Basal ganglia morphology links the metabolic syndrome and depressive symptoms ☆

Ikechukwu C. Onyewuenyi [a], Matthew F. Muldoon [b], Israel C. Christie [c], Kirk I. Erickson [a], Peter J. Gianaros [a,*]

[a] Department of Psychology, University of Pittsburgh, 3131 Sennott Square, 210 South Bouquet Street, Pittsburgh, PA 15260, United States
[b] Heart & Vascular Institute, University of Pittsburgh School of Medicine, Old Engineering Hall, Room 506, Pittsburgh, PA 15260, United States
[c] MD Anderson Cancer Center, The University of Texas, 1515 Holcombe Blvd., Houston, TX 77030, United States

HIGHLIGHTS

• Pallidal volume associated with the metabolic syndrome and depressive symptoms
• The metabolic syndrome associated with depressive symptoms
• Pallidal volume mediated this association

ARTICLE INFO

ABSTRACT

The metabolic syndrome (MetS) is a clustering of cardiovascular and cerebrovascular risk factors that are often comorbid with depressive symptoms. Individual components of the MetS also covary with the morphology of basal ganglia regions that are altered by depression. However, it remains unknown whether the covariation between the MetS and depressive symptomatology can be accounted for in part by morphological changes in the basal ganglia. Accordingly, we tested the hypothesis that increased depressive symptoms among individuals with the MetS might be statistically mediated by reduced gray matter volume in basal ganglia regions. The presence of the MetS was determined in 147 middle-aged adults using the criteria of the National Cholesterol Education Program, Adult Treatment Panel III. Basal ganglia volumes were determined on an a priori basis by automated segmentation of high-resolution magnetic resonance images. Depressive symptoms were assessed using the Patient Health Questionnaire. Even after controlling for demographic and other confounding factors, having the MetS and meeting more MetS criteria covaried with reduced globus pallidus volume. Meeting more MetS criteria and reduced pallidal volume were also related to depressive symptoms. Moreover, the MetS-depression association was statistically mediated by pallidal volume. In summary, reduced globus pallidus volume is a neural correlate of the MetS that may partly account for its association with depressive symptoms.

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1. Introduction

The metabolic syndrome (MetS) refers to a clustering of cardiovascular and cerebrovascular risk factors within the same individual. These risk factors include atherogenic dyslipidemia, elevated blood pressure, central adiposity, hypertriglyceridemia, and glucose dysregulation. Importantly, the MetS confers risk for cardiovascular and cerebrovascular disease morbidity and mortality more so than the risk conferred by any of its individual components [1–7]. The MetS also confers risk for type 2 diabetes mellitus, as well as associated impairments in cognitive function and adverse mental health outcomes, particularly dysphoric mood and major depressive disorder [8–10]. As reviewed below, several lines of evidence suggest that the MetS may be linked to risk for these diverse health outcomes via alterations in brain systems that are jointly involved in energy balance, metabolism, cardiovascular regulation, cognitive processes, and emotional functions that support hedonic or appetitive behaviors [11–14]. At present, however, the specific brain systems that may plausibly link the MetS to often comorbid health outcomes are incompletely understood [15–17]. Accordingly, a primary goal of the present study was to examine whether the basal ganglia may represent one such system, as there is cumulative evidence showing that aspects of basal ganglia morphology in particular relate to individual components and clinical sequelae of the MetS [18–20], as well as to comorbid health conditions that are specifically characterized by depressive symptomatology [21].

* Corresponding author. Tel.: +1 412 383 5078; fax: +1 412 383 5458.
E-mail address: gianaros@pitt.edu (P.J. Gianaros).

To elaborate, a growing number of structural neuroimaging studies show that individuals who exhibit features and correlates of the MetS also exhibit morphological changes in the basal ganglia, particularly reduced tissue volumes. For example, increased adiposity among otherwise healthy individuals has been linked to volumetric reductions in the putamen and globus pallidus of the basal ganglia [18,20]. In extension, individuals with type 2 diabetes mellitus – a condition that is often consequent to the MetS – also exhibit reduced volumes in the caudate, putamen, and globus pallidus [18]. Recent work has shown further that glucose dysregulation covaries with reduced volume in striatal and pallidal regions [19]. Together, these findings indicate that components of the MetS (e.g., adiposity, glucose dysregulation) and endpoints of the MetS (e.g., type 2 diabetes) associate with morphological, particularly volumetric, changes in basal ganglia regions. However, despite such prior associations, there are two questions that still remain open: (i) does the MetS itself, as opposed to its individual components, relate to reduced volumes within the basal ganglia, particularly among people without a clinical condition (e.g., type 2 diabetes)? And (ii), does meeting more MetS criteria – above and beyond its adiposity and glucose dysregulation components – relate to reduced volumes within the basal ganglia in a graded fashion? Findings bearing on these questions would help to address the issue of whether there is a neural (volumetric) correlate of the MetS that is not confounded by the presence of a clinical disorder, which would have further implications for understanding associated variation in and risk for mental health symptoms and health conditions that are often comorbid with this syndrome.

More precisely, and as noted above, the neural correlates of the MetS that may in turn relate to risk for comorbid conditions, particularly those associated with depressive symptoms, remain incompletely understood. This is despite prior speculations and indirect evidence that the MetS and depression may share common pathophysiological features within the brain [9], with structural or morphological changes in the basal ganglia being one specific and possibly shared feature [22]. In support of this possibility, reduced tissue volumes within the basal ganglia have been associated with major depression in postmortem samples [23,24]. Moreover, previous magnetic resonance imaging (MRI) studies on patients with major depressive disorder (MDD) have reported volumetric reductions in striatal and pallidal regions [21,25–28]. Secondly, case study evidence indicates that selective bilateral lesions to the globus pallidus result in depressive symptoms and concurrent weight gain [29]. In view of such findings and prior work relating individual MetS components and its clinical consequents to reduced basal ganglia volumes [18–20], it appears plausible that structural or morphological aspects of the basal ganglia may relate to the covariation between the MetS and depressive symptoms.

