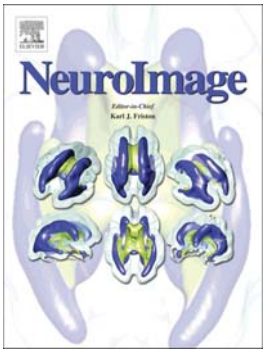

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Associations between Dopamine D2 Receptor Availability and BMI Depend on Age

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Abstract

Objective: The dopamine D2/3 receptor subtypes (DRD2/3) are the most widely studied neurotransmitter biomarker in research on obesity, but results to date have been inconsistent, have typically involved small samples, and have rarely accounted for subjects' ages despite the large impact of age on DRD2/3 levels. We aimed to clarify the relation between DRD2/3 availability and BMI by examining this association in a large sample of subjects with BMI spanning the continuum from underweight to extremely obese.

Subjects: 130 healthy subjects between 18 and 81 years old underwent PET with [18F]falllypride, a high affinity DRD2/3 ligand.

Results: As expected, DRD2/3 availability declined with age. Critically, age significantly interacted with DRD2/3 availability in predicting BMI in the midbrain and striatal regions (caudate, putamen, and ventral striatum). Among subjects under 30 years old, BMI was not associated with DRD2/3 availability. By contrast, among subjects over 30 years old, BMI was positively associated with DRD2/3 availability in the midbrain, putamen, and ventral striatum.

Conclusion: The present results are incompatible with the prominent dopaminergic hypofunction hypothesis that proposes that a reduction in DRD2/3 availability is associated with increased BMI, and highlights the importance of age in assessing correlates of DRD2/3 function.

Keywords: BMI, obesity, dopamine D2 receptor, striatum, midbrain, aging

1. Introduction

Obesity and its complications are the leading causes of preventable death in the U.S. (1). With over one-third of adults and nearly one-fifth of children meeting criteria for obesity, the need to understand the causes and consequences of obesity has never been greater (2). Research exploring the brain's contributions to obesity have suggested the possible importance of dopamine functioning, which has been associated with body mass index (BMI), food intake, anticipatory response to reward, and responses to food restriction and other weight loss measures (3-7). Within the dopamine system, the dopamine D2/3 receptor subtypes (DRD2/3) have been the most widely studied biomarker in relation to obesity. The Taq1A minor (A1) allele of the DRD2/3 gene is associated with lower DRD2/3 density and has been found to exist in higher frequencies in obese subjects (8-11). A landmark study in 2001 reported that striatal DRD2/3 availability was reduced in extremely obese subjects relative to control subjects and that striatal DRD2/3 availability correlated negatively with BMI in obese subjects (3). These findings gave rise to the prominent dopaminergic hypofunction hypothesis of obesity wherein it is speculated that reduced DRD2/3 availability plays a central role in a reduced response to the hedonic value of food that leads to compensatory overconsumption (3, 12).

Since the initial proposal of the dopaminergic hypofunction account of obesity, several findings suggest that the relation between DRD2/3 and obesity might not reflect a simple reduction in DRD2/3 availability. A few studies with large sample sizes have reported no association between Taq1A and markers of

obesity such as BMI (13-15). As a genetic marker, Taq1A polymorphism offers only an indirect assessment of DRD2/3 expression and explains only part of the variance of DRD2/3 availability. *In vivo* assessment with PET provides a more direct index of DRD2/3 availability, but to date reports of associations between BMI and DRD2/3 availability measured with PET have also been inconsistent (3, 5, 16-21). Although a few PET studies supported the initial finding that lean subjects had greater DRD2/3 availability than higher BMI subjects (5, 18, 20-23), several studies observed no relation (5, 17, 24-26) or a correlation in the opposite direction (16, 18, 19, 26, 27) (Table 1). Several factors may have contributed to these inconsistencies in the current literature. Most of the studies observing lower binding in obesity compared extremely obese subjects (BMI > 40) with normal weight subjects, and these findings may not generalize to other BMI ranges, especially in light of evidence that extreme obesity may reflect a state of aberrance distinct from other BMI categories (28). Additionally, the sample sizes of most PET studies were small, with most having less than two dozen subjects. Because of their wide confidence intervals in estimating correlations (29), these small sample sizes have likely limited the potential of past PET studies to provide clarity on this association.

The present study aimed to clarify the relation between DRD2/3 availability and BMI by examining this often-cited association in a large sample of subjects. To ensure adequate statistical power, we assessed 130 subjects, which is more than three times the sample size of the previous largest PET study examining this question. Instead of contrasting an extremely obese group with a

normal weight group, we included subjects spanning the range from mildly underweight to extremely obese in order to examine how DRD2/3 availability relates to the whole BMI spectrum. Lastly, in contrast to the majority of previous PET studies that utilized the tracer [11C]raclopride, here we used [18F]fallypride, which has higher affinity for DRD2/3 than [11C]raclopride and yields higher target-to-background signal for DRD2/3 availability (30).

