Striatal dopamine transporter changes after glucose loading in humans

Kyoungjune Pak PhD1 | Seongho Seo PhD2,3 | Keunyoung Kim PhD1 |
Myung Jun Lee MD4 | Myung Jun Shin PhD5 | Sunghwan Suh PhD6 |
Hyung-Jun Im PhD7 | Jung-Jun Park PhD8 | Seong-Jang Kim PhD9 | In Joo Kim PhD1

1Department of Nuclear Medicine and Biomedical Research Institute, Pusan National University Hospital, Busan, Republic of Korea
2Department of Neuroscience, College of Medicine, Gachon University, Incheon, Republic of Korea
3Neuroscience Research Institute, Gachon University, Incheon, Republic of Korea
4Department of Neurology, Pusan National University Hospital, Busan, Republic of Korea
5Department of Rehabilitation Medicine, Pusan National University Hospital, Busan, Republic of Korea
6Department of Internal Medicine, Dong-A University College of Medicine, Busan, Republic of Korea
7Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Republic of Korea
8Division of Sport Science, Pusan National University, Busan, Republic of Korea
9Department of Nuclear Medicine, Pusan National University Yangsan Hospital, Yangsan, Republic of Korea

Correspondence
Dr Kyoungjune Pak MD, PhD, Department of Nuclear Medicine, Pusan National University Hospital, 179 Gudeok-ro, Seo-gu, Busan 49241, Republic of Korea.
Email: ilikechopin@me.com

Funding information
National Research Foundation of Korea, Grant/Award Numbers: 2017M3C7A1044367, 2017R1A1B03029352, 2017R1D1A1B03032325

Peer Review
The peer review history for this article is available at https://publons.com/publon/10.1111/dom.13872.

Abstract
Aims: The dopamine transporter (DAT) actively translocates dopamine that is released from the presynaptic neurons across the membranes of nerve terminals into the extracellular space. We hypothesized that glucose loading-induced changes in striatal DAT levels could be associated with food intake in humans.

Materials and methods: An intravenous bolus injection of 18F-FP-CIT was administered after infusion of glucose or placebo (normal saline), and emission data were acquired over 90 minutes in 33 healthy males. For a volume-of-interest-based analysis, an atlas involving sub-striatal regions of ventral striatum (VST), caudate nucleus and putamen was applied. DAT availability and binding potential (BPND) were measured using a simplified reference tissue method with cerebellum as the reference.

Results: The glucose-loaded BPND from the VST negatively correlated with body mass index (BMI), whereas the placebo-loaded BPND from the VST did not. After loading with glucose, there were substantial increases in BPNDs: 18.3%, 71.7% and 34.0% on average in the VST, caudate nucleus and putamen, respectively.

Conclusion: Striatal DAT changes after glucose loading, and BMI is associated with glucose-loaded DAT availability, not with placebo-loaded DAT availability. DAT might have a role in the reward system of eating behavior.

KEYWORDS
dopamine plasma membrane transport proteins, glucose, obesity, reward

Kyoungjune Pak and Seongho Seo contributed equally to this study.

Received: 30 July 2019 | Revised: 20 August 2019 | Accepted: 28 August 2019
DOI: 10.1111/dom.13872

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1 | INTRODUCTION

Obesity rates have nearly tripled worldwide since 1975 and obesity has become one of the major public health threats.1 Obesity arises from energy intake that chronically exceeds energy expenditure.2 Among other factors, the brain plays a critical role in controlling this energy balance.2 Food intake is controlled by a homeostatic system in the hypothalamus and the hedonic reward system, which are closely linked with each other.3 Dopamine is a neurotransmitter that plays a major role in motivation and reward pathways.4 Feeding induces dopamine release in the striatum, and dysfunction of the dopaminergic reward system can lead to overeating, which significantly overlaps with drug addiction.5

There is no direct method of measuring dopamine levels in the human brain. Therefore, molecular neuroimaging using dopamine receptor (DR) radiopharmaceuticals was adopted in order to understand the dopaminergic pathway in the brain. When compared with lean controls, DR availability in individuals with moderate obesity was observed to be higher than that in individuals with severe obesity.6,7 However, results among studies are inconsistent, and the role of DR in obesity is uncertain. In addition, DR availability acquired by positron emission tomography (PET) is sensitive to the endogenous concentrations of dopamine,8 which can complicate interpretation of results.

