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Cerebral serotonin transporter binding is inversely related to body mass index ☆

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ABSTRACT

Overweight and obesity is a health threat of increasing concern and understanding the neurobiology behind obesity is instrumental to the development of effective treatment regimes. Serotonergic neurotransmission is critically involved in eating behaviour; cerebral level of serotonin (5-HT) in animal models is inversely related to food intake and body weight and some effective anti-obesity agents involve blockade of the serotonin transporter (SERT). We investigated in 60 healthy volunteers body mass index (BMI) and regional cerebral SERT binding as measured with [^{11}C]DASB PET.

In a linear regression model with adjustment for relevant covariates, we found that cortical and subcortical SERT binding was negatively correlated to BMI (−0.003 to −0.012 BP_{ND} unit per kg/m^2). Tobacco smoking and alcohol consumption did not affect cerebral SERT binding.

Several effective anti-obesity drugs encompass blockade of the SERT; yet, our study is the first to demonstrate an abnormally decreased cerebral SERT binding in obese individuals. Whether the SERT has a direct role in the regulation of appetite and eating behaviour or whether the finding is due to a compensatory downregulation of SERT secondary to other dysfunction(s) in the serotonergic transmitter system, such as low baseline serotonin levels, remains to be established.

Introduction

Being overweight or obese is a condition characterized by abnormal or excessive accumulation of body fat and it is associated with increased risks for developing diseases, such as type-2 diabetes, cardiovascular diseases, and certain types of cancer. For this reason, and because the frequency of obesity increases alarmingly world-wide the epidemic of obesity is today considered one of the most severe threats to human health. Globally, approximately 1.6 billion adults weight too much, and WHO has estimated that by 2015, this number will be 2.3 billion (WHO 2006).

Body mass index (BMI), defined as the body weight divided by the squared height, is a commonly used measure for the nutrition state of an individual (Molarius and Seidell, 1998). Individuals with a BMI of more than 25 kg/m^2 are designated as being overweight, whereas people with a BMI larger than 30 kg/m^2 are obese. Although the neurobiological mechanism behind overeating is only partly understood, the serotonergic neurotransmission is known to be involved in eating behaviour and regulation of body weight: low brain levels of the monoamine serotonin (5-hydroxytryptamine, 5-HT) are related to elevation of self-administration of food in animals (Breisch et al., 1976; Saller and Stricker, 1976; Waldbillig et al., 1981). Furthermore, 5-HT agonism has been related to weight loss in obese human subjects (Bever and Perry, 1997) whereas depletion of 5-HT has been associated to an increase in food intake in women with bulimia nervosa (Weltzin et al., 1995).

The evolutionarily highly conserved serotonin transporter (SERT) is a pre- and extrasynaptically localized membrane protein (Miner et al., 2000) that regulates the serotonin transmission via its reuptake of released 5-HT thereby modulating the extracellular fluid 5-HT concentrations. Drugs that increase the extracellular 5-HT through inhibition of SERT also inhibit food intake both in animals (Blundell, 1984; Simansky, 1996) and in humans (Olausson et al., 2002; Halford et al., 2005; Johnson, 2008). In addition, studies of SERT knockout mice have uncovered SERT as a candidate gene for human obesity, e.g., SERT mutant (SCL6A4−/−) mice become obese (Murphy and Lesch, 2008) and in obese and overweight individuals, recent

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evidence points to a decreased expression of the gene encoding for SERT (Sookoian et al., 2007; Fuemmeler et al., 2008).