Accordingly, the present study examined the structural neural correlates of the MetS, with an a priori focus on anatomically defined regions of interest (ROIs) in the basal ganglia. Hence, based on prior associations between MetS components and basal ganglia volumes [18–20], we predicted that otherwise healthy individuals with the MetS, as well as those meeting more MetS criteria, would show reduced gray matter volume in basal ganglia ROIs, including the striatum (nucleus accumbens, putamen, caudate nucleus) and the globus pallidus, beyond the variance accounted for by demographic and confounding health-related factors. In addition, insofar as the MetS often exhibits comorbidity with depression [9], we next examined whether the presence of the MetS and meeting more MetS criteria would covary with depressive symptomatology. We predicted that greater depressive symptoms would be observed in individuals with the MetS relative to those without the syndrome, and among individuals meeting an increasing number of MetS criteria. Lastly, we explored the possibility that volumetric changes in basal ganglia regions might account for (statistically mediate) any observed association between the MetS and depressive symptoms.

2. Materials and methods

2.1. Participants

Participants were 155 otherwise healthy adults (78 men, 77 women; mean age, 40.7 ± 6.2 SD, range $= 30$–50 years) who were recruited by mass mailings to residents of Allegheny County, PA, USA. Inclusion criteria included no history of (1) cardiovascular disease (including treatment for or diagnoses of hypertension, stroke, myocardial infarction, congestive heart failure, and atrial or ventricular arrhythmias); (2) prior neuro-surgery or neurological disorder; (3) any current treatment for or self-reported psychiatric disorder; (4) typical consumption of more than 15 alcoholic beverages per week; (5) daily use of corticosteroid inhaler; (6) current use of psychotropic, lipid lowering, or any cardiovascular medication, including any medication to control blood pressure; (7) metal implants or exposure; (8) colorblindness; and (9) claustrophobia. All participants demonstrated right-handedness, as assessed by the Edinburgh Handedness Inventory [30]. Women were excluded if pregnant (as verified by urine test).

The ethnicity of the sample was Caucasian/White (70.3%), African American/Black (21.9%), Asian (5.8%), and multiracial or other (1.9%). We have previously reported on the functional neural correlates of stressor-evoked baroreflex suppression [31] and insulin resistance [32] among a subset of participants from this study. All participants provided informed consent prior to completing study protocols, which were approved by the University of Pittsburgh Institutional Review Board.

All analyses were based on a final sample size of 147 participants (76 men, 71 women). This reduced sample size was due to missing variables needed to compute MetS criteria (n = 3), missing or poor quality structural neuroimaging data (n = 4), and a score on the Patient Health Questionnaire (PHQ-9) for one participant that was 3 SD above the mean of the sample (see below). These excluded participants did not differ from the remaining sample in age, sex, metabolic factors studied here or in basal ganglia ROI volumes.

2.2. Assessment of the MetS and its components

Participants arrived for a neuroimaging protocol between 7:00 AM and 11:00 AM, after an 8-hour fast. This also included abstaining from exercising and consuming caffeine, alcohol, and tobacco products. Prior to neuroimaging, participants' seated, resting BP was measured from the non-dominant arm with an oscillometric device (Critikon Dinamap 8100, Johnson & Johnson, Tampa, FL). Participants provided 3 BP measures taken 2 min apart after a ~20 min acclimation period, with the average of the last 2 of the 3 BP readings serving as the resting systolic (SBP) and diastolic (DBP) blood pressures. Participants' waist circumference (in inches) was measured at the level of the umbilicus to the nearest 1/2 in at end expiration. After each participant's height, weight, and waist circumference were measured, a research nurse performed blood draws. Procedures to determine fasting serum lipids and glucose levels were performed in the Department of Primary Care Laboratory Services (Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center). Concentrations of total cholesterol and triglycerides were measured by a CHOL and triglyceride GPO reagent, respectively, using an enzymatic, timed-endpoint method on the SYNCHRON LX System (Beckman Coulter, Inc., Brea, California; [33–37]. The concentration of high-density lipoprotein (HDL) cholesterol was measured with a HDLD reagent on the SYNCHRON LX System, which uses an enzymatic, time-endpoint method to uniquely facilitate a detergent that solubilizes only the HDL lipoprotein particles (Beckman Coulter, Inc., Brea, California; [33,36]). Low-density lipoprotein (LDL) cholesterol concentrations were estimated by using the Friedewald calculation [38].

The MetS was defined by the criteria of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III [39,40]. According to NCEP criteria, the MetS is defined as the presence of three or more

of the following: (1) serum triglycerides ≥ 150 mg/dL; (2) fasting serum glucose ≥ 110 mg/dL; (3) waist circumference ≥ 102 cm in men or ≥ 88 cm in women; (4) SBP ≥ 130 or DBP ≥ 85 mm Hg; (5) serum HDL cholesterol < 40 mg/dL in men or < 50 mg/dL in women. Given our screening criteria, we adopted a conservative NCEP guideline such that use of medication for hypertension or hyperglycemia was not factored into defining the presence of the MetS in this cohort. This conservative definition of the MetS ensured that presence of disease or medication use did not confound our results.

2.3. Magnetic resonance imaging

Imaging data were acquired on a 3Tesla Trio TIM whole-body scanner (Siemens, Erlangen, Germany), equipped with a 12-channel phased-array head coil. For each participant, we obtained T_1-weighted magnetization-prepared rapid gradient echo (MPRAGE) neuroanatomical images over 7 min 17 s by these parameters: field of view = 256×208 mm, matrix size = 256×208 mm, time to repetition = 2100 ms, time-to-inversion = 1100 ms, time to echo = 3.29 ms, and flip angle = $8°$ (192 slices, 1 mm thick, no gap). MPRAGE images were used to derive volumetric measures described below.