Furthermore, although the relation between DRD2/3 availability and BMI has received considerable attention in recent years, few studies have examined how this relation may change with age, particularly in humans. The dopamine system undergoes significant changes during aging, and some associations between dopamine and reward functions differ at different life stages (31, 32). Given that both BMI and DRD2/3 availability change across the lifespan, age represents a major potential confound in this literature. As obesity occurs in all stages of life, knowledge of how age influences the link between DRD2/3 availability and BMI may impact the development of effective prevention or treatment for obesity. Indeed, if the dopamine hypofunction hypothesis is correct, one might expect the negative relation between BMI and DRD2/3 receptors to increase with age, given a greater time span for the influence of receptors on BMI and the natural decline in DRD2/3 receptors that occurs with aging. We therefore examined the interaction of age on DRD2/3 availability and BMI to assess how this association changes across the lifespan.

2. Methods

2.1 Subjects

130 healthy subjects (age: 35.6 ± 18.2 years, 72 females, BMI: 25.5 ± 4.8) participated in this study. Among the 4 major BMI categories, 3 subjects were underweight (BMI<18.5), 63 were in the normal range (BMI=18.5-24.9), 46 were overweight (BMI=25-29.9), and 18 were obese (BMI>30; 3 of 18 reached criteria for extreme obesity with BMI >40). Subjects were part of three separate studies examining different questions in our lab. Two studies involved subjects between 18 and 30 years old. The third study included subjects from 18 to 81 years old. Together there were 73 subjects under 30 years old and 57 subjects over 30 years old. Subjects were excluded if they reported any history of psychiatric illness in a screening interview (a Structured Clinical Interview for DSM-IV Diagnosis (33) was also available for all subjects and confirmed no history of major Axis I disorders). Subjects were also excluded if they had any history of head trauma, any significant medical condition, or any condition that would interfere with MRI (e.g. claustrophobia or metal implants). Subjects with major medical disorders including diabetes and/or abnormalities on a comprehensive metabolic panel or complete blood count were excluded. Subjects were also excluded if they reported a history of substance abuse, current tobacco use, alcohol consumption greater than 8 ounces of whiskey or equivalent per week, use of psychostimulants (excluding caffeine) in the past 6 months, or any psychotropic medication in the last 6 months other than occasional use of benzodiazepines for sleep. Any illicit drug use in the last 2 months was grounds for exclusion, even in subjects who did not otherwise meet criteria for substance

abuse. Urine drug tests were administered, and subjects testing positive for the presence of amphetamines, cocaine, marijuana, PCP, opiates, benzodiazepines, or barbiturates were excluded. Written informed consent was obtained from all subjects. This study was approved by the Institutional Review Board at Vanderbilt University and performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

2.2 Physical exam

Weight and height were measured by a clinician during each subject's physical exam, which was conducted to ensure that subjects met eligibility for MRI and PET scanning.

2.3 MRI data acquisition

Structural MRI scans were performed on two identically configured 3 Tesla Phillips Achieva scanners located at the Vanderbilt University Institute for Imaging Science (VUIIS). T1-weighted high-resolution 3D anatomical scans (1×1×1mm resolution) were obtained for each participant to aid coregistration and normalization of PET images.

2.4 PET data acquisition

PET imaging was performed on a GE Discovery STE scanner located at Vanderbilt University Medical Center. The scanner has axial slices of 3.25 mm and in-plane pixel dimensions of 2.3 x 2.3 mm (with estimated FWHM of 4.5-5.5

mm near the center of the field of view). [18F]fallypride ((S)-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3[18F]fluoropropyl)-2,3- dimethoxybenzamide) was produced in the radiochemistry laboratory attached to the PET unit, following synthesis and quality control procedures described in US Food and Drug Administration IND 47,245. [18F]fallypride is a substituted benzamide with very high affinity to D2/D3 receptors (34).

3D emission acquisition scans were performed following a 5.0 mCi slow bolus injection of [18F]fallypride (specific activity greater than 3000 Ci/mmol). CT scans were collected for attenuation correction prior to each of the three emission scans, which together lasted approximately 3.5 hours, with two 15-minute breaks for subject comfort. PET images were reconstructed with decay correction, attenuation correction, scatter correction, and calibration.

2.5 [18F]fallypride binding potential (BP_{ND}) image calculation

Voxelwise D2/D3 binding potential images were calculated using the simplified reference tissue model, which has been shown to provide stable estimates of [18F]fallypride BP_{ND} (35). The cerebellum was the reference region because of its relative lack of D2/D3 receptors (36). The cerebellar reference region was obtained from an atlas provided by the ANSIR laboratory at Wake Forest University. Limitations in PET spatial resolution introduce blurring and cause signal to spill onto neighboring regions. Because the cerebellum is located posterior and adjacent to the midbrain, the location of dopamine neurons, only the posterior ¾ of the cerebellum was included in the ROI to avoid

contamination of [18F]fallypride signal from the midbrain. The cerebellum ROI also excluded voxels within 5mm of the cortex to prevent contamination of cortical signals. The putamen ROI, drawn according to guidelines by Mawlawi et al. (37) on the MNI brain, served as the receptor rich region in the analysis. The putamen, unlike other striatal ROIs, is not adjacent to any ventricle so the putamen ROI is free from ventricle-related partial volume effects. The cerebellum and putamen ROIs were registered to each subject's T1 image using FSL non-linear registration of the MNI template to individual subject T1. T1 images and their associated cerebellum and putamen ROIs were then coregistered to the mean image of all realigned frames in the PET scan using FSL-FLIRT (http://www.fmrib.ox.ac.uk/fsl/, version 6.00). Emission images from the 3 PET scans were merged temporally into a 4D file. To correct for motion during scanning and misalignment between the 3 PET scans, all PET frames were realigned using SPM8 to the frame acquired 10min post injection (www.fil.ion.ucl.ac.uk/spm/). Model fitting and BP_{ND} calculation were performed using the PMOD Biomedical Imaging Quantification software (PMOD Technologies, Switzerland). Binding potential images represent the ratio of specifically bound ligand ([18F]fallypride in this study) to its free concentration.