In spite of these intriguing findings, only two in vivo molecular imaging studies of the SERT have been performed on overweight/obese subjects and both studies have employed non-selective monoamine transporter radioligands. In the first single photon emission tomography (SPECT) study, midbrain SERT binding was found to be lower in binge eating obese women than in non-binging obese women (Kuikka et al., 2001). At re-examination after 8–24 months of SSRI treatment SERT binding in the binge eating obese subjects was increased (Tammela et al., 2003). In a recent [^{123}I]nor-β-CIT SPECT study including 16 monozygotic twin pairs, twins with a BMI higher than their monozygotic co-twins were found to have higher SERT binding (Koskela et al., 2008). In two independent large samples of healthy human subjects we previously detected a positive association between BMI and global neocortical 5-HT$_{2A}$ receptor binding (Adams et al., 2004; Erritzoe et al., 2009) supporting the notion that overweight subjects might have lower cerebral 5-HT levels (Roth et al., 1998; Cahir et al., 2007).

With the introduction of [^{11}C]DASB as a selective PET radioligand for SERT, reproducible quantification has become possible in multiple brain regions, even without arterial sampling (Houle et al., 2000a,b; Ginovart et al., 2001; Frankle et al., 2006; Kim et al., 2006). In this study we investigated the cerebral SERT binding using [^{11}C]DASB PET in a large group of humans representative of a Western population in terms of BMI.

Materials and methods

Participants and interviews

Sixty adult subjects (23 females, mean age 35.7 ± 18.2 years, and mean BMI 26.5 ± 5.9 kg/m^2) were included in the study. Seven of the subjects had BMI above 30 and were thus classified as obese. Written informed consent was obtained according to the declaration of Helsinki II, and the study had been approved by the Copenhagen Region Ethics Committee ((KF) 01-124/04, (KF) 01-156/04, and (KF) 01 2006-20, with amendments).

All subjects had a normal neurological examination and were lifetime naïve to antidepressants and antipsychotics. Nine-teen women were below the age of 55 years, their menstrual phase was not validated by ultrasound scanning of ovaries and consequently, menstrual phase was not considered in the analysis. Also, according to Jovanovic et al. (2009), there is no difference in cerebral SERT or 5-HT1A receptor binding between the follicular and late luteal phases. None of the subjects had stimulant abuse or history of neurological or psychiatric disorders. On the day of the PET scan, the subjects were screened for psychiatric symptoms using the Symptom Check List Revised (SCL-90-R) (Derogatis, 1994). None of the subjects were depressed according to the cutt-offs from Danish normative data (Olsen et al., 2006). The Danish version of the 240-item NEO PI-R self-report personality questionnaire (Hansen and Mortensen, 2004) was also filled out on the day of the PET scan. This questionnaire evaluates the personality dimensions of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness.

Subjects were interviewed about use of alcohol and tobacco smoking habits using an in-house made questionnaire. Based on their alcohol consumption, the subjects were divided into the following 3 categories: (1) subjects who drank maximum 2 units of alcohol per week (n = 15); (2) subjects who drank 3 to 9 units of alcohol per week (n = 24); 3) subjects who drank 10 or more units of alcohol per week (n = 21). With regard to tobacco smoking, the subjects were divided into a group of tobacco smokers (n = 12) and a group of non-smokers (n = 48).

PET imaging

PET scans were performed with an 18-ring GE-Advance scanner (General Electric, Milwaukee, WI, USA), operating in 3D acquisition mode, and producing 35 images slices with an interslice distance of 4.25 mm. Following a 10-min transmission scan, a dynamic 90-min-long emission recording was initiated after intravenous injection over 12 s of 475 ± 92 MBq (range: 246–601) [^{11}C]DASB with a specific radioactivity of 30 ± 16 GBq/μmol (range: 9–82). The emission recording consisted of 36 frames, increasing progressively in duration from 10 s to 10 min. The attenuation and decay corrected recordings were reconstructed by filtered back projection using a 6-mm Hanning filter.