2.3.1. Volumetric processing

For the a priori segmentation and volumetric analysis of the left and right basal ganglia ROIs in line with study hypotheses (i.e., caudate nucleus, putamen, nucleus accumbens, and globus pallidus), we used the Oxford University Centre for Functional MRI of the Brain (FMRIB) Integrated Registration and Segmentation Tool (FIRST) in the FMRIB Software Library (FSL) version 4.0. FIRST is a semi-automated model-based subcortical segmentation tool that relies on a Bayesian framework, as well as shape and appearance models obtained from manually segmented images provided by the Center for Morphometric Analysis, Massachusetts General Hospital (Boston, MA). Volumetric labels are parameterized by a three-dimensional deformation of a surface model based on multivariate Gaussian assumptions. Specifically, FIRST searches through linear combinations of shape modes of variation for the most probable shape given the intensity distribution in the T_1-weighted image (for a more detailed description of this method, see [41,42]).

This volumetric processing method runs a two-stage affine registration to a standard space template (Montreal Neurological Institute space) with 1 mm resolution using 12 degrees of freedom and a subcortical mask to exclude voxels outside of subcortical regions. Second, the caudate nucleus, putamen, nucleus accumbens, and globus pallidus were segmented with 30, 40, 50, and 40 modes of variation, respectively. Modes of variation were optimized based on a leave-one-out cross-validation using the training set [41]. Finally, boundary correction was implemented for each structure to classify boundary voxels as belonging to the structure or not using a statistical probability threshold (z score > 3.00; $p < 0.001$). The volume for each structure was then measured in mm^3. Segmentations from each participant were visibly checked for any significant errors that could have occurred during the segmentation process (no errors were noted). The correlations between left and right hemisphere basal ganglia volumes across the entire sample were strong (rs ranged from 0.70 to 0.90, $ps < 0.001$). Hence, for volumetric analyses, we used the average of participants' bilateral basal ganglia ROI volumes to reduce the number of statistical tests and because no laterality was hypothesized regarding the associations between the MetS, basal ganglia structures, and depression. Lastly, total brain volume was determined for each subject by calculating the sum of gray, white, and cerebrospinal fluid using the automated segmentation toolbox in FSL version 4.0. All volume measures (i.e., basal ganglia ROIs, total brain volume) were standardized (z-score transformed) to aid interpretation. All normalized basal ganglia ROI volume values were normally distributed. Supplemental Fig. 1 illustrates the basal ganglia ROIs.

2.4. Assessment of depressive symptoms

Depressive symptoms were measured with the 9-item Patient Health Questionnaire (PHQ-9). The questionnaire was designed to aid in screening for affective disorders in primary care settings [43]. The PHQ-9 compares favorably with structured diagnostic interviews, particularly for major depressive disorder, (MDD) in both clinical populations as well as those with coronary heart disease [44–46]. This is in part due to the fact that each item on PHQ-9 assesses the frequency of depressive symptoms as per the 9 diagnostic criteria for MDD in *Diagnostic and Statistic Manual of Mental Disorder Fourth Edition* (DSM-IV). The PHQ-9 also demonstrates acceptable reliability and validity as a measure of depression severity [45]. In the present study, the PHQ-9 displayed acceptable internal consistency ($\alpha = 0.64$).[1]

Participants reported the frequency of experiencing 9 depressive symptoms during the past two weeks, ranging from "not at all" (scored 0), "several days" (scored 1), "more than half the days" (scored 2), and "nearly every day" scored 3. Thus, possible scores on the PHQ-9 were 0–27. For our sample, PHQ-9 scores ranged from 0–6, which suggests mild depressive symptoms on average (see Table 1). This is consistent with our screening criteria, which excluded subjects with reported histories or current experiences of mood related disorders. Based on a recommended and established PHQ-9 cut-off score ≥ 10, we excluded one participant (PHQ-9 = 13) in the distribution from all analyses for possible major depression [45,46] as well as being more than 3 SDs above the group mean. There was another PHQ-9 outlier score that was 3 SDs above the group mean (PHQ-9 = 9); however, this score did not exceed the established clinical PHQ-9 cut-off score cited above. Hence, this score was winsorized by transforming the data point to 3 SDs from the group mean. This transform improved the normality of the PHQ-9 distribution, enabling parametric testing.

2.4.1. Long-term test-retest stability of PHQ-9

Test-retest assessment of the PHQ-9 was performed on a subset of participants (n = 94) from the 155 participants who completed the PHQ-9 on two occasions, separated by a ~3 year delay. These reliability analyses were performed to determine the stability of PHQ-9. We found that PHQ-9 scores at Time 1 and Time 2 were significantly correlated ($r = 0.50$, $p < 0.01$), indicating a moderately stable dimension of individual difference assessed by this depression questionnaire. Additional analyses determined the stability of the PHQ-9 using SPSS 20 to compute the intra-class correlation coefficient (ICC) with a two-factor mixed-effects model and type consistency. The PHQ-9 showed moderate consistency over the 3 years (ICC = 0.59, $p < 0.01$), which further suggests the long-term stability of scores on this questionnaire.

2.5. Assessment of covariates

Associations between the MetS, basal ganglia volumes, and depressive symptoms may be accounted for by (or confounded with) several factors, including age, sex, race (non-white versus white), years of education, and smoking status (i.e., categorically defined as 0 = non-smoker, 1 = smoker). These factors were treated as covariates. Of note, all covariates were chosen a priori based on existing research implicating them in association with the MetS, basal ganglia morphology, or psychological wellbeing. First, the prevalence of the MetS among U.S. adults is known to vary by age, sex, and race [47]. Second, education and smoking habits have been associated with the presence of the MetS [48,49]. Third, consistent with the age-, sex-, and smoking-related differences in the

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[1] The alpha coefficient of the PHQ-9 is important to consider when interpreting our findings among an otherwise healthy sample. Hence, the magnitude of this scale's internal consistency can be expected to influence the magnitude of any observed covariation between depressive symptoms and the MetS or basal ganglia volumes. However, we note that significant associations were still observed here, despite the moderate value of this alpha coefficient of the PHQ-9.

Table 1
Descriptive characteristics of the sample.