2.6 [18F]fallypride BP_{ND} and BMI correlations

Relations between [18F]fallypride BP_{ND} and BMI were assessed with both voxelwise and ROI approaches. In all voxelwise and ROI analyses, gender was entered as a covariate of no-interest. Unless explicitly stated, age was also

entered as a covariate of no interest. In voxelwise analyses, implemented in SPM8, BMI, age, and gender were regressed against [18F]fallypride BP_{ND} with familywise error correction and small volume correction with a striatal mask consisting of all three striatal ROIs (caudate, putamen, and ventral striatum). We also ran voxelwise analyses without small volume correction to examine associations between BMI and [18F]fallypride BP_{ND} in extrastriatal brain areas. In ROI analyses, mean binding potential in the midbrain and 3 striatal ROIs were extracted to regress against BMI with age and gender as covariates of no interest. The midbrain and striatal ROIs were drawn in MNI standard space using previously described guidelines (37-39) and registered to PET images using the same transformations for cerebellum registration to PET images (Fig. 1).

To examine effects of age on the relation between BP_{ND} and BMI, we reran the ROI regressions described above with an age by BP_{ND} interaction term in the models predicting BMI, with gender as a covariate of no interest. In these analyses age in years was entered as a continuous variable spanning the entire age range of the sample. To more fully characterize the nature of the observed interaction, we further divided subjects into those under and those over 30 years old. We note that the selection of age 30 as a dividing line for grouping subjects is arbitrary, but it was consistent with the preexisting cutoff point for the two studies of young adults included in these analyses. To verify that this grouping captured the interaction and to better understand its spatial representation within the striatum, we performed a follow-up voxelwise analysis contrasting regression slopes for BMI and BPnd between the two age groups (<30 vs. > 30). We then

reran regressions between BP_{ND} in each ROI and BMI for each age group separately. We converted regression results into Pearson's r for ease of comparison across the two age groups. Finally, we performed voxelwise analyses regressing BMI on BP_{ND} separately for the under and over 30 year old groups to characterize the distribution of associations within the striatum.

3. Results

3.1 BP_{ND} and BMI across all ages

Voxelwise analyses (controlling for age and gender) did not identify any brain region showing a significant association between BP_{ND} and BMI at the whole brain level (N=130). To confirm this result in DRD2/3 rich areas, we performed ROI analyses regressing BP_{ND} from the 3 a priori striatal ROIs and the midbrain ROI against BMI, again controlling for age and gender. We applied Bonferroni correction to counteract the issue of multiple comparisons with 4 ROIs and utilized a corrected significance threshold of $p<0.0125$. In these more targeted analyses, BMI demonstrated a positive association with putamen BP_{ND} at the p-corrected threshold ($r=0.27$, $p=0.002$), with putamen BP_{ND} explaining 7.5% of the variance in BMI. We note that the observed relation between BMI and putamen BP_{ND} was positive, which stands in sharp contrast to the predictions of the dopamine hypofunction hypothesis. No relation was observed between BMI and midbrain, ventral striatum, or caudate BP_{ND} across the whole age range (Table 2.A).

BMI increased with age ($r=0.27$, $p<0.002$) while BP_{ND} substantially decreased with age ($r<-0.6$, $p<10^{-10}$ for all ROIs). In light of evidence that age was related to both BP_{ND} and BMI, we sought to examine whether the relationship between BMI and BP_{ND} differs across adulthood. We therefore tested for an interaction of age (in years) and BP_{ND} on BMI across all 130 subjects. The interaction of age and BP_{ND} on BMI was significant in all 3 striatal ROIs at the p-corrected threshold ($r=0.23$, $p=0.009$ for putamen, $r=0.22$, $p=0.011$ for caudate, and $r=0.23$, $p=0.010$ for ventral striatum) and in the midbrain at the p-uncorrected threshold ($r=0.20$, $p=0.023$) (Table 2.B). There was not an additional main effect of putamen BP_{ND} on BMI ($p=0.425$) after including the significant age by BP_{ND} interaction in the model. In other words, rather than a consistent relation between putamen BP_{ND} and BMI across ages, the relationship (or lack thereof) differed based on the age of the subjects.

The above interaction analyses treated BMI as the dependent variable based on the causal direction implied by the dopaminergic hypofunction hypothesis in which D2/D3 receptor levels influence BMI. However, an alternative hypothesis could be that obesity leads to declines in D2/D3 receptor availability. We tested for this reverse causal direction in a regression model in which BMI and age interact to predict BPnd, These analyses did not reveal significant interactions in any of the ROIs (all p-values >0.5).

To characterize the interaction, we divided the sample into those above or below age 30. Voxelwise analysis contrasting regression slopes for BMI and BPnd between the under and over 30-year-old groups confirmed that the relation

between BP_{ND} and BMI differed between those under and those over 30 years old, particularly in the caudate and putamen (Fig. 2A).

