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Protein and Gene Markers of Metabolic Dysfunction and Inflammation Together Associate with Functional Connectivity in Reward and Motor Circuits in Depression

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Abstract

Bidirectional relationships between inflammation and metabolic dysfunction may contribute to the pathophysiology of psychiatric illnesses like depression. Metabolic disturbances drive inflammation, which in turn exacerbate metabolic outcomes including insulin resistance. Both inflammatory (e.g. endotoxin, vaccination) and metabolic challenges (e.g. glucose ingestion) have been shown to affect activity and functional connectivity (FC) in brain regions that subserve reward and motor processing. We previously reported relationships between elevated concentrations of endogenous inflammatory markers including C-reactive protein (CRP) and low corticostriatal FC, which correlated with symptoms of anhedonia and motor slowing in major depression (MD). Herein, we examined whether similar relationships were observed between plasma markers related to glucose metabolism (non-fasting concentrations of glucose, insulin, leptin, adiponectin and resistin) in 42 medically-stable, unmedicated MD outpatients who underwent fMRI. A targeted, hypothesis-driven approach was used to assess FC between seeds in subdivisions of the ventral and dorsal striatum and a region in ventromedial prefrontal cortex (VS-vmPFC), which was previously found to correlate with both inflammation and symptoms of

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anhedonia and motor slowing. Associations between FC and gene expression signatures were also explored. A composite score of all 5 glucose-related markers (with increasing values reflecting higher concentrations) was negatively correlated with both ventral striatum (VS)-vmPFC ($r=-0.33$, $p<0.05$) and dorsal caudal putamen (dcP)-vmPFC ($r=-0.51$, $p<0.01$) FC, and remained significant after adjusting for covariates including body mass index ($p<0.05$). Moreover, an interaction between the glucose-related composite score and CRP was observed for these relationships ($F[2,33]=4.3$, $p<0.05$) whereby significant correlations between the glucose-related metabolic markers and FC was found only in patients with high plasma CRP (>3 mg/L; $r=-0.61$ to -0.81, $p<0.05$). Insulin and resistin were the individual markers most predictive of VS-vmPFC and dcP-mPFC FC, respectively, and insulin, resistin and CRP clustered together and in association with both LV-vmPFC and dcP-vmPFC in principal component analyses. Exploratory whole blood gene expression analyses also confirmed that gene probes negatively associated with FC were enriched for both inflammatory and metabolic pathways (FDR $p<0.05$). These results provide preliminary evidence that inflammation and metabolic dysfunction contribute jointly to deficits in reward and motor circuits in MD. Future studies using fasting samples and longitudinal and interventional approaches are required to further elucidate the respective contributions of inflammation and metabolic dysfunction to circuits and symptoms relevant to motivation and motor activity, which may have treatment implications for patients with psychiatric illnesses like depression.

Keywords

glucose metabolism; inflammation; insulin; C-reactive protein; functional connectivity; fMRI; gene expression

1. Introduction

A significant portion of patients with depression (30–50% depending on the sample) have increased inflammatory markers in both the peripheral blood and cerebrospinal fluid, including inflammatory cytokines and the acute phase reactant C-reactive protein (CRP) (Dantzer et al., 2008; Felger et al., 2018; Goldsmith et al., 2016b; Zunszain et al., 2013). Both obesity and its associated metabolic disturbances are important drivers of systemic inflammation, in part through activation of resident macrophages around expanding adipocytes (Park et al., 2005; Weisberg et al., 2003). However, increases in circulating insulin and free fatty acids also activate macrophages (Ieronymaki et al., 2019a; Ieronymaki et al., 2019b), and the associated release of inflammatory cytokines is a major contributor to insulin resistance (Hirosumi et al., 2002; Hotamisligil et al., 1994; Shi et al., 2006). Accordingly, a high percentage of patients with depression also exhibit evidence of metabolic dysfunction including insulin resistance (IR) (Hamer et al., 2019; Li et al., 2016).

Of further clinical relevance to depression is the mounting evidence that increased inflammation, obesity and metabolic disturbances have all been associated with reduced responsiveness to standard antidepressant medications (Furman et al., 2018; Haroon et al., 2018b; Jha et al., 2018; Strawbridge et al., 2015; Vogelzangs et al., 2014). With regard to novel antidepressants, work from our group for example showed that, in addition to inflammatory pathways, differential expression of genes related to glucose and lipid

metabolism were predictive of the subsequent antidepressant response to the tumor necrosis factor (TNF) antagonist, infliximab (Mehta et al., 2013), which was observed in depressed patients with higher levels of plasma CRP (Raison et al., 2013). These findings were validated by measurement of circulating glucose and lipid-related biomarkers (Bekhbat et al., 2018), and both metabolism-related genes and plasma biomarkers were affected by administration of infliximab in these patients, further exemplifying bidirectional relationships between inflammation and metabolism.