MR imaging

MR imaging of the brain was acquired on a Siemens Magnetom Trio 3 T MR scanner with an eight-channel head coil (In vivo, FL, USA). High-resolution 3D T1-weighted, sagittal, magnetization prepared rapid gradient echo (MPRAGE) scan of the head and 2D T2-weighted, axial, Turbo Spin Echo (TSE) scans of the whole brain were acquired. Both T1 and T2 images were corrected for spatial distorsions due to non-linearity in the gradient system if the scanner (Jovicich et al., 2006) using the Gradient Non-Linearity Distorsion Correction software distributed by the Biomedical Informatics Research Network (http://www.nbirn.net). Subsequently, non-uniformity correction was performed with two iterations of the N3 program (Sled et al., 1998). The resulting T1 images were intensity normalized to a mean value of 1000.

To enable extraction of the PET Volume of Interest (VOI)-signal from gray matter voxels only, MR images were segmented into gray matter, white matter, and cerebrospinal fluid tissue classes using SPM2 (Welcome Department of Cognitive Neurology, University College London, UK) and the Hidden Markov Random Field (HMRF) model as implemented in the SPM2 VBM toolbox (http://dbm.neuro.uni-jena.de/vbm/). This was done for the subcortical high-binding region and for neocortex, but not for midbrain because the segmentation within this region is not considered reliable. Therefore all midbrain voxels were included in the analysis. A brain mask based on the gradient non-linearity corrected T2 image was created in order to assure exclusion of extra-cerebral tissue.

Data analysis

Movement correction and co-registration

To correct for movements during the [^{11}C]DASB PET scan, all frames from 10 to 36 were aligned using AIR 5.2.5 (Woods et al., 1992). The frames acquired for the first 2 min did not contain sufficient information to be reliably aligned. Before alignment, each frame was filtered with a 12-mm Gaussian filter and thresholded at the 80% fractile of the voxel count values in the image. These parameters were chosen by visual inspection of the thresholded images to ensure that they included the bran gray matter voxels. The rigid transformation was estimated for each frame to a selected single frame with enough structural information (frame 26: 20–25 min post injection) using the scaled least squares cost-function in AIR. Subsequently, single frames were resliced and converted to a dynamic Analyze image file format.

The [^{11}C]DASB image (based on an average of frame 10–36) was co-registered to the individual MR image using the AIR algorithm (Woods et al., 1992). The quality of each co-registration was assessed visually in three planes and adjusted when needed (this was needed in 3 cases).

VOI analysis

Volumes of interest (VOIs) were automatically delineated on each subject's MR image in a user-independent fashion with the Pvelab software package (freely available on www.nru.dk/downloads) (Svarer et al., 2005). For each of the 10-template VOI sets, a 12-parameter affine transformation and a warping field were calculated between the template MR image and the individual MR image for a

subject. Having obtained the MR/PET co-registration for the same individual as described above, the template VOI sets are then transferred to the dynamic PET image space for each subject, using the identified transformation parameters. From the VOI sets, a probability map was created for each subject and a common VOI set was generated for each individual subject. These VOI sets were then used for automatic extraction of gray matter time activity curves (TAC) for each of the VOIs. The TAC extracted for the cerebellum, excluding the cerebellar vermis (Kish et al., 2005), was used as the reference tissue input for kinetic modeling.

As a robust measure of cerebral SERT binding, a high-binding subcortical region consisting of 3 paired regions with high, homogenous binding (Houle et al., 2000a,b) was computed as the volume-weighted average of binding in caudate, putamen and thalamus. Together with the midbrain region and a neocortical region, this high-binding region served as the primary VOI. The neocortical region consisted of a volume-weighted average of the following 8 cortical regions: Orbitofrontal cortex, medial inferior frontal cortex, superior frontal cortex, superior temporal cortex, medial inferior temporal cortex, sensory motor cortex, parietal cortex, and occipital cortex. The delineation of all VOIs except the midbrain have been described previously (Svarer et al., 2005). Midbrain was defined in the ACPC plane (the plane of the anterior and posterior commissure) as the superior limit and the boarder between the inferior colliculi and the superior cerebellar peduncle as the inferior limit. In the 2–3 most superior slices where the peduncle is less well defined, only tegmentum and tectum were included in the region. Within the high-binding subcortical region and neocortex, the ratio between gray matter volume and the sum of the white plus gray matter volumes was computed.