We further characterized the interactions by examining the relation between BP_{ND} and BMI separately for subjects under 30 years old and for subjects over 30 years old. We note that these follow-up analyses were performed not to separately test for relations observed in the above analyses, but to clarify the nature and direction of the observed interaction.

We did not observe an interaction of gender and BPnd on BMI (all p-values > 0.3), suggesting that although the Dunn et al. study included only females, relations between BPnd and BMI do not vary by gender. Furthermore, we controlled for gender in all our analyses.

3.2 BP_{ND} and BMI among subjects under 30 years old

ROI analyses of subjects under age 30 (with gender and age entered as covariates of no interest) revealed no significant associations between BMI (range: 17.5–36.3, mean=24.1) and BP_{ND} in the midbrain and all striatal ROIs (Table 3). Similarly, voxelwise analysis did not identify any brain region showing a significant (small-volume-corrected) association between BMI and BP_{ND} in this young adult age group.

3.3 BP_{ND} and BMI among subjects over 30 years old

Age did not correlate with BMI (range: 19.9–44.5, mean=27.4) in this age group (r=–0.07, p=0.603). By contrast, BP_{ND} decreased with age in all ROIs (r=–

0.46, p<0.001 for midbrain, r=–0.57, p<0.001 for putamen, r=–0.81, p<0.001 for caudate, r=–0.41, p=0.002 for ventral striatum). ROI analyses (again controlling for age and gender) confirmed that BMI was significantly positively associated with BP_{ND} in the midbrain and all 3 striatal ROIs, although only at the p-uncorrected threshold for the caudate (Fig. 3 and Table 3). Three subjects in this age group were in the extremely obese category (BMI>40) and may have magnified the correlations between BMI and BP_{ND}. To verify that these subjects did not unduly influence the results, we excluded these 3 subjects and reran the analyses. Without subjects having BMI over 40, the positive correlations between BMI and BP_{ND} remained significant in the putamen at the p-corrected threshold and ventral striatum at the p-uncorrected threshold (3). Finally, to better understand the spatial distribution of the observed association, we performed voxelwise analysis examining the association between BMI and BP_{ND} controlling for age and gender. This analysis revealed that BP_{ND} was positively associated with BMI in the caudate and putamen (Fig. 2B) in an area that largely overlapped with the area identified in the voxelwise analysis testing for the interaction with age group and BMI (Fig. 2A).

Concerning a path of influence between age, BPnd, and BMI, since age was not correlated with BMI, age did not have a direct effect on BMI and can only affect BMI via BPnd, which did correlate with BMI. Therefore, among these variables, the path of influence is likely of age affecting BPnd which affects BMI.

3.4 Cutoff point

To confirm that the differential relationship between BMI and BPnd among the two age groups was not an artifact of the 30 year old cutoff point (which was the age cutoff in two of the studies from which data were drawn), we also analyzed the data using a median split (which corresponded to a 26 year old cutoff point) and a mean split (which corresponded to a 36 year old cutoff point). The pattern of results did not change. With a median split, there was again no significant relation between BMI and BPnd among subjects in the age group below the median split point (n=65) (all p-values > 0.20). Among subjects in the age group above the median split point (n=65), BMI again positively correlated with BPnd significantly in all striatal ROIs ($r=0.48$, $p<0.0001$ for putamen, $r=0.27$, $p=0.030$ for caudate, $r=0.33$, $p=0.009$ for ventral striatum) and at trend level in the midbrain ($r=0.24$, $p=0.060$). With a 36 year old cutoff point, we also did not observe any significant relationship between BMI and BPnd in the under 36-year old group (n=83, all p-values>0.10). Among subjects over 36 years old, putamen BPnd remained positively associated with BMI ($r=0.33$, p-value=0.023) at the smaller sample size (n=47). These additional analyses further confirmed the findings that relations between BMI and BPnd differed across age groups.

4. Discussion

In the largest study to date to assess DRD2/3 availability *in vivo* in obesity and weight research, we observed relations between DRD2/3 availability and BMI that were dependent upon the age of the subjects studied. The interaction between age and DRD2/3 availability in predicting BMI was significant in the

midbrain and all three striatal ROIs. Among subjects under 30 years old, BMI was not associated with DRD2/3 availability in the striatum or the midbrain. However, among subjects over 30 years old, BMI was positively associated with DRD2/3 availability in both the midbrain and the striatum.

The dopaminergic hypofunction hypothesis of obesity states that lower dopamine function leads to deficits in neural reward responses, resulting in compulsive eating and consequently obesity (6, 40). Early reports of lower DRD2/3 density, indexed with the Taq1A gene, in obese subjects and negative associations between DRD2/3 availability and BMI led to the proposal that reduced DRD2/3 availability plays a causal role in altered reward processing in obesity (3, 8, 22). The present results are incompatible with the dopaminergic hypofunction hypothesis, at least as typically formulated in regards to DRD2/3. In young adults, when dopamine function is presumably optimal, individual differences in DRD2/3 availability showed no relation to BMI. Our observation of a positive association between DRD2/3 availability and BMI in adults over age 30 runs directly counter to the predictions of the dopaminergic receptor hypofunction model.