This relationship between inflammation and metabolism and antidepressant response is not surprising considering that both systemic inflammation and metabolic disturbances like insulin resistance can have consequences on organs and tissues such as the brain. Indeed, numerous studies in humans and laboratory animals have demonstrated that administration of inflammatory stimuli can impact dopamine release and affect the neural activation of key basal ganglia and prefrontal regions that subserve reward processing, motivation, and motor speed (Brydon et al., 2008; Capuron et al., 2012; Eisenberger et al., 2010; Felger et al., 2015; Felger et al., 2013; Felger and Treadway, 2017; Harrison et al., 2016; Moieni et al., 2019; Yohn et al., 2016). Likewise, oral glucose challenge has been shown to alter the cerebral blood flow and functional connectivity (FC) of cortical and limbic brain regions including basal ganglia (Al-Zubaidi et al., 2018; Jastreboff et al., 2016; Page et al., 2013; van Opstal et al., 2018).

Increasing data from our group and others has demonstrated evidence that endogenous inflammation in patients with major depression (MD) is also associated with alterations in the structure and function of reward and motor-relevant cortical and subcortical structures (Felger et al., 2016b; Haroon et al., 2018a; Mehta et al., 2018a; Meier et al., 2016; Opel et al., 2019; Savitz et al., 2012; Yin et al., 2019). For example, our previous work using resting-state functional magnetic resonance imaging (rfMRI) showed that plasma concentrations of CRP (as well as inflammatory cytokines and their soluble receptors) were negatively corelated with FC in dopaminergic corticostriatal reward and motor circuits in association with anhedonia and motor slowing in whole-brain analyses (Felger et al., 2016a; Yin et al., 2019). Using a targeted, hypothesis-driven FC approach, reduced FC between the left ventral striatum (VS) and right dorsal caudal putamen (dcP) and the ventromedial prefrontal cortex (vmPFC) corelated with CRP (Felger et al., 2016b). Corticostriatal FC involving VS was in turn selectively associated with anhedonia, a core symptom of depression, and FC involving dcP predicted motor slowing, a prominent symptom in depression and other psychiatric disorders. Symptoms of anhedonia and motor slowing have been associated with alterations in reward and motor circuits involving the basal ganglia and prefrontal cortex (Drysdale et al., 2017; Dunlop and Nemeroff, 2007; Treadway and Pizzagalli, 2014) and with inflammation (Felger and Treadway, 2017; Medeiros et al., 2020). However, whether deficits in reward and motor circuits are similarly associated with highly related markers of metabolic dysfunction in MD has not been examined.

Herein, we examined relationships between non-fasting levels of glucose-related plasma metabolic markers (Bekhbat et al., 2018) and targeted FC in reward and motor circuits found to be related to anhedonia and motor slowing in our previous work (Felger et al., 2016b; Mehta et al., 2020b). The glucose-related metabolic panel from our previous work was

comprised of glucose and insulin as well as the adipokines leptin, resistin, and adiponectin, which are associated with glucose homeostasis and insulin resistance as well as with depression (Bryson et al., 1999; Lehto et al., 2010; Li et al., 2016; Singh and Saxena, 2010). Multivariate linear models and principal components analyses (PCA) including CRP were used to determine whether metabolic and inflammatory markers exhibited joint or independent relationships with FC. Because gene expression has been previously associated with brain structure in MD and is predictive of antidepressant response (Cattaneo et al., 2016; Cattaneo et al., 2013; Mehta et al., 2013; Mora et al., 2018; Savitz et al., 2013), we also examined whether inflammatory and metabolic whole blood immune cell gene expression pathways were associated with FC.

