentanil \(BP_{ND}\) were observed in any region.

To rule out the possible effect of smoking on receptor availability, we reanalyzed the data excluding the smokers. This analysis yielded results for MOR and D2R that were similar to those for the whole sample population, confirming that decreased MOR in obese subjects is not due to smoking. We also compared the \(BP_{ND}\) values between the obese smokers and obese nonsmokers, and found no significant differences.

Even though obesity was not associated with D2R availability per se, we next asked whether MOR and D2R availabilities would have a joint contribution to an individual’s BMI. To this end, we conducted a regression analysis where we predicted BMIs with regional MOR and D2R availability, running a separate regression model for each striatal ROI. For all tested ROIs, MOR \((p < 0.05)\) but neither D2R availability nor interaction between MOR and D2R availability \((p > 0.05)\) predicted an individual’s BMI.

Morbidly obese subjects scored significantly higher on the scales measuring pathological eating (DEBQ restrained and emotional eating) and food addiction (YFAS; Table 2). They also scored significantly lower in the BIS scale measuring behavioral inhibition, but the groups did not differ from each other in depression (BDI-II), trait anxiety (STAI), behavioral activation (BAS), or food craving (FCQ) scores. The STAI scores were negatively associated with MOR availability in anterior cingulate cortex, middle cingulate cortex, and posterior cingulate cortex \((r \leq -0.38, p < 0.05)\). The DEBQ restrained eating score correlated negatively with MOR availability in ventral striatum, amygdala, and thalamus \((r \leq -0.38, p < 0.05)\), and YFAS scores were negatively associated with MOR availability in dorsal caudate \((r = -0.39, p < 0.05)\). However, these analyses were not significant after adjusting for multiple comparisons using the Bonferroni procedure. No significant associations between D2R availability and questionnaire scores were found. The full volume group differences in MOR availability were in general similar, albeit with slightly weaker effects when each of these factors was included as covariates in the SPM analyses. Including these covariates in the analysis of D2R availability did not alter the corresponding results.

Finally, the meta-analysis (Fig. 5) confirmed that, even though the overall effect size for BMI on D2R availability was positive \((r = 0.14)\), its 95% CI overlapped with zero \((-0.11 \text{ to } 0.40)\), suggesting that there was no effect of BMI on D2R availability. However, moderator analysis using Wald-type test for model coefficients revealed that the effect size had a quadratic relationship between the BMI of the patients studied \((QM(1) = 4.3439, p = 0.04)\), suggesting that only extreme obesity may lead to lowered D2R availability \((p > 0.05)\).

### Discussion

Cerebral MOR availability was lowered in morbidly obese patients in brain regions implicated in reward processing, including ventral striatum, orbitofrontal cortex, amygdala, putamen, insula, and anterior cingulate, while D2R availability remains unaltered. Altered MOR availability was also paralleled with alterations in affect-driven eating, as indicated by elevated self-reported food addiction and restrained eating behavior. Critically, food addiction and restrained eating scores were also associated with MOR availability, suggesting that the lowered MOR availability is directly linked with the tendency to compulsively eat regardless of internal state of hunger or satiety.

Prior work has established that the opioid system is involved in the pathophysiology of addictive disorders by causing altered sensations of pleasure, but it is also involved in hedonic and motivational processing of food (Pečiňa and Smith, 2010). Opi-
Perturbation of the dopaminergic system in obesity. (A) Inverse correlation between BMI and the effects of the D2R agonist [11C]raclopride on regional dopamine receptor availability (Haltia et al., 2008). (B) Schematic illustration of the proposed compensatory reward system in obesity. (C) Frontal cortical dopamine system in obesity showing increased D2R availability (Carpenter et al., 2013). (D) Relative expression of dopamine receptors compared to control conditions in obesity (Le Merrer et al., 2009). (E) Phasic dopamine release in obesity (Small et al., 2003).

Exposure to high-calorie foods is known to activate the dopaminergic reward system (Berridge, 2009). However, obesity is associated with decreased response to reward-related stimuli, which may explain the blunted response to high-calorie foods observed in obese subjects. This blunted response is thought to be mediated by increased D2R availability, which may lead to enhanced dopamine signaling in the hypothalamic area of the brain (Le Merrer et al., 2009). This increased dopamine signaling can result in compulsive eating behavior, which is a hallmark of obesity.

Limitations
Because the present study involved only female subjects, we cannot rule out sex effects. Furthermore, we did not control for the cycle phase in the current study, but the phase of the menstrual cycle has been shown to affect the response to reward-related stimuli (Le Merrer et al., 2009).
cycle was distributed evenly (data not shown). Even though our sample was sizeable, it is possible that more pronounced differences associated with the obese phenotype could be established in larger studies. Finally, it must be borne in mind that the present cross-sectional study cannot reveal whether obesity causes MOR downregulation or vice versa.

Conclusions
Morbid obesity is associated with decreased MOR availability in the brain, while D_2R availability remains unaltered. We propose that the endogenous opioid system is a key component underlying human obesity, whereas the function of the dopaminergic system is less profound. The neurochemical changes associated with obesity are partially distinct from those observed in patients with addictive disorders and substance abuse. Future longitudinal studies should examine whether decreased MOR function is a trait phenomenon reflecting a vulnerability to develop obesity by overeating, or a direct and possibly reversible consequence of obesity on the brain.

References

Obesity and Brain Receptors