Quantification of non-displaceable tracer uptake.

The outcome parameter from the [^{11}C]DASB-PET study is the non-displaceable binding potential, designated BP_{ND} (Innis et al., 2007). Cerebellum (as defined above) was used as a reference region, representing non-specific binding only. We used a modified reference tissue model designed specifically for quantification of [^{11}C]DASB (MRTM/MRTM2) as described and evaluated by Ichise et al. (2003) using the software PMOD version 2.9, build 2 (PMOD Technologies): A fixed washout constant, designated k2', was calculated for each individual as an average of k2 in caudate, putamen and thalamus relative to cerebellum using MRTM. Subsequently, k2' was inserted into MRTM2 and BP_{ND} was calculated for the VOIs relative to cerebellum.

Voxel-based analysis

The data was prepared for voxel-wise analysis by use of the SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm). MR images were spatially normalized to the MNI template in SPM8, and the estimated warp fields were applied to the DASB images. DASB images were smoothed with a12-mm Gaussian kernel prior to analysis. In order to control for structural differences and mis-registration across subject, we included GM segmentations in the analysis using the Biological Parametric Mapping software package (Casanova et al., 2007). Gray matter (GM) segmentations were smoothed with 8 mm Gaussian filter to approximate the smoothness of the DASB images. The GM segmentations was further warped into MNI space and smoothed with 12 mm Gaussian kernel. Inclusion of GM segmentations in the statistical modeling facilitates a correction for anatomical variability and mis-registration on a voxel-by-voxel basis. In the general linear model the dependent variable was DASB binding while age, gender, BMI, openness to experience daylight and GM were entered as covariates. Statistical significance was chosen at 0.05 Family Wise Error corrected. Non-significant covariates were eliminated sequentially.

Statistics

The association between BMI and SERT binding in the three VOIs was analyzed in a linear regression model with adjustment for age, gender, minutes of daylight on the scan date at the latitude of Copenhagen (http://aa.usno.navy.mil/data/docs/Dur_OneYear.php/), and openness to experience. Daylight was included in this full model because of its association with SERT binding (Praschak-Rieder et al., 2008). Subjects were scanned between fall 2005 and spring 2008, and the average daylight time on the day of PET scan was 683 (range: 422 to 1052) minutes. Openness to experience was included because of relation to SERT binding shown in 50 subjects overlapping with the individuals in the present study (Kalbitzer et al., 2009). Age has been previously been found negatively related to [^{11}C]DASB binding (Meyer et al., 2001; Frokjaer et al., 2009). In a subsequent analysis, model simplification was performed by backward elimination with cut-off at a p-value of 0.05. To examine if our primary observation was influenced by BMI-related differences in gray matter, we tested the linear association between the cortical and subcortical gray matter ratio and BMI. Variance homogeneity and normality were validated graphically. The linearity of quantitative variables was assessed by including second order terms in the models which in all cases were statistical non-significant. Finally, in a post-hoc analysis the association between SERT binding and tobacco smoking and alcohol consumption was evaluated in both the full and in the reduced model.

We expected a non-linear association between age and BMI and hence the inclusion of both predictors in a linear model should not cause severe problems with co-linearity. This was confirmed by examination of variance inflation factors as well as ridge regression estimates. Ridge regression is a penalized maximum likelihood method (L2 quadratic penalizing term) (Hoerl and Kennard, 1970). In the presence of severe multicollinearity the mean squared error of the ridge regression estimator is smaller compared to the maximum likelihood estimator. Only minor differences were detected between the ridge and maximum likelihood estimates.

Group-comparisons between normal-weighted (BMI of max 25 kg/m^2) and overweight (BMI of more than 25 kg/m^2) subjects were performed with a t-test. Comparisons included age, injected mass of cold ligand, specific activity of [^{11}C]DASB, openness to experience, daylight minutes, the area under the cerebellar time-activity curves normalized to the injected dose, and the reference tissue wash-out k2'.