	Full sample (n = 147)	Individuals meeting NCEP MetS criteria (n = 24)	Individuals not meeting NCEP MetS criteria (n = 123)
Age, yrs	40.5 ± 0.5	40.8 ± 1.3	40.5 ± 0.6
Sex, m/f	76/71	12/12	64/59
Smoking status,			
never/former/current	97/28/22	14/4/6	83/24/16
Education, yrs	17.2 ± 0.3	16.8 ± 0.6	17.3 ± 0.3
Systolic BP, mm Hg[*]	120.1 ± 0.8	128.3 ± 1.8	119.5 ± 0.9
Diastolic BP, mm Hg[*]	73.4 ± 0.8	77.9 ± 2.0	72.5 ± 0.8
BMI, kg/m^2[*]	27.1 ± 0.4	32.1 ± 0.9	26.1 ± 0.4
Waist, in[*]	35.6 ± 0.4	41.2 ± 0.6	34.5 ± 0.4
Hip, in[*]	42.1 ± 0.5	45.2 ± 0.7	41.5 ± 0.5
Triglycerides, mg/dL[a][*]	74.0 (63.0)	147.0 (142.0)	70.0 (41.0)
HDL cholesterol, mg/dL[*]	49.6 ± 1.4	37.1 ± 1.1	52.0 ± 1.5
Glucose, mg/dL[a][*]	87 (12.0)	96.0 (23.0)	86.0 (12.0)
Verbal IQ	113.8 ± 0.7	112.2 ± 2.0	114.2 ± 0.8
PHQ-9, total score	1.0 ± 0.1	1.3 ± 0.4	0.9 ± 0.1
Basal ganglia gray matter volumes, mm^3			
Caudate nucleus	6950.1 ± 69.7	6748.1 ± 160.6	6989.5 ± 76.9
Putamen	9360.3 ± 85.8	9162.3 ± 260.9	9398.9 ± 89.1
Nucleus accumbens	860.0 ± 16.0	807.6 ± 40.41	870.3 ± 17.3
Globus pallidus[*]	3264.5 ± 28.3	3130.7 ± 71.1	3290.6 ± 30.4
Total brain tissue volume, mm^3 (gray, white, cerebrospinal fluid)	1,429,104.0 ± 11,545.0	1,424,036.1 ± 32,289.1	1,430,092.6 ± 12,338.1

Note. NCEP = National Cholesterol Education Program; BP = blood pressure; mm Hg = millimeters of mercury; BMI = body mass index; HDL = high-density lipoprotein; PHQ-9 = 9-item Patient Health Questionnaire; Verbal IQ = Estimated Verbal IQ score from the North American Adult Reading Test (Blair and Spreen, 1989).
Values are mean ± standard error of the mean, unless indicated otherwise.
[a] Median (interquartile range).
[*] Significant group difference between presence and absence of the MetS, $p < 0.05$.

MetS, basal ganglia areas are susceptible to gray matter changes that vary by sex and age [50,51] in addition to smoking relapse [52]. Finally, depressive symptoms show sex, race, and age differences [53–55].

2.6. Statistical analyses

2.6.1. Demographic and cardio-metabolic variables

We used descriptive statistics to characterize participants with and without the MetS on age, years of education, smoking status, body mass index (BMI), and MetS components. Specifically, differences in demographic and cardio-metabolic risk factors as a function of presence of the MetS were analyzed using *t*-tests for continuous variables and chi-square test for categorical variables (see Table 1).

2.6.2. Volumetric variables

Several regression models were used to determine the effect of the MetS on basal ganglia volumes, adjusting for age, sex, race, education, smoking status, and total brain volume as covariates. The relationships between the MetS and volumes in basal ganglia ROIs were assessed first by regressing a variable coding the presence/absence of the MetS on standardized (mean = 0; standard deviation = 1) average bilateral volumes for the nucleus accumbens, caudate nucleus, putamen, and globus pallidus in a single logistic regression model. Accordingly, the results reflect unique relationships between the MetS and basal ganglia volumes in a single model, as opposed to relationships revealed in separate models for each basal ganglia ROI, which would inflate type I error. We also conducted an additional logistic regression model whereby the presence/absence of the MetS was based on individuals meeting criteria for MetS (i.e., 3 or more criteria) that were compared against a subset of individuals meeting no criteria for any MetS component. Next, we examined associations between basal ganglia volumes and the number of MetS criteria met across the full sample of 147 participants in a similarly structured negative binomial model — regressing the number of MetS criteria met (i.e., criteria counts) on basal ganglia volumes. Hence, for these negative binomial models, the number of MetS criteria met (ranging from 0–5) was regressed on basal ganglia

brain volumes together in one model with covariate adjustment. We also regressed a modified number of MetS criteria met (ranging from 0–3+) variable on basal ganglia volume to ensure that the few individuals that met criteria for 4 or 5 components did not influence the results.

Exploratory logistic regression analyses were also performed on the individual components of the MetS (i.e., low HDL cholesterol or elevated blood pressure, plasma glucose, triglycerides, or waist circumference) by regressing the presence/absence of each MetS component on basal ganglia volumes to determine which components were most closely related to basal ganglia volumes. Similar exploratory analyses with linear regression models were also performed on continuous measures of each syndrome component. Logarithmic transformations were applied to triglyceride values to correct distributional skew, and outliers (i.e., 3 SD above or below the respective means) were removed from blood glucose (n = 4) and HDL cholesterol distributions (n = 1).

An alpha of 0.05 was used for all tests of statistical significance, while an alpha within the range of 0.06–0.10 was taken as a trend. To aid interpretation and clearly convey effect sizes, positive logistic regression coefficients are presented alongside odds ratios (OR; the exponentiated regression coefficient, e^B), and negative logistic regression coefficients alongside inverse odds ratios (IOR; the inverse of the exponentiated regression coefficient, $1/e^B$). Negative binomial results are presented alongside the exponentiated regression coefficients, and these can be interpreted as the change in number of criteria met per unit change in predictor of interest. All analyses were conducted using SPSS version 20.