The causal factors leading to the positive association between BMI and DRD2/3 in the older age range but not the younger age range are not immediately clear. If the associations were driven by DRD2/3 receptor availability influencing food consumption, one would predict that this would already have an impact on BMI in young adults. One study using both PET tracers [11C]-(+)-PHNO and [11C]raclopride observed a relationship between BMI and [11C]-(+)-

PHNO BPnd but not [11C]raclopride BPnd. Citing evidence that [11C]-(+)-PHNO is more sensitive to dopamine D2 receptor affinity state, the authors proposed that the relation between BMI and [11C]-(+)-PHNO BPnd reflects increasing D2 receptor affinity with higher BMI. Normal aging is associated with numerous changes in dopamine function (31). It may be that beyond a certain age, changes in DRD2 affinity state cause the positive relation between BPnd and BMI to be more prominent and observable with high affinity DRD2/3 ligands, such as 11C]-(+)-PHNO and [18F]fallypride (16, 19, 26). Future studies specifically examining DRD2 affinity and BMI across the lifespan would provide insight into this possibility.

It is possible that methodological issues contribute to some of the inconsistencies that have emerged across studies. In contrast with previous PET studies that examined associations between DRD2/3 availability and BMI, we used the high affinity ligand [18F]fallypride to assess DRD2/3 availability. Previous PET studies that examined the role of DRD2/3 availability in obesity often used the lower affinity ligand [11C]raclopride (3, 5, 21). [11C]raclopride binding is more likely to be displaced by endogenous dopamine release (30, 41), so [11C]raclopride binding potential reflects the combined effects of DRD2/3 availability and dopamine release to a greater degree than [18F]fallypride (42), possibly complicating the interpretation of correlations with [11C]raclopride binding potential especially since dopamine release has been associated with obesity in rodents (43, 44). It is noteworthy that the landmark PET study in 2001 that reported a negative relationship between BMI and DRD2/3 availability used

[11C]raclopride (3), whereas PET studies using the higher affinity DRD2/3 ligand [18F]fallypride either reported positive associations between BMI and DRD2/3 availability in the striatum (19) or both positive and negative associations in the striatum, suggesting regional specificity within the striatum. Future studies assessing dopamine release independent of DRD2/3 availability and DRD2 affinity will be necessary to determine whether previous reports of negative associations between [11C]raclopride binding potential and BMI stemmed from a link between BMI and dopamine release and/or DRD2 affinity.

Dopamine plays a critical role in reward processes, including responses to food cues and food intake. Obese individuals habituate to food reward at a slower rate than lean individuals, and high reward sensitivity contributes to overeating (45, 46). If DRD2 availability has a causal influence on reward sensitivity, our findings of a positive association between DRD2/3 availability and BMI suggest that across much of adulthood, higher DRD2/3 availability may affect bodyweight by increasing or maintaining reward sensitivity for food.

Our study is different from some of the previous studies in that we did not focus on extremely obese subjects but instead included individuals along a broad BMI continuum (20). It could be that extreme obesity represents a condition that is distinct from other BMI categories and has a different relationship with DRD2/3 availability; several studies have reported that relations with DRD2/3 availability were different for obese subjects and non-obese subjects (22, 23, 26). Indeed, it has been proposed that, rather than dopaminergic hypofunction influencing BMI, increased dopamine release associated with overeating downregulates

dopamine receptor function, leading to the previously observed negative correlation between DRD2/3 availability and BMI in extremely obese subjects but not normal weight subjects (3, 46). There are not enough subjects meeting criteria for extreme obesity in the present study to examine this possibility. However, in our study, positive associations between BMI and DRD2/3 availability among adults over age 30 were as high or higher with extremely obese subjects in the analyses than without. One would have expected the positive association to be reduced when extremely obese participants are included in the analysis if there was a negative association among extremely obese subjects or if there was an inflection point above which the relation between BMI and DRD2/3 reverses. It is notable in this regard that Dunn et al. (2012) found similar positive associations with BMI in an independent sample that included 14 obese women (mean BMI = 40). Taken together, these findings fail to provide evidence for a differential relationship between BMI and DRD2/3 in obese participants. Future clinical studies of the dopamine system exploring differences between extreme obesity and other BMI categories would help answer this question.

The present results suggest the need to play close attention to age when considering relations between dopamine, weight and obesity. While past studies often provide evidence that their obese and nonobese groups do not significantly differ in age, given the robustness of the association between age and DRD2/3 BP_{ND}, even relatively modest differences in age could substantially impact results. It is also of note that studies in this area (including the present one) generally pay

little attention to the representativeness of samples. To qualify for the studies conducted in our lab, participants had to have no major medical problems (other than obesity), and had to pass a physical exam and blood work (complete blood count and comprehensive metabolic panel). With aging, fewer and fewer potential participants are likely to meet such criteria, and therefore there is a potential bias when selecting healthy subjects in older age groups. This problem is not unique to this study, but may nevertheless influence findings from any study with strict exclusion criteria.

Our findings of a differential relationship between DRD2/3 availability and BMI in different age groups were observed with three different cutoff points for dividing subjects into a younger group and an older group. However, we have not attempted to determine a specific inflection point, whether it is at age 30 vs. age 26 or age 36. The age group categorization was primarily used here to characterize the nature of the observed statistical interaction using the continuous variables. It may prove valuable in future studies to determine if there is a specific age or age range after which the link between BMI and DRD2/3 availability changes. A final caveat is that, like the other PET studies in the field, the present study utilized a cross-sectional design and thus cannot speak to the causality of the current findings. The present study examined the dopaminergic hypofunction hypothesis, which postulates that lower dopamine function as reflected in DRD2 receptors leads to compensatory food intake and consequently obesity. However, it may be that obesity changes dopamine function. Longitudinal data are needed to address this question of causality.