A p-value<0.05 was considered statistically significant. p-Values, parameter estimates with standard errors (SE) and 95% confidence limits, and degrees of freedom (DF) are reported when appropriate. We used SAS software (SAS Institute Inc.) version 9.1.3 for statistical analysis.

Results

Fig. 1 shows the averaged parametric BP_{ND} image of the SERT binding. In the full linear model where the BMI effect on SERT binding was adjusted for age, gender, daylight minutes, and openness to experience, BMI correlated statistically significant negatively to SERT binding in neocortex (-0.003 BP_{ND} unit per kg/m^2 (SE: 0.001, 95% confidence limits: -0.005 to -0.001), $p=0.017$) and in the subcortical high-binding region (-0.009 BP_{ND} unit per kg/m^2 (SE: 0.004, 95% confidence limits: -0.018 to -0.0003), $p=0.042$). In midbrain, the inverse relationship was borderline significant only (-0.010 BP_{ND} unit per kg/m^2 (SE: 0.005, 95% confidence limits: -0.021 to 0.001), $p=0.066$).

The overweight individuals (BMI$>$25, $n=36$) did not differ from normal-weighted subjects (BMI$\leq$25, $n=24$), with regard to injected mass (69.9$\pm$34.7 vs. 79.5$\pm$40.7 ng per kg body weight, $p=0.349$), [^{11}C]DASB specific activity (29$\pm$15 vs. 31$\pm$17 GBq/μmol, $p=0.558$), openness score (113$\pm$19 vs. 120$\pm$18, $p=0.184$), number of daylight minutes on the day of the individual PET scan (700$\pm$236 vs. 657$\pm$233,

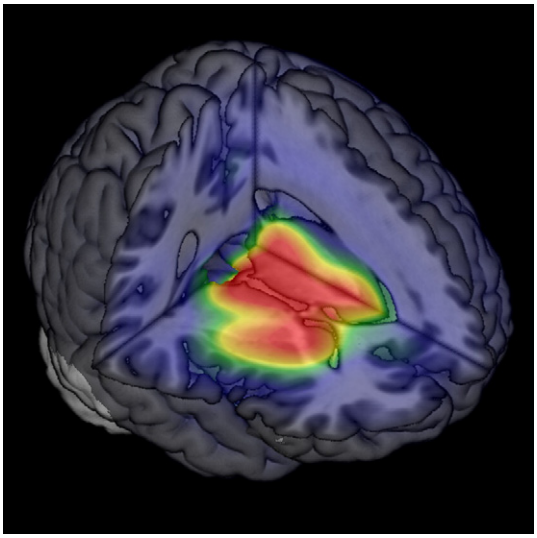

Fig. 1. Parametric image of averaged BP_{ND} values for all 60 subjects. Average BPnd values: thalamus 1.7; caudatus 1.4; putamen 1.7; midbrain 1.8, neocortex 0.2.

$p = 0.486$), area under the cerebellar time-activity curves normalized to the injected dose (136145 ± 29535 vs. 146157 ± 32961, $p = 0.236$), or in the reference tissue wash-out k2′ (0.054 ± 0.009 vs. 0.056 ± 0.008, $p = 0.433$). There was a tendency for overweight subjects to be slightly older than the normal-weighted controls (38.9 ± 19.0 vs. 31.0 ± 16.2 years, $p = 0.090$).

After backward elimination of parameters from the full linear model (see above) that did not contribute significantly to the model description, an even stronger inverse correlation between BMI and SERT binding was found in all three VOIs. A description of as well as the regional data from this backward regression analysis is presented in Table 1, and high-binding subcortical SERT BP_{ND} is plotted against BMI in Fig. 2.