The dopamine transporter (DAT) is located on the plasma membrane.9 It actively translocates dopamine that is released from the presynaptic neurons across the membranes of nerve terminals into the extracellular space.10 Unlike DR, the DAT might be insensitive to the synaptic dopamine concentration, as no change in DAT availability was observed after depletion or release of dopamine.11,12 Although the DAT is a major target of various pharmacologically active drugs, its role in obesity has not been established. The majority of previous studies reported no significant correlation between DAT availability and body mass index (BMI),13-15 and the DAT was not thought to be involved in the neurobiology underlying obesity in humans.13 However, according to animal studies, insulin increases the levels and activity of DAT mRNA and, thereby, enhances the clearance of dopamine from the synapse.16

We hypothesized that glucose loading might change striatal DAT availability in humans. Therefore, we investigated the potential role of the DAT by exploring (a) the association between BMI and DAT availability and (b) the effect of glucose loading on DAT availability in humans.

2 | METHODS

2.1 | Participants

The study was approved by the institutional review board of Pusan National University Hospital (PNUH-1707-019-057). All participants signed an informed consent form prior to participation. Thirty-three healthy, non-obese males were recruited. Participants who experienced more than 10% change in weight over six months, who were heavy smokers, or who had a history of drug abuse, brain injury, neuropsychological disorders or endocrine disorders were excluded. On the day of each visit, participants were instructed to fast overnight for at least 12 hours and to abstain from smoking and alcohol consumption. The participants visited the study site between 11:00 am and 12:00 pm to avoid the effect of diurnal variations in dopamine.

2.2 | Study design

Each participant visited the study site twice, on separate days, for two PET scans. During each visit, height (m) and weight (kg) were measured and BMI was calculated as weight/height−2. Bilateral antecubital veins were cannulated: one for blood sampling and for injection of 18F-FP-CIT, and the other for glucose or placebo infusion. Participants were blinded and randomly assigned to either glucose or placebo infusions. For 10 minutes, 300 mg/kg of glucose in a 50% solution was administered. Placebo (normal saline) was administered at the same speed and volume.17 The serum glucose level (mg/dL) and insulin level (μU/mL) were measured before and after the infusions of glucose and placebo. The serum glucose level was determined by an enzymatic reference method using hexokinase with Glucose HK Gen.3 (Roche Diagnostics GmbH, Mannheim, Germany). The serum insulin level was determined by an electrochemiluminescence immunoassay method using Elecsys Insulin (Roche Diagnostics GmbH). An intravenous bolus injection of 18F-FP-CIT (210.9 ± 16.3 MBq) was administered after infusion of glucose or placebo. Emission data were acquired over 90 minutes with 50 frames of progressively increasing durations (15 s × 8 frames, 30 s × 16 frames, 60 s × 10 frames, 240 s × 10 frames and 300 s × 6 frames) using the Biograph 40 Truepoint PET/CT (Siemens Healthcare, Knoxville, Tennessee). Dynamic PET data were collected in the three-dimensional mode, with 148 slices with image sizes of 256 × 256 and pixel sizes of 1.3364 × 1.3364 mm². These were reconstructed by filtered back projection using a Gaussian filter. The study design is illustrated in Figure 1.