5. Conclusions

The present findings in a large sample of adults demonstrate the importance of age in the relationship between DRD2/3 availability and BMI. Although there was no relation between DRD2/3 and BMI in young adults, a positive relationship emerged later in adulthood. These data provide no support for the idea that lower DRD2/3 plays a casual role in weight gain, and indicate the strong need to incorporate age into the analysis and interpretation of data in the field.

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Figure Captions

Fig 1. ROIs and binding potential (BP_{ND}) images. A) Striatal (top) and midbrain (bottom) ROIs used for extracting BP_{ND}. B) One under 30-year-old subject's and C) one over 30-year-old subject's [^{18}F]fallypride BP_{ND} images in native PET space. BP_{ND}, which declined with age, was highest in the striatum (top) and the midbrain (bottom). Note that the BP_{ND} maps use different scales to reflect the large differences in BP_{ND} values in striatal and extrastriatal brain regions.

Fig 2. Voxelwise results. A) Age group by BMI interaction analysis showed that the relation between BMI and BP_{ND} differed between those under and those over 30 years old in both the caudate and putamen (peak t-stat=5.21, peak coordinate: x=-32, y=-6, x=-2). B) Among subjects over 30 years old, BMI was positively associated with BP_{ND} in bilateral caudate and bilateral putamen (peak t-stat=5.39, peak coordinate: x=10, y=6, z=14). Results were small-volume-corrected with a striatal mask.

Fig 3. BMI and BP_{ND} among subjects over 30 years old. ROI analyses confirmed that BMI correlated positively with BP_{ND} in the midbrain, putamen, and ventral striatum at the p-corrected threshold, and the caudate at the p-uncorrected threshold.