For the subcortical high-binding region, there were no statistically significant interactions between age and BMI or between daylight minutes and BMI. In midbrain we found a main effect of gender on SERT binding, with females having a higher SERT binding. There were no significant interactions between BMI and openness, or between BMI and age, or BMI and gender. For neocortex, there was no significant interaction between age and BMI. BMI was categorized into 3 categories when looking for interactions with another continuous variable. No significant correlation was detected between BMI and grey matter ratio in any VOI.

No effect of tobacco smoking or alcohol consumption on SERT binding was found in any of the three VOIs. This was true both for the full model with inclusion of all co-variates, and for the reduced model.

Discussion

Overweight and obesity is a health threat of increasing concern and understanding the neurobiology behind obesity is instrumental to the development of effective treatment regimes. Some effective anti-obesity treatments encompass blockade of the SERT; yet, our study is the first to demonstrate an abnormally decreased cerebral SERT binding in obese individuals. In a sample of 60 healthy people, representative of a Western population in terms of BMI, we found a negative correlation between BMI and in vivo SERT binding in all three investigated brain regions.

A negative correlation between BMI and cerebral SERT binding has also been noted in a preliminary report (Matsumoto, 2008) based on 25 healthy humans. In contrast, twins with higher BMI were found to have higher SERT binding, as measured with [^{123}I]nor-β-CIT SPECT, than their monozygotic twin sibling with lower BMI (Koskela et al., 2008). In the latter study, however, BMI did not correlate with SERT binding in the entire group of 31 subjects and [^{123}I]nor-β-CIT is not considered an optimal radiotracer for measurements of SERT.

Table 1
The main effect on regional SERT binding of significant parameters after backward-elimination. For each VOI, the following regressors were all evaluated in a in a multiple linear regression model with SERT binding as the dependent variable: BMI, age, gender, minutes of daylight on the scan date at the latitude of Copenhagen, and openness to experience. Regressors that were not significantly contributing to the model (p-value>0.05) were eliminated one by one (the regressor with highest p-value first). The remaining significant regressors are presented for each VOI in the table.

Region	Estimate ± Standard Error	95% Confidence limits	p-Value
Caud–Put–Thal			
BMI	−0.008 ± 0.004 (BP_{nd} unit per kg/m^2)	−0.016 to −0.001	$p = 0.027$
Age	−0.003 ± 0.001 (BP_{nd} unit per year)	−0.006 to −0.001	$p = 0.006$
Daylight minutes	−0.0002 ± 0.00009 (BP_{nd} unit per min)	−0.0004 to −0.000001	$p = 0.048$
Midbrain			
BMI	−0.012 ± 0.005 (BP_{nd} unit per kg/m^2)	−0.022 to −0.001	$p = 0.027$
Age	−0.004 ± 0.001 (BP_{nd} unit per year)	−0.007 to −0.001	$p = 0.019$
Openness	−0.004 ± 0.002 (BP_{nd} unit per Op. unit)	−0.007 to −0.001	$p = 0.014$
Gender	−0.176 ± 0.059 (BP_{nd} unit; ref: female)	−0.294 to −0.057	$p = 0.004$
Global neocortex			
BMI	−0.003 ± 0.001 (BP_{nd} unit per kg/m^2)	−0.005 to −0.001	$p = 0.003$
Age	−0.001 ± 0.0003 (BP_{nd} unit per year)	−0.002 to −0.001	$p < 0.001$

The negative correlation between BMI and in vivo cerebral SERT binding could be due to genetic and/or environmental factors influencing primarily SERT or it could be caused by a primary change in 5-HT level with a secondary change in SERT. These options are considered below.