2.3 | Image analysis

For a volume-of-interest (VOI)-based analysis, an averaged image (0–10 minutes after injection) was created from dynamic PET frames and spatially normalized to a 15O-Water PET template in statistical parametric mapping 5 (Wellcome Trust Centre for Neuroimaging, UK). To
extract time-activity curves (TACs) of VOIs from full dynamic PET scans, the Oxford-GSK-Imanova striatal atlas from the FMRIB Software Library v5.0 (https://fsl.fmrib.ox.ac.uk/fsl) was applied. This is an atlas involving sub-striatal regions of ventral striatum (VST), caudate nucleus and putamen, segmented according to anatomical structure and manually delineated on the non-linear MNI 152 template.\(^{18}\) DAT availability, expressed in terms of binding potential (BP\(_{ND}\)), was measured by analyzing TACs using a simplified reference tissue method\(^{19}\) with cerebellum as the reference tissue. Percent changes in BP\(_{ND}\) were calculated as follows: \((\text{Glucose-loaded BP}_{ND} - \text{Placebo loaded BP}_{ND}) / \text{Placebo-loaded BP}_{ND} \times 100\%\). Image analysis was done using pmod version 3.6 (PMOD Technologies LLC, Zurich, Switzerland).

### 2.4 Statistical analysis

Normality was assessed using the D’Agostino and Pearson normality test. The paired t-test was used to compare the glucose-loaded BP\(_{ND}\) and placebo-loaded BP\(_{ND}\) of each participant, and to compare glucose/insulin levels before and after glucose loading. Pearson correlation analysis was used to determine the association between BMI and the BP\(_{ND}\). All analyses were conducted using Prism v7.0d (GraphPad Software Inc, La Jolla, California).

### 3 RESULTS

Thirty-three healthy males, with an age range of 20 to 31 years and mean age of 24.5 ± 2.8 years, were included in the study. Mean BMI of the study group was 23.1 kg/m\(^2\). Glucose and insulin levels were increased after glucose loading (both \(P < 0.0001\)). Participant characteristics are summarized in Table 1.

In VOI-based analyses, the average BP\(_{ND}\) of the VST, caudate nucleus and putamen were 4.49 ± 1.99, 3.98 ± 1.90 and 5.96 ± 1.96, respectively, for participants loaded with glucose, and were 4.61 ± 1.61, 3.25 ± 1.97 and 5.25 ± 2.14, respectively, for participants loaded with placebo (Figure 2). BP\(_{ND}\) of the VST, caudate nucleus and putamen were increased after glucose loading in 15, 19 and 21 participants, respectively. Interestingly, glucose-loaded BP\(_{ND}\) from the VST negatively correlated with BMI (\(r = -0.4159; P = 0.0161\)), whereas, placebo-loaded BP\(_{ND}\) from the VST did not show any significant association with BMI (\(r = -0.1355; P = 0.4521\)) (Figure 3). Both glucose-loaded and placebo-loaded BP\(_{ND}\)S from the caudate nucleus (\(r = 0.0067; P = 0.9703\) and \(r = -0.1219; P = 0.4992\), respectively) and the putamen (\(r = -0.1038; P = 0.5652\) and \(r = -0.0708; P = 0.6953\), respectively) did not show any significant correlation with BMI. However, after loading with glucose, there were substantial increases in BP\(_{ND}\)S: 18.3%, 71.7% and 34.0% on average in the VST, caudate nucleus and putamen, respectively, although the paired t-test did not reveal significant differences in BP\(_{ND}\)S in the VST (\(P = 0.7828\)), caudate nucleus (\(P = 0.0763\)) and putamen (\(P = 0.1094\)) (Figure 4).

### 4 DISCUSSION

To the best of our knowledge, this is the first human study to investigate the association of DAT availability with glucose loading. The results indicate, first, that BMI is associated with DAT availability from the VST after glucose loading, whereas such an association is not present with placebo loading, and second, that the average increase in DAT availability after glucose loading was more than 18%.