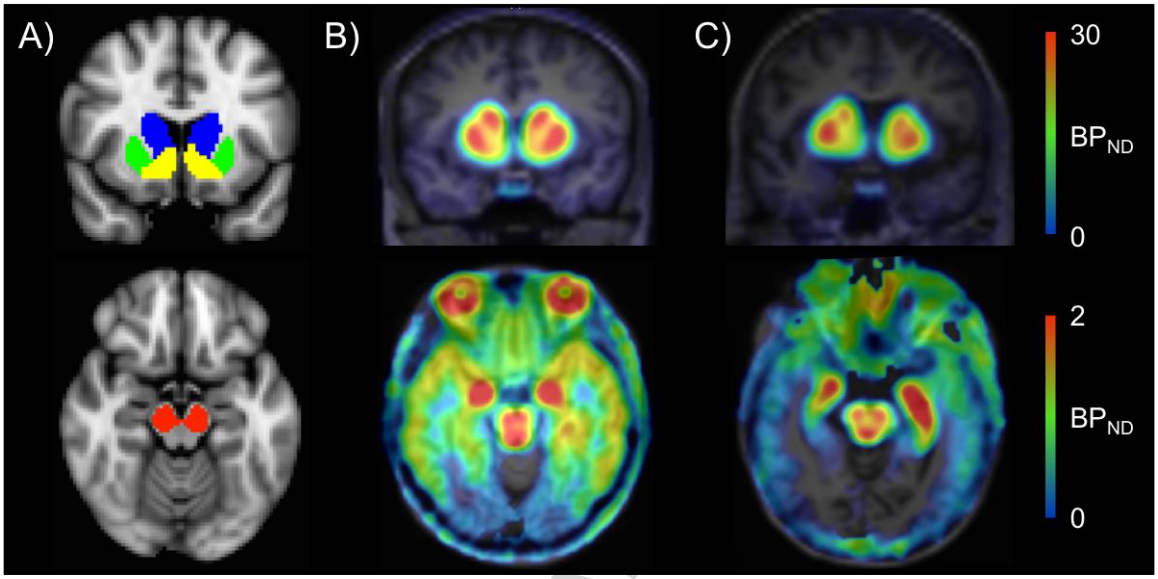

Fig. 1

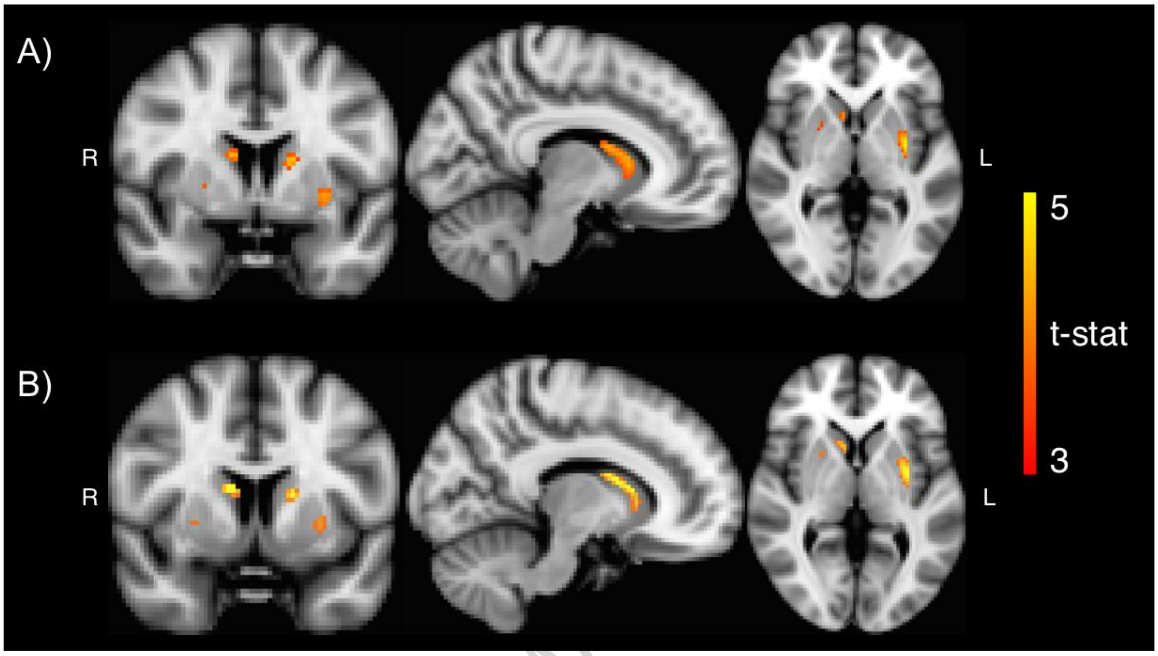

Fig. 2

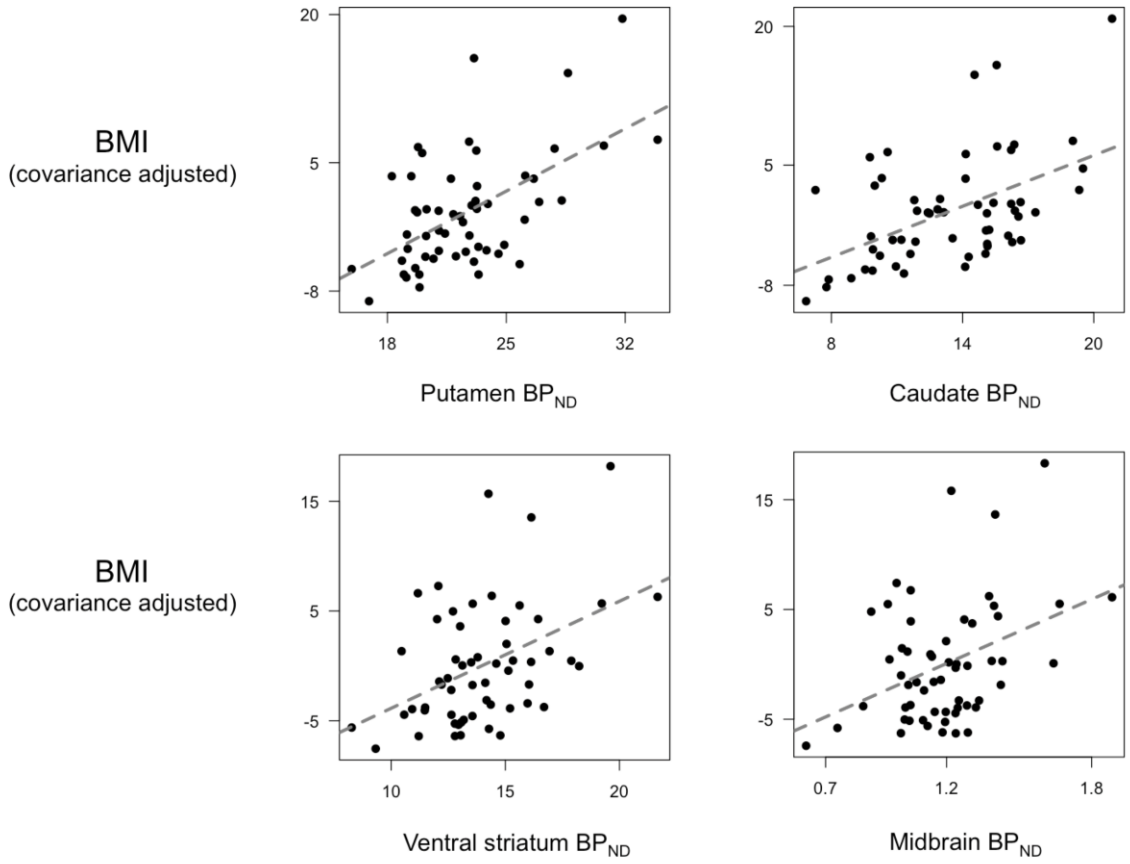

Fig. 3

Table 1: Previous PET studies reporting associations between BMI and DRD2/3 availability