2.6.3. Depression variables

Similar regression models were used to test whether depressive symptoms were associated with the MetS (i.e., presence/absence, number of criteria met). Hence, logistic and negative binomial regression models were used to predict the presence/absence of the MetS and the number of criteria (i.e., 0–5, 0–3+) as a function of depressive symptoms, adjusting for age, sex, race, smoking, and education. In light of prior reports linking MDD and depressive symptoms to altered basal ganglia morphology [21,23–29], we also examined the relationship
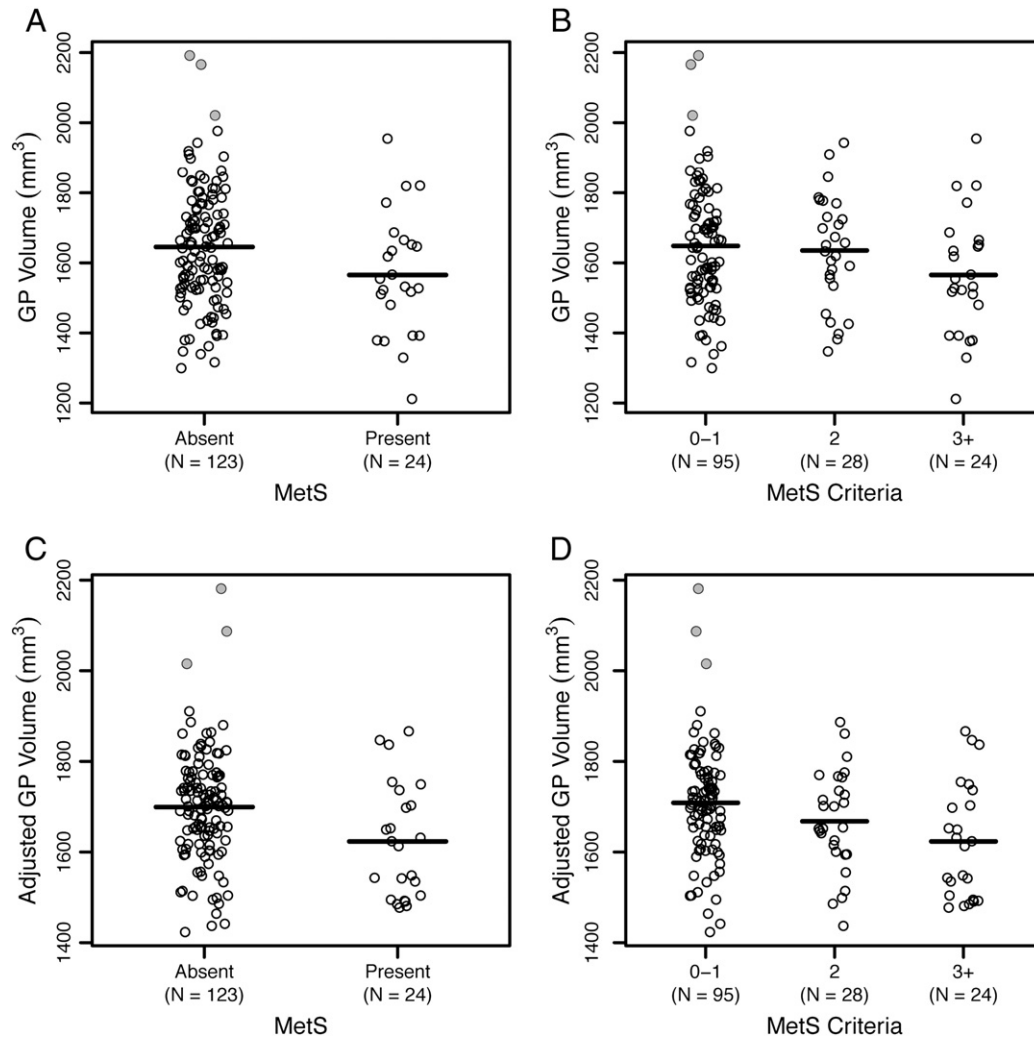
Fig. 1. Panels A and B plot unadjusted globus pallidus volume as a function of absence or presence of the metabolic syndrome and the number of metabolic syndrome criteria met. Panels C and D plot globus pallidus volumes that are adjusted for model covariates (age, gender, race, smoking status, and education, as well as gray matter, white matter and cerebral spinal fluid volumes). Significant group differences in globus pallidus volume were explained by individuals with the metabolic syndrome showing smaller pallidal volume. The shaded data points are the three outliers identified across both the pallidal and putamen volumes.

between depressive symptoms and each basal ganglia ROI in separate linear regression models, whereby PHQ-9 scores were regressed on basal ganglia ROI volumes with covariate adjustment.

2.6.4. Exploratory mediation

To test whether basal ganglia volumes might meditate observed associations between the MetS and depressive symptoms, we used the product-of-coefficients strategy that tests the significance of the indirect effect (i.e., the path through a proposed mediator) by bootstrapping the product of the effect of the independent variable on the mediator (path a), and the effect of the mediator on the dependent variable accounting for the effect of the independent variable (path b). However, the sampling distribution of the indirect effect that is determined through the ab product of coefficients is subject to violations of normality when the possibility of mediation is present [56]. Hence, Preacher and Hayes [57] recommend using a bootstrapping approach whereby 5000 estimates of ab are used to calculate confidence intervals (CIs) and standard errors around the indirect effect. This bootstrapped mediation analysis approach was performed with the *INDIRECT macro* designed for SPSS [57].