Obesity results from an imbalance between energy intake and expenditure,\(^{1}\) and the brain is the primary centre controlling this balance.\(^{20}\) Eating behavior is regulated by the homeostatic and hedonic systems of the brain.\(^{3}\) The hypothalamus plays a central role in maintaining the physiologic requirements of the body through regulatory neuropeptides such as leptin, ghrelin and orexin.\(^{16,21}\) Also, the regulation of eating behavior is involved with a reward system.\(^{16}\) Neurotransmitters, such as dopamine, opioid and serotonin, mediate the hedonic functions of this reward system.\(^{3,22}\) Among them, dopamine plays a major role in modulating motivation and in reward processing.\(^{23}\) There are two major hypotheses regarding the role of dopamine. The first hypothesis, dopamine hyper-responsiveness, explains the hypersensitivity to rewards that is related to an increased salience of food, leading to the excessive intake of highly palatable foods.\(^{24,25}\) The second hypothesis, reward deficiency syndrome, maintains that individuals who are insensitive to rewards overeat to increase their endogenous dopamine levels.\(^{24,25}\) Hence, a nonlinear relationship of an inverted parabola has been proposed between DR and BMI based on previous studies. In mild obesity, the change in DR

### TABLE 1 Characteristics of participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Characteristics of participants</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>24.5 ± 2.8</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>23.1 ± 2.2</td>
</tr>
<tr>
<td>Glucose-loaded</td>
<td></td>
</tr>
<tr>
<td>VST</td>
<td>4.49 ± 1.99</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>3.98 ± 1.90</td>
</tr>
<tr>
<td>Putamen</td>
<td>5.96 ± 1.96</td>
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<tr>
<td>Placebo-loaded</td>
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<tr>
<td>VST</td>
<td>4.61 ± 1.61</td>
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<tr>
<td>Caudate nucleus</td>
<td>3.25 ± 1.97</td>
</tr>
<tr>
<td>Putamen</td>
<td>5.25 ± 2.14</td>
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Note: Data are expressed as number of participants or mean ± standard deviation. Abbreviations: BP\(_{ND}\), binding potential; VST, ventral striatum.
is not significant. However, with the onset of moderate obesity, the responsiveness of dopamine increases until the BMI rises to approximately 35–40 kg/m², following which a reward deficiency occurs in severe obesity.3,24

As it is not currently possible to measure directly the concentration of dopamine in the human brain, molecular imaging using radiopharmaceuticals to assess biomarkers in vivo has been adopted. The DR has been investigated widely in the field of neuroimaging with regard to obesity. However, the association between obesity and the DR remains unclear as the result of lack of agreement among previous studies. A lower availability of DR in obese individuals than in non-obese individuals was reported for the first time by Wang et al.6 However, the opposite was also observed, as well as the absence of a difference in availability of DR between obese and non-obese individuals.26,27 This inconsistency could be attributed to the characteristics of the radiopharmaceuticals. For example, in individuals who were scanned with both 11C-PHNO and 11C-raclopride, different associations between the radiopharmaceuticals and obesity were found, even in the same individual.28 In addition, the distribution of BMI in each study might have had an effect on this disagreement. As obesity can be defined as a BMI of 30 or more, individuals with both moderate and severe obesity can be included in the obese group in each study.6,26,29 Moreover, as a fundamental limitation of DR availability acquired as assessed by PET scans is that endogenous dopamine competes with the radiopharmaceuticals for binding with the DR,8 lower DR availability can be interpreted as either higher release of endogenous dopamine or downregulation of DR. DR availability cannot determine whether obesity is associated with changes in endogenous dopamine concentration or with the expression of DR. In addition, the majority of studies regarding DR availability were done without food stimulation (in the preprandial state) or eating (in the postprandial state). The prediction of DR availability or endogenous dopamine concentration in the preprandial or postprandial state, from baseline DR availability, might be difficult in both obese and non-obese individuals. The average striatal DR availability showed a statistically insignificant decrease of 3.0%–7.4% with stimulation via the smell and taste of food.30,31 Although the average striatal DR availability did not change significantly after eating, DR availability from the dorsal striatum decreased by 6.8%–12.4%.32 When loaded with glucose, striatal DR availability decreased by 7.0%–8.2%.17 Therefore, based on studies in the preprandial or postprandial state, the changes in DR availability are minimal, the maximal decrease being 12.4%.