Authors	Age (mean ± SD years)	BMI (mean ± SD)	Ligand	Findings	Age effects ?
Positive BMI-BPnd associations					
Caravaggio et al. 2015	26 healthy normal: 30 ± 7, range 20-45	24 ± 3	PET-[11C]PHNO	Ventral striatal BPnd positively related to BMI.	No effect of age on BMI or BPnd.
Cosgrove et al. 2015	12 healthy normal: 28 ± 6, range 20-37	28 ± 5	PET-[11C]PHNO	Caudate BPnd positively related to BMI.	No effect of age on BMI or BPnd.
Dunn et al. 2010	14 obese: 40 ± 8	40 ± 5	PET-[18F]fallypride	Caudate BPnd positively related to BMI.	na
	8 control: 40 ± 9	23 ± 2			
Guo et al. 2014	20 obese: 35 ± 2, range 18-45	36 ± 1	PET-[18F]fallypride	Dorsal/lateral striatal BPnd positively related to BMI.	No effect of age on BMI or BPnd. Age used as nuisance covariate.
	23 control: 28 ± 1, range 18-45	22 ± 1			
Yasuno et al. 2001	16 healthy normal: 26 ± 5, range 21-35	range 20-26	PET-[11C]FLB 457	Amygdala BPnd positively related to BMI.	na
Negative BMI-BPnd associations					
de Weijer et al. 2011	15 obese: 38 ± 7, range 26-49	47 ± 7	SPECT-[123I]IBZM	Striatal BPnd lower in obese than control.	No effect of age on BPnd. Age used

					as nuisance covariate.
	15 control: 28 ± 10, range 20-60	22 ± 2			
Frank et al. 2005	10 recovered anorexics: 24 ± 5	22 ± 3	PET-[11C]raclopride	Antero-ventral striatal BPnd higher in recovered anorexics than control.	No effect of age on BPnd.
	12 control: 27 ± 6	23 ± 2			
Guo et al. 2014	20 obese: 35 ± 2, range 18-45	36 ± 1	PET-[18F]fallypride	Ventromedial striatal BPnd negatively associated with BMI	No effect of age on BMI or BPnd. Age used as nuisance covariate.
	23 control: 28 ± 1, range 18-45	22 ± 1			
Haltia et al. 2007	12 obese: 25 ± 2.5	33 ± 5	PET-[11C]raclopride	Striatal and thalamus BPnd lower in obese than control	na
	12 control: 26 ± 4.5	22 ± 1			
Steele et al. 2010	5 gastric bypass patients: 20-38 (mean 32) 5 control: mean 22	40-53 (mean 45) mean 21	PET-[11C]raclopride	BPnd increased after gastric bypass surgery.	na
Volkow et al. 2008*	10 obese: 36 ± 10, range 20-55	51 ± 5	PET-[11C]raclopride	Striatal BPnd lower in obese than control.	na
	12 control: 33 ± 8, range 20-55	25 ± 3			
Wang et al. 2001	10 obese: 39 ± 7, range 26-54	51 ± 5	PET-[11C]raclopride	Striatal BPnd lower in obese than control. BMI correlated negatively with BPnd in obese.	Age negatively correlated with BPnd in

					control. Age used as nuisance variable.
	10 control: 38 ± 6, range 25-45	25 ± 3			
No BMI-BPnd associations					
Caravaggio et al. 2015	35 healthy normal: 31 ± 9, range 20-47	23 ± 3	PET-[11C]raclopride	No correlation between ventral striatal BPnd and BMI.	No effect of age on BMI or BPnd
Eisenstein et al. 2013	15 obese: 33 ± 6, range 25-41	40 ± 5	PET-[11C]NMB	No striatal BPnd diff. between obese and control. Striatal BPnd not correlated with BMI.	Putamen BPnd negatively correlated with age
	15 control: 30 ± 6, range 22-40	23 ± 2			
Karlsson et al. 2015	13 obese: 39 ± 11	42 ± 4	PET-[11C]raclopride	No BPnd diff. between obese and control in any brain region.	na
	14 control: 45 ± 13	23 ± 3			
Steele et al. 2010	5 gastric bypass patients: 20-38 (mean 32)	40-53 (mean 45)	PET-[11C]raclopride	No striatal BPnd diff. between patients and control.	na
	5 control: mean 22	mean 21			

* reanalysis of data from Wang et al. 2001

Table 2: Correlations between BP_{ND} and BMI across all ages

A) Model without BP_{ND} by age interaction	r	p-value
midbrain	0.10	0.244
putamen	0.27	0.002**
caudate	0.16	0.063
ventral striatum	0.13	0.134

B) Model with BP_{ND} by age interaction			BP_{ND} by age interaction	
	r	p-value	r	p-value
midbrain	-0.12	0.179	0.20	0.023*
putamen	-0.07	0.425	0.23	0.009**
caudate	-0.10	0.284	0.22	0.011**
ventral striatum	-0.13	0.146	0.23	0.010**

* p-uncorrected , ** p-corrected

Table 3: Correlations between BP_{ND} and BMI for under and over 30 year old groups

	all BMI values		BMI < 40	
	r	p-value	r	p-value
Under 30 years old (N=73)				
midbrain	-0.10	0.393	na	na
putamen	-0.09	0.446	na	na
caudate	-0.15	0.224	na	na
ventral striatum	0.00	0.978	na	na
Over 30 years old (N=57)				
midbrain	0.35	0.010**	0.26	0.058
putamen	0.48	0.000**	0.35	0.011**
caudate	0.33	0.015*	0.22	0.125
ventral striatum	0.4	0.002**	0.31	0.028*

* p-uncorrected , ** p-corrected

Highlights

- • Age interacted with DRD2 availability in predicting BMI.
- • Among subjects under 30 years old, BMI was not associated with DRD2 availability.
- • Among subjects over 30 years old, BMI positively associated with DRD2 availability.
- • Present results are incompatible with the dopaminergic hypofunction hypothesis.
- • Results highlight the importance of age in assessing correlates of DRD2 function.