At the age of approximately 3 months, SERT knockout mice become obese Holmes et al., 2002; Warden et al., 2005; Murphy and Lesch, 2008). In addition, two recent publications have shown an association between obesity and the short allele of the SLC6A4 HTTLPR polymorphism (Sookoian et al., 2007) (Fuemmeler et al., 2008). Considering that molecular imaging studies have indicated that cerebral SERT binding is higher among homozygotic carriers of

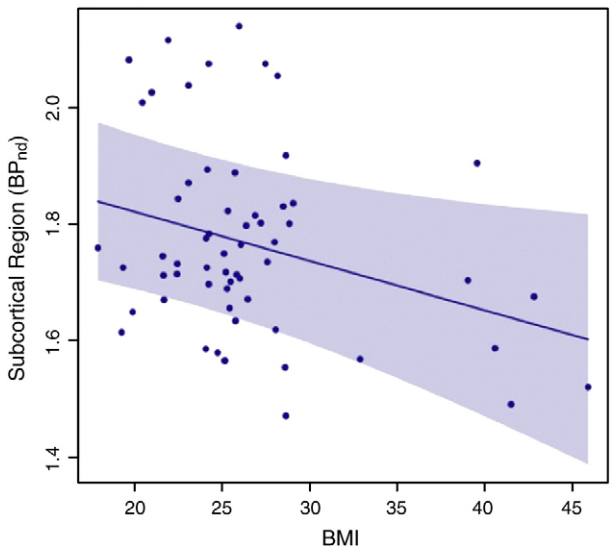

Fig. 2. Plot of BMI vs. subcortical high-binding (caudatus–putamen–thalamus) SERT binding. The plotted BP_{ND} values are the partial residuals with 95% pointwise confidence limits from the linear model with BMI, age (centered), and daylight minutes as co-variates.

the long 5-HTTLPR allele who also have the A variation at rs25531 (Praschak-Rieder et al., 2007; Reimold et al., 2007; Kalbitzer et al., 2009) then there is some support for genetic basis for our finding. Adaptive changes of the serotonergic neurotransmission to environmental factors during early development could also take place. For example, protein restriction during early development leads to an attenuation of the inhibitory action of 5-HT on food intake (Lopes de Souza et al., 2008).

It also is possible that our finding of an inverse relationship between cerebral SERT binding and BMI is mediated through genetically and/or environmentally determined interindividual differences in extracellular 5-HT levels. As mentioned above, manipulations that decrease brain 5-HT neurotransmission in animals lead to elevated self-administration of food whereas treatments that increase 5-HT levels induce satiety which subsequently lead to decreased food intake and weight loss (Saller and Stricker, 1976; Waldbillig et al., 1981; Blundell, 1984; Simansky, 1996; Leibowitz and Alexander, 1998). It has been shown that serotonergic acting agents, especially when injected directly into the hypothalamus, suppress carbohydrate consumption while having little or no effect on the ingestion of protein or fat (Leibowitz and Alexander, 1998). Likewise, carbohydrate ingestion leads to increased circulating levels of the 5-HT amino acid precursor, tryptophan (Fernstrom et al., 1975; Noach, 1994; Wurtman and Wurtman, 1995), as well as increased hypothalamic and raphe nuclei 5-HT (Leibowitz and Alexander, 1998). Thus, 5-HT as a feedback on eating, serves to terminate the meal and yield a state of satiety. As obese subjects tend to have a preference for carbohydrate-rich food (Weltzin et al., 1994; Wurtman and Wurtman, 1995) it is possible that these subjects have a disturbed 5-HT-mediated feedback. The observation of low cerebrospinal fluid levels of serotonin metabolites in women with primarily abdominal obesity (Strombom et al., 1996) and the demonstration of increased food intake in women suffering from bulimia nervosa after lowering brain 5-HT levels by tryptophan depletion support that also in humans, low cerebral synaptic 5-HT levels increases appetite and food intake (Weltzin et al., 1995; Smith et al., 1999). Since 5-HT levels cannot readily be determined in vivo in the human brain there are no clinical data available on the association between cerebral 5-HT levels and SERT binding. There is, however, evidence from animal studies to support that a decrease in cerebral 5-HT levels leads to a down-regulation of SERT density; chronic (i.e., after 2 weeks) 5-HT depletion in animals leads to downregulation of SERT binding (Rattray et al., 1996; Rothman et al., 2003) although one study found no change in SERT binding after 8 days of 5-HT depletion (Dewar et al., 1992). Our observation of a negative association between BMI and SERT binding concurs well with the previously observed positive association between BMI and 5-HT_{2A} receptor binding (Erritzoe et al., 2009), in that both associations could be jointly explained by lower brain 5-HT levels in individuals with a high BMI. Also, a primary (genetic or environmental) decrease in SERT would be more likely to elicit higher extracellular levels of 5-HT, which–as reviewed above–is thought to be associated with satiety.