In these analyses, we computed two mediation models with the INDIRECT macro for each operational definition of the MetS under study (i.e., presence/absence, number of criteria met). The presence of the MetS or the number of MetS criteria met served as independent variables in separate models, depressive symptoms as measured
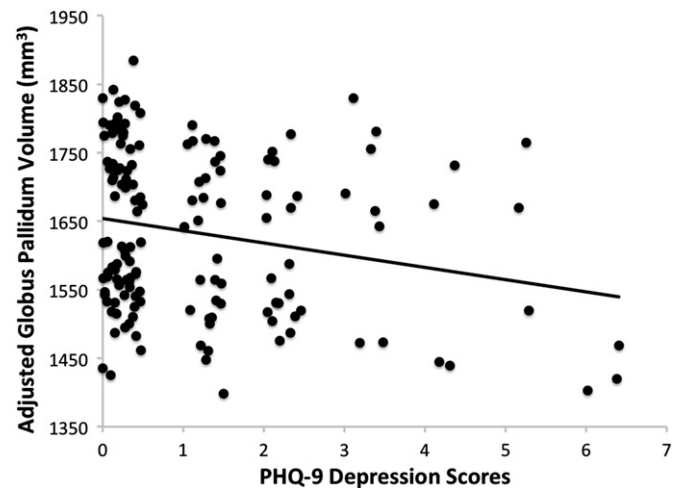

Fig. 2. Scatterplot of the association between the volume of globus pallidus (adjusted for age, gender, race, smoking status, education, and total brain tissue volume) and PHQ-9 depression scores.
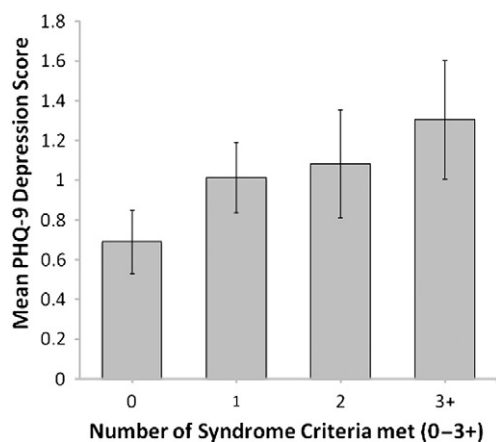
Fig. 3. The association between number of metabolic syndrome (MetS) components and PHQ-9 depression score. Subjects with three, four, and five MetS components were combined.

by the PHQ-9 served as the dependent variable, and basal ganglia volume(s) served as mediators. Age, sex, education, smoking status, race, and total brain volume were included as confounding covariates. Specific to modeling the indirect effect for number of criteria met, we first constructed k-1 dummy variables, with k being the number of possible criteria met. To model the indirect effect for number of criteria met, we first constructed k-1 dummy variables, with k being the number of possible number of criteria met. Next, each dummy variable was entered as an independent variable while the others were entered as covariates. This provided a test of the indirect effect of each category relative to reference category (i.e., meeting zero MetS criteria) in our dummy-coding scheme.

3. Results

3.1. Prevalence of MetS

There were 24 participants who met criteria for having the MetS and 123 subjects who did not. Thus, the prevalence of the MetS in this otherwise healthy and un-medicated sample was 16.3%, which is consistent with expected rates of the MetS demonstrated in national samples and similar community samples recruited from Allegheny County, Pennsylvania [49,58,59]. Table 1 shows descriptive statistics for the participants' demographic and other characteristics.

Among the 24 *with* the MetS, 18 met three criteria, 4 met four criteria, and 2 met five criteria. Further, among these individuals meeting criteria for the MetS, the following frequencies were observed across syndrome components: low HDL cholesterol ($n = 23$), elevated waist circumference ($n = 21$), elevated systolic ($n = 13$) and diastolic ($n = 6$) blood pressure, elevated triglycerides ($n = 12$), and elevated fasting glucose ($n = 10$). Among the 123 *without* the MetS, 51 met zero criteria, 44 met one criterion, and 28 met two criteria. The following frequencies were observed across syndrome components among those who were *without* the MetS: low HDL cholesterol ($n = 40$), elevated waist circumference ($n = 25$), elevated systolic ($n = 15$) and diastolic ($n = 12$) blood pressure, elevated triglycerides ($n = 7$), and elevated fasting glucose ($n = 7$).

3.2. MetS and basal ganglia volume

The presence of the MetS was associated with reduced gray matter volume within the basal ganglia. Hence, after accounting for demographic and health-related variables, reduced gray matter volume in the globus pallidus in particular was associated with an increased odds of having the MetS ($B = -0.83$, $SE = 0.37$, $IOR = 2.30$, $p = 0.03$). Specifically, a decrease of one standard deviation in globus pallidus volume was

associated with 2.30 times increased odds of meeting criteria for the MetS. Because more than half of the individuals *without* the MetS met criteria for some MetS components (i.e., 72/123 met criteria for one or two components; see Section 3.1), we ran an additional logistic regression model comparing individuals *with* the MetS to a subset of individuals who did not meet criteria for any MetS component. The group differences were still present, such that reduced pallidal gray matter volume was specifically associated with a greater odds of having the MetS ($B = -0.93$, $SE = 0.48$, $IOR = 2.54$, $p = 0.05$). Similarly, negative binomial models revealed that only globus pallidus volume covaried inversely with the number of MetS criteria met while simultaneously accounting for all other basal ganglia ROI volumes ($B = -0.27$, $SE = 0.11$, $e^B = 0.76$, $p = 0.02$), indicating that one standard deviation decrease in globus pallidus volume was associated with 76% increase in the number of MetS criterion met.[2] By contrast, neither the presence of the MetS nor the number of MetS criteria met covaried positively or negatively with the volume of other basal ganglia ROIs; namely, the caudate, putamen, and nucleus accumbens ($ps > 0.34$). Fig. 1 shows the associations of pallidal volume to the presence of the MetS and the number of MetS criteria met.

With respect to individual MetS components, reduced globus pallidus volume was associated with elevated waist circumference ($B = -0.78$, $SE = 0.32$, $IOR = 2.18$, $p = 0.02$) and low HDL cholesterol ($B = -0.56$, $SE = 0.27$, $IOR = 1.75$, $p = 0.04$) while there was a trend for elevated fasting glucose ($B = -1.15$, $SE = 0.52$, $IOR = 3.15$, $p = 0.10$). And although gray matter volume in the putamen did not covary with either the presence of the MetS or the number of MetS criteria met, reduced putamen volume was associated with elevated blood pressure ($B = -0.74$, $SE = 0.35$, $IOR = 2.10$, $p = 0.04$). Additional analyses also examined the association between continuous cardio-metabolic parameters defining the syndrome and basal ganglia volume. After adjusting for demographic and health-related factors, continuous measures of waist circumference ($B = -0.12$, $t = -1.88$, $p = 0.06$) and HDL cholesterol ($B = 0.12$, $t = 1.82$, $p = 0.07$) showed marginal associations with pallidal volume.