2. Methods

2.1 Participants

Forty-eight participants (18–65 years) with a primary diagnosis of major depressive disorder or bipolar disorder, current episode depressed as determined by Structured Clinical Interview for Diagnostic and Statistical Manual-IV-TR (SCID-IV) were studied, as previously described (Felger et al., 2016b), all of whom had rfMRI, CRP and gene expression data. Forty-two of these 48 participants also had additional plasma samples available for analysis of metabolic markers. Briefly, subjects were free of psychotropic medications or medications known to affect the immune system, and were tested for drugs of abuse at screening and on the day of the scan. Medications for other medical conditions were allowed per patients' treating physicians, although patients were required to be medically-stable as determined by medical history, physical exam and laboratory testing (see Supplementary Information). No patients had non-fasting glucose levels out of normal range and only one subject was taking a medication used to treat diabetes. CRP was assessed 2–4 time at screening (1–4 weeks apart) to ensure stable levels of inflammation (two values within 25%). Participants with active infections were excluded until medically-stable. Subjects were recruited from a parent study on phenotyping depressed patients with increased inflammation (ClinicalTrials.gov NCT01426997) which involved a single inpatient visit to the Emory Clinical Research Network clinic to collect MRI, biomarker, behavioral and sleep data. All procedures were approved *a priori* by the Institutional Review Board of Emory University. All participants provided written informed consent.

2.2 Resting-state fMRI data acquisition and preprocessing

Data was acquired on a 3T Magnetom Trio scanner (Siemens, USA) with a 20-channel head coil at the Emory Center for Systems Imaging in the afternoon (3PM±2 hours). Anatomic images ($1mm^3$ isotropic) were obtained using a T1-weighted, magnetization prepared rapid gradient echo (MPRAGE) sequence (Mugler and Brookeman, 1990). Wakeful rfMRI images were acquired using a Z-SAGA pulse sequence for recovering susceptibility signal losses regularly seen in T_2-weighted fMRI (Heberlein and Hu, 2004) (See Supplementary Information). Analysis of FC was conducted with AFNI (http://afni.nimh.nih.gov/). Pre-processing (Li et al., 2019b; Li et al., 2017; Yin et al., 2019) included voxel-wise outlier detection (~5.5× median absolute deviation), despiking, slice timing correction, volume alignment, anatomy-to-EPI co-registration (Saad et al., 2009), nuisance signal (head motion,

cerebrospinal fluid, and white matter) regression, band pass filtering (0.009Hz<f<0.08Hz), and Gaussian (FWHM=5mm) spatial smoothing. Regarding head motion, no participant had movement >3.4mm/degree in translation/rotation, consistent with previous studies (Felger et al., 2016b; Oathes et al., 2015; Wacker et al., 2009). Individual's 4D fMRI data was spatially normalized into a standard stereotaxic space, the Montreal Neurological Institute (MNI) template, with 1mm^3 resolution.

2.3 Analysis of functional connectivity

Associations between circulating metabolic and gene expression markers and corticostriatal resting-state FC were examined using targeted VS and dcP to vmPFC FC relationships previously identified to be associated with both inflammation and behavior (anhedonia and motor slowing, respectively)(Felger et al., 2016b). Targeted seed-to-ROI FC was calculated as signal correlations between spherical seeds (r=3mm) centered on the left inferior VS (including nucleus accumbens; MNI coordinates: x=−14, y=8, z=−9) or the right dcP (x=28, y=1, z=3)(Capuron et al., 2012; Di Martino et al., 2008; Felger et al., 2016b) and a ROI in vmPFC (MNI coordinates: x=0, y=44, z=−8 and volume=1408mm^3). This vmPFC ROI was previously reported to be associated with neural activation in response to receipt of reward versus loss in a meta-analysis of neuroimaging studies (Diekhof et al., 2012) and with decreased cortico-limbic FC in our previous work (Felger et al., 2016b; Mehta et al., 2018b; Mehta et al., 2020b). From this vmPFC ROI, subject-level FC scores (Fisher's Z values) were extracted for correlation with metabolic markers and CRP (Felger et al., 2016b; Mehta et al., 2018b; Mehta et al., 2020b).

2.4 Blood collection

Whole blood was collected on the day after the scan through indwelling catheters after participants had at least 30 minutes of rest. For plasma isolation, blood was collected into EDTA-containing vacutainer tubes approximately three hours from a meal provided at the research clinic (3pm±1hour). Plasma was obtained by centrifugation at 1000×g for 10 minutes at 4°C, aliquoted into siliconized polypropylene tubes, and stored at −80°C until assay. Whole blood for gene expression analysis was collected in Tempus Blood RNA Tubes (Applied Biosystems, Carlsbad, CA) at 9am and stored at −80°C until RNA extraction by the Emory Cancer Genomics Core for microarray analysis.