No relationship between SERT binding and the degree of alcohol consumption was detected in our study of these non-alcoholic healthy subjects. The absence of heavy alcohol consumption in our sample may explain why we did not confirm the suggested dysregulation of 5-HT neurotransmission in alcohol abusers (Heinz et al., 2004; Feinn et al., 2005). Further, we did not detect any effect of smoking on SERT binding.

Potential confounds such as differences between overweight/obese and normal weighted subjects apart from body weight should be considered. In theory, a false-negative correlation could occur if subjects with high BMI had higher non-specific binding than those with low BMI. However, as a proxy for non-specific binding, neither the area under the cerebellar time-activity curves normalized to the injected dose nor reference tissue wash-out, k2, differed between groups indicating that differences in non-specific binding did not explain group differences in specific SERT binding. A group difference in injected mass could potentially also influence the outcome; if more cold mass was administered to the overweight/obese subjects a larger fraction of the transporters would be blocked yielding a false low BP_{ND} within these subjects. The injected mass did not differ between the groups and thus could not explain the finding. Also, there were no group-differences between openness to experience or number of daylight minutes on the day of the PET scan and none of these parameters interacted with BMI in the linear model.

Our study has some limitations. First, when reporting SERT binding from cortical areas with [^{11}C]DASB PET it should be emphasized that because of the relatively low SERT binding in these areas the interindividual variability is high and the signal to noise ratio is low. However, in a test–retest study using the same method as used in our study ([^{11}C]DASB-PET and MRTM2), except for longer scan time, a high reliability of cortical [^{11}C]DASB binding was found (temporal 0.82, occipital 0.85, and frontal 0.55)(Kim et al., 2006). Secondly, because we conducted comparisons between BMI and three different brain regions a multiple comparison correction should be considered. In the reduced model (data presented in Table 1), only the relationship between BMI and neocortical BP_{ND} (corrected $p = 0.009$) survives the correction (corrected $p = 0.81$ in the two other ROIs). A Bonferroni correction is, however, regarded as too conservative, since the measures are not independent (Erritzoe et al., 2010). Finally, the binding potential was included as the dependent variable in the statistical analysis. In the present study, as well as in our prior analysis of 5-HT_{2A} receptor binding (Adams et al., 2004; Erritzoe et al., 2009) we chose the same approach. One could argue, however, that the causality between BMI and serotonergic neurotransmission should be reverted and that a more meaningful model would therefore include BMI as the dependent variable. When we analyzed our data with a linear regression model with adjustment for age and gender and with BMI as explained by SERT binding, we confirmed a statistically significant negative association in neocortex and the averaged caudate–putamen–thalamus region, and a trend for a negative association in midbrain. However, with this approach, the potential feedback mechanism from changes in eating behaviour and body weight on the 5-HT neurotransmission is disregarded.

Conclusion

We found an inverse correlation between cerebral SERT binding and BMI. Whether the SERT has a direct role in the regulation of appetite and eating behaviour or whether the finding is due to a compensatory downregulation of the transporter secondary to other dysfunction(s) in the serotonergic transmitter system, such as low baseline serotonin levels, is not yet established. To further address the causality, exploration in a longitudinal set-up with intervention would be needed; e.g., to study the effect on brain serotonergic markers in response to a substantial weight loss.

Disclosure/conflict of interest statement

The authors declare that, except for income received from primary employers, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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