3.3. MetS and depressive symptoms

Depressive symptoms were positively associated with the number of MetS criteria met (Fig. 3). Hence, after adjusting for age, sex, race, smoking, and education, greater severity in depressive symptoms was associated with meeting more MetS criteria ($B = 0.10$, $SE = 0.05$, $e^B = 1.11$, $p = 0.03$). In an additional model wherein number of criteria met was defined as 0–3+, the dose–response relationship between number of criteria met and depressive symptoms trended toward significance ($B = 0.09$; $SE = 0.05$, $e^B = 1.09$; $p = 0.07$). Contrary to prior research [60], however, we did not observe a significant association between presence/absence of the MetS and depressive symptoms, even in unadjusted analyses ($ps \geq 0.16$).

3.4. Basal ganglia volume and depressive symptoms

Reduced globus pallidus volume covaried with higher depressive symptoms ($B = -0.16$, $t = -2.52$, $p = 0.01$), after adjusting for total brain tissue volume (Fig. 2). This association between depressive

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[2] In secondary analyses, similar models indicated that the presence of MetS was independently related to left ($B = -0.66$, $SE = 0.31$, $IOR = 1.94$, $p = 0.03$) and right ($B = -0.71$, $SE = 0.32$, $IOR = 2.04$, $p = 0.02$) globus pallidus volume after adjusting for demographic and health-related factors, and total brain tissue volume. Further, the number of MetS criteria met was also associated with left ($B = -0.18$, $SE = 0.10$, $e^B = 0.84$, $p = 0.06$) and right ($B = -0.23$, $SE = 0.10$, $e^B = 0.79$, $p = 0.02$) pallidal volume, although marginally with left regions. Again, the correlation between right and left globus pallidus volume was strong ($r = 0.69$, $p < 0.01$) which validated the use of average bilateral globus pallidus volume. Nonetheless, laterality effects did not appear to drive the observed volumetric associations.

symptoms and pallidal volume persisted after additional adjustment for demographic and health-related covariates ($p = 0.04$).

3.5. Mediation testing

The significant pattern of results between (i) number of MetS criteria met and globus pallidus volume and (ii) globus pallidus volume and depressive symptoms indicates that variation in pallidal gray matter volume may represent a candidate mediator linking number of MetS criteria met and depressive symptoms. Despite the absence of a total effect between presence of the MetS and depressive symptoms, we did test the hypothesis of a possible indirect effect through globus pallidus volume in accordance with prior recommendations regarding mediation testing [61]. We found a significant indirect effect with respect to presence/absence of the MetS and depressive symptoms through globus pallidus volume (ab indirect effect $= 0.130$; $SE = 0.094$; 95% CI $= 0.0041$, 0.398; $p < 0.05$). Similarly, we found that association of number of syndrome criteria met on depressive symptoms was mediated by globus pallidus volume (ab indirect effect $= 0.147$, $SE = 0.108$; CI $= 0.0090$, 0.4862 $p < 0.05$). This indirect effect was specific to meeting three syndrome criteria. Based on the direction of the paths, this mediation effect indicates that individuals with the MetS and/or meeting criteria for three MetS components may exhibit increased depressive symptoms as statistically mediated by decreases in pallidal regions of the basal ganglia.

3.6. Ancillary analyses

We identified 3 potential outlier cases (see Fig. 1) exhibiting pallidal and putamen gray matter volumes that were 3 SD above the mean of their respective brain volume distributions. Ancillary analyses were conducted to determine whether removal of these outliers would influence our observed results regarding the significant MetS-pallidal or pallidal-depressive symptom associations. Removal of these outliers did not alter any of the respective associations between the MetS, globus pallidus volume, and depressive symptoms; gray matter volume in the putamen was not associated with MetS or depressive symptoms after outlier adjustment. Hence, our reported findings persisted after accounting for the influence of these cases.

4. Discussion

The first novel finding of this study was that individuals with the MetS showed reduced gray matter volume in the globus pallidus, independent of demographic and health-related variables. Second, individuals meeting more MetS criteria showed a graded reduction in globus pallidus volume — suggesting a dose–response association, rather than a threshold effect of meeting binary MetS diagnostic criteria (i.e., presence vs. absence). However, neither the presence of the MetS nor the number of MetS criteria met associated significantly with gray matter volume in other basal ganglia regions. Third, as consistent with prior work [62], we found that individuals meeting more MetS criteria reported more depressive symptoms. Fourth, reduced globus pallidus volume mediated the association between meeting more MetS criteria and depressive symptoms. Finally, we found an indirect mediation effect of the MetS (i.e., presence, absence) on depressive symptoms through reduced globus pallidus volume, even though we observed no statistically significant total effect of the MetS on depressive symptoms. Taken together, there appears to be regional specificity within the basal ganglia – namely in the globus pallidus – with respect to volumetric reductions that are associated with the MetS and meeting more MetS criteria among otherwise healthy individuals. It is possible that these volumetric changes in the globus pallidus may correspond to a neural correlate that is common to the MetS and depressive symptomatology. However, the causal directions of association between the MetS,

depressive symptomatology, and globus pallidus volume are unclear because of the correlational design of the present study.