2.6 Measurement of glucose-related metabolic markers and CRP

Plasma markers related to glucose metabolism were measured in the Biomarker Core Lab of the Foundation for Atlanta Veterans Education and Research. Plasma leptin, resistin and adiponectin were measured by ELISA (Boster Biological, Pleasanton, CA). Assay detection limits were 10 pg/ml, 3 pg/ml and 0.06 ng/ml, respectively. Plasma glucose (colorimetric), insulin (immunoturbidometric) and high sensitivity (hs)-CRP (immunoturbidimetric) were assayed using reagents from Sekisui Diagnostics (Exton, PA) implemented on the AU480 chemistry analyzer (Beckman Coulter). Mean inter- and intra-assay coefficients of variation were reliably <10% (Bekhbat et al., 2018; Felger et al., 2016b; Le et al., 2000; Mehta et al., 2013). To examine the shared contribution of all glucose and insulin-related circulating metabolic markers to the relationship with FC, a composite score was created from the sum of Z-scores of all markers, a common method for examining the contribution of multiple

related metabolic (Agostinis-Sobrinho et al., 2017; Eisenmann, 2008; Erdembileg et al., 2015) or inflammatory (Erdembileg et al., 2015; Felger et al., 2018; Haroon et al., 2020; Hopkins et al., 2012; Mehta et al., 2020a) markers or risk factors, and referred to as the glucose-related composite score.

2.7 Microarray

Extraction of RNA was performed using Ambion Tempus RNA Spin kit (Thermo Fisher, Asheville, NC, USA) according to manufacturer instructions. RNA concentrations and A260/280 ratio (Tecan iControl, Life Sciences, Switzerland) and sample quality (Agilent Bioanalyzer 2100 RNA Nano assay, Agilent, Santa Clara, CA, USA) was determined. Each sample was linearly amplified by TotalPrep-96 RNA Amplification Kit for Array Analysis (Illumina, San Diego CA, USA). On the same day, all samples were hybridization to Illumina Human HT-12 Expression BeadChips using the Whole-Genome Gene Expression Direct Hybridization Assay (Illumina). BeadChips were scanned on the Illumina HiScan to determine raw probe fluorescence intensity.

2.8 Gene by functional connectivity associations and pathway analyses

Raw expression data were quantile-normalized in Illumina Genome Studio and 16,969 probes passed the filter criteria of Illumina probe detection p-value of <0.01 in at least 10% of the samples. Data were deposited in NCBI Gene Expression Omnibus as series GSE135524. The average signal intensity data were $\log_2$-transformed in R (http://www.R-project.org) (Mehta et al., 2013; Xiao et al., 2016). The association between FC Z-scores and gene expression was assessed while adjusting for age, sex, race and body mass index (BMI) using linear models in R (Barfield et al., 2012; Xiao et al., 2016). To explore relationships between FC and gene pathways, a set of probes was first identified to be associated with FC based on a threshold of $|R_{partial}|>0.30$ (after controlling for covariates in multiple linear regression) consistent with a biologically relevant, medium effect size (Cole et al., 2003; Han et al., 2016; Mellon et al., 2016; Miller et al., 2018; Ross et al., 2019; Torres et al., 2013), combined with nominal $p<0.05$ (Guo et al., 2006; Han et al., 2016; Torres et al., 2013), which together has been shown to be more reliable across pathway analyses than use of FDR p-value alone (Cole et al., 2003; Guo et al., 2006; Patterson et al., 2006). Identified genes were then applied to the stringency of secondary statistical analyses (Cole et al., 2003; Guo et al., 2006; Patterson et al., 2006) at FDR<0.05 to assess their functional annotation within curated pathways and networks in GeneGo MetaCore (St. Joseph, MI, USA) (Ekins et al., 2007; Han et al., 2016; Mehta et al., 2013). Of note, the percentage of peripheral blood immune cell subsets from complete blood count (CBC) was stable across the range of FC Z-scores (STable 1).