The role of the DAT in obesity has been investigated previously. Although synaptic dopamine concentrations are regulated by the DAT, it is insensitive to the synaptic dopamine concentration, unlike

FIGURE 2  Average BP_{ND} after glucose and placebo loading

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DR, and it is affected neither by the depletion of dopamine via the tyrosine hydroxylase inhibitor\textsuperscript{11} nor by the release of dopamine by the DR antagonist.\textsuperscript{12} With the exception of one study,\textsuperscript{33} most baseline studies have shown no significant association between DAT availability and BMI.\textsuperscript{13-15} Consistent with previous studies, DAT availability in the placebo-loaded participants (baseline) did not show any significant correlation with BMI in this study. However, eating behavior is a complicated and incompletely understood process, resulting from a combination of visual stimulation, olfactory stimulation, expectation, gastric distension and glucose levels.\textsuperscript{17} In this study, we used the glucose infusion to reflect food intake, and we focused on the effect of an increase in glucose level on DAT availability. Interestingly, DAT availability in the VST after glucose loading negatively correlated with BMI. The VST plays a key role in processing reward cues and in motivating reward-seeking behavior.\textsuperscript{28} As DATs take up synaptic dopamine into the presynaptic neuron, individuals with a lower BMI may experience higher clearance of synaptic dopamine, resulting in lower endogenous concentrations of dopamine in the VST, which stops food intake. As DAT availability is not affected by endogenous dopamine concentrations, DAT availability in the VST after glucose loading might be directly connected with eating behavior. This connection is seen despite the narrow range in BMI among participants in this study. From animal studies, it is known that insulin can act on insulin receptors to amplify dopamine uptake by the DAT through the PI3 kinase signaling pathway, which enhances the surface expression of DATs, and also the release of dopamine in the striatum,

**FIGURE 3** Correlation between body mass index (BMI) and BP\textsubscript{ND} of the ventral striatum (A, D), caudate nucleus (B, E) and putamen (C, F) after glucose and placebo loading.

**FIGURE 4** Paired t-test of BP\textsubscript{ND} of ventral striatum (A), caudate nucleus (B) and putamen (C) between glucose loading and placebo loading.
which might influence food-related rewards.\textsuperscript{34,35} Therefore, the change in DAT availability might be related to the increase in insulin level after glucose loading. In this study, the average change in DAT availability was more than 18\%, which is much higher than the average change in DR availability, or in dopamine concentrations, seen in previous studies.\textsuperscript{17}

There are several limitations to this study. First, the sample size of 33 healthy males was small. Second, only males were studied, to exclude the effect of sex, as sex hormones are known to regulate appetite and eating behavior.\textsuperscript{36} Third, the BPND\textsuperscript{S} measured did not distinguish between DAT density and affinity. Moreover, both direct comparisons with DR availability in the same individuals and further studies to investigate the role of DAT in obesity are necessary.

We have highlighted that 1) striatal DAT changes after glucose loading, and 2) BMI is associated with glucose-loaded DAT availability, not with placebo-loaded DAT availability. After loading with glucose, substantial increases in striatal DAT availability were observed and DAT might have a role in the reward system concerning eating behavior.

ACKNOWLEDGMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by the Ministry of Education (2017R1D1A1B03032932/2017R1D1A1B03033235) and by the Brain Research Program through the National Research Foundation of Korea, funded by the Ministry of Science and ICT (2017M3C7A1044367). The authors thank the research nurse, Yoon Jeong Joo for her valuable work and skillful assistance in this study.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

P. K. and S. S. were responsible for the study design. P. K., K. K. and L. M. J. were responsible for conducting the study and data collection. K. K., L. M. J., S. M. J., S. S., I. H. J., P. J. J. and P. K. were responsible for analysis. P. K., K. S. J. and K. I. J. wrote the manuscript.

ORCID

Kyoungjune Pak https://orcid.org/0000-0001-5051-1894
Seongho Seo https://orcid.org/0000-0001-7894-0535
In Joo Kim https://orcid.org/0000-0003-1765-0774

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