Despite this design limitation, our novel finding of an inverse association between the MetS and pallidal volume appears consistent with prior animal and human evidence linking functional alterations within the basal ganglia to impairments in metabolism and energy balance [63]. This finding also appears consistent with those showing that individual components and correlates of the MetS, including adiposity [18], impaired fasting glucose [18], and insulin resistance [19], are associated with structural changes within the pallidal region of the basal ganglia. This finding also adds to an emerging literature indicating the MetS as a whole, as opposed to its individual components, relates to adverse brain changes and associated impairments in cognitive functions [17]. We note, however, that although we did not find adiposity or glucose associations with the volumes of other basal ganglia regions (e.g., putamen; [20]), other studies have reported similar null associations [64–66]. By contrast, our findings did replicate previous work [18], insofar as we found that both high fasting glucose and central adiposity (elevated waist circumference) covaried with reduced globus pallidus volume. Thus, in light of our findings and prior work, it may be that the link between the MetS and globus pallidus volume more closely reflects the associations of adiposity and glucose dysregulation with this region, as compared with other basal ganglia regions and other features and correlates of the MetS [67]. This interpretation is consistent with cumulative evidence that adiposity and glucose dysregulation are likely to be key etiological factors in the development of the MetS [39]. However, we cannot rule out the possibility that the MetS association with reduced globus pallidus volume is mediated by factors other than glucose dysregulation or central adiposity (e.g., systemic inflammatory mechanisms). Although, it is noteworthy that we found individuals meeting more MetS criteria exhibited a graded reduction in the gray matter volume of the globus pallidus, which suggests that (i) the other syndrome components are also linked to structural changes in this region, and (ii) the aggregate clustering of multiple MetS components exhibited a dose–response or graded association with the gray matter volume of this region, above-and-beyond just the presence of adiposity, glucose elevations, or other isolated MetS components per se. The latter observation appears consistent with work showing that the clustering of cardio-metabolic risk factors accounts for unique variation in MRI indicators of gross brain atrophy [68,69], and it adds to this work by showing an apparently specific neural correlate of the MetS (reduced globus pallidus volume) that is still observed after accounting for gross or total brain tissue volume.

The potential functional implications of reduced gray matter volume in the globus pallidus may relate to the role of this region in regulating physiologic processes relevant to the development of the MetS [13]. For instance, neurochemical and animal lesion studies suggest an influence of the globus pallidus on glucose metabolism [70], with pallidal lesions inducing hyperglycemia in rats [71]. More recent work has also shown that lesions to ventral regions of the pallidum lead to elevated glucose levels coupled with impaired glucose control following a regulatory challenge [72]. Critically, such pallidal lesions produce hyperglycemic levels that are enduring and comparable to pre-diabetic states in humans, states that are engendered by the MetS. Additional human case study evidence has also shown that a patient with lesions to the globus pallidus gained 20-lbs [29]. Although speculative, it may be that the gray matter changes in the globus pallidus linked to the MetS and, more specifically, impaired fasting glucose and adiposity might reflect alterations in energy balance functions served by the globus pallidus [73].

We also found that reduced pallidal volume was associated with more depressive symptomatology, which agrees with prior work [14,28]. Existing models of depression indicate that the globus pallidus and other basal ganglia regions are components of prefrontal and limbic circuits important for goal-directed behaviors and executive cognitive control processes that support emotion and mood regulation [74].

Interestingly, altered pallidal function has been associated with inhibited thalamo-cortical stimulation of prefrontal areas that mediate the regulation of negative affect [74,75]. Thus, the anatomical connections of the globus pallidus support the possibility that structural or volumetric changes in this region may relate to the pathophysiology of depression or depression risk by affecting emotion regulation functions supported by prefrontal and limbic circuits [75]. And here, our results appear to extend previous clinical findings by demonstrating that depressive-related variation in the gray matter volume of the globus pallidus might be best conceptualized along on a continuum that extends to otherwise healthy individuals. In view of such a conceptualization, it may be that volumetric and other features of basal ganglia morphology could reflect a so-called endophenotype associated with vulnerability to or risk for depression. In support of this possibility, two recent studies have proposed that depression vulnerability among otherwise healthy individuals might relate to volumetric reductions in the basal ganglia [76,77]. However, further research bearing on this issue is needed to determine whether the volumetric or other morphological changes in the pallidum emerge *before* or *after* the onset of depressive symptoms — especially in the context of comorbid metabolic dysregulation and MetS development [22].

In this context, it is noteworthy that in the present study individuals exhibiting more depressive symptoms also met more MetS criteria, which agrees with prior work on otherwise healthy adults [62,78,79]. Given that the MetS involves diverse physiological changes (e.g., systemic inflammatory states) that are associated with and implicated in the symptomatic expression of depression, it appears reasonable to expect that depressive symptoms would be more prevalent among individuals meeting more MetS criteria [80]. Our mediation findings extend prior findings linking peripheral physiological changes associated with the MetS and depressive symptomatology by identifying a potential mediating factor: reduced globus pallidus volume. As noted previously, this observation is consistent with prior findings and speculations that the MetS and depressive symptoms may share common pathophysiological features [22], including alterations in central neurotransmitter systems that covary with metabolic dysregulation [58,59] and risk for depressive symptoms [81], as well as volume reductions in basal ganglia regions [74,82]. Again, however, the causal directions of associations and temporal ordering of depressive symptoms, volumetric changes, and depressive symptoms need to be tested in future longitudinal research.

In closing, there are several remaining study limitations and specific future directions that should be acknowledged. First, our present study does not permit inferences regarding the *mechanisms* that link the MetS to globus pallidus volume. Second, although our findings were specific to the globus pallidus, previous studies have implicated volumetric and white matter changes in medial temporal and other regions as a possible neural correlates of the MetS among adolescents [83]. Thus, future studies on adults should employ multimodal and voxel-wise methods that permit an examination of such changes in regions outside of the basal ganglia. This suggestion is reinforced by findings showing that individual components of the MetS covary with gray matter changes in the regions of the frontal and temporal lobes [18–20,64,84,85]. Third, our exclusion criteria likely truncated variability in depressive symptoms to a range that is more restricted than that observed in prior studies of the MetS and depressive symptoms [60]. In extension, the use of more structured instruments and interviews to systematically assess depressive symptoms may better capture sub-clinical variation in self-reported symptoms in otherwise healthy community samples such as ours. Finally, an unmeasured third-factor explanation, such as a shared genetic or heritable factor, may have accounted for the observed co-expression of the MetS, globus pallidus volume reductions, and depressive symptoms, which may not be functionally or causally related. Thus, longitudinal study designs that incorporate imaging assessments, as well as more comprehensive measures of mood-related symptoms, energy metabolism, and health

behaviors (e.g., food intake and physical activity) are needed to extend the present findings to better understand the mechanisms and functional implications of the associations between the MetS, depressive symptoms, and gray matter volume changes in the basal ganglia.

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