2.9 Statistics

Clinical characteristics were summarized using mean and standard deviation (SD) for continuous variables and percent for categorical variables, which were examined with respect to both the glucose composite score and plasma CRP. Associations between the glucose composite score and FC Z-scores were assessed in linear regression models with and without clinical covariates that may modify relationships between peripheral biomarkers and brain circuits, including age, sex, race, and body mass index (BMI). To determine

whether relationships between metabolic markers and FC depended on inflammation, an interaction term was created for the glucose composite score and level of plasma CRP concentration (as per American Heart Association/Center for Disease Control definitions of low, moderate and high risk for cardiovascular and metabolic disease, <1, 1–3 and >3 mg/L CRP coded as 1, 2 and 3, respectively)(Ridker, 2003), which was examined in multivariate general linear models including glucose scores and CRP with and without clinical covariates. To determine which individual metabolic markers contributed most to the relationships between glucose scores and FC, individual metabolic markers and FC Z-scores were entered into backward and forward linear regression models using the same criteria for entry and removal. Consistent with previous reports (Bekhbat et al., 2018; Felger et al., 2018; Haroon et al., 2014; Raison et al., 2009; Torres et al., 2013), markers were natural log (ln) transformed to improve normality for parametric statistical modeling. PCA was also used to determine whether individual metabolic markers and CRP clustered together and with FC Z-scores. Varimax rotation with Kaiser Normalization was used to simplify factor structure, as determined by Eigen values >1, and only individual variable contributions of >0.5 qualified for loading components (Grimsholm et al., 2005). Tests of significance were two-tailed, conducted in IBM SPSS Statistics 24.

3. Results

3.1 Sociodemographic, clinical, and biological characteristics of the sample

Characteristics of the study sample, including demographic variables as well as concentrations of CRP and metabolic markers from 48 patients (42 with metabolic markers), are summarized in Table 1. CRP was significantly correlated with the glucose composite score ($r=0.374$, $p=0.015$), as well as with a number of the individual metabolic markers, including insulin ($r=0.454$, $p=0.003$), leptin ($r=0.532$, $p<0.001$), and resistin ($r=0.391$, $p=0.01$). BMI was also significantly correlated with CRP ($r=0.652$, $p<0.001$), the glucose composite score ($r=0.426$, $p=0.005$), leptin ($r=0.623$, $p<0.001$), and insulin ($r=0.369$, $p=0.016$). As such, BMI was included in all analyses involving covariates to control for the potential confounding of additional factors related to metabolism and/or obesity that were not measured herein.

3.2 Relationships between glucose-related metabolic markers and functional connectivity in reward and motor circuits and their interaction with CRP

Glucose composite scores, with increasing values representing higher concentrations of each marker (Figure S1) and reflective of greater metabolic dysfunction, were negatively associated with both VS to vmPFC (VS-vmPFC; $r=-0.330$, $p=0.033$) and dcP-vmPFC ($r=-0.508$, $p=0.001$) FC (Figure 1). Both relationships remained significant after adjusting for covariates ($p<0.05$ for VS-vmPFC, $p<0.01$ for dcP-vmPFC). To determine whether associations between glucose-related metabolic markers and FC depended on inflammation, relationships between FC and an interaction term for the glucose composite score by level of plasma CRP concentration (low <1, moderate 1–3 and high >3 mg/L) was examined in multivariate general linear models including the glucose composite score and plasma CRP level. Main effects were observed for CRP level ($F[4,74]=3.2$, $p=0.018$) and the glucose score by CRP interaction term ($F[2,33]=4.3$, $p=0.017$); the interaction term (but not level of

plasma CRP, p=0.122) remained significant (p<0.05) when controlling for clinical covariates, and univariate sub-analyses indicated that the interaction term was significant for VS-vmPFC FC (F[1,33]=4.7, p=0.037) with a trend for dcP-vmPFC FC (F[1,33]=3.9, p=0.058). To illustrate these results, correlations between glucose scores and FC were examined and plotted for each level of CRP, and indicated that significant relationships were observed only in patients with high CRP (>3 mg/L; n=11) for both VS-vmPFC (r=−0.613, p=0.045) and dcP-vmPFC (r=−0.813, p=0.002) FC (Figure 2).

3.3 Individual metabolic markers, insulin and resistin cluster together and with CRP in association with functional connectivity in reward and motor circuits

Although no individual metabolic markers were associated with VS-vmPFC connectivity at p<0.05 (all r=−0.002 to −0.221), insulin was found to be the strongest predictor of VS-vmPFC FC (r=−0.221, p =0.160) in backward and forward linear regression models using the same criteria for entry and removal. Resistin was the strongest predictor of dcP-vmPFC FC (r=−0.422, p = 0.005), as confirmed in backward and forward linear regression using the same criteria for entry and removal. Insulin also exhibited a non-significant trend to correlate with dcP-vmPFC FC (r=−0.272, p=0.081). To confirm and extend the above results from linear regression indicating a metabolism (glucose composite score) by inflammation (plasma CRP) interaction with respect to their relationship with FC, PCA was used to examine whether metabolic markers and CRP clustered together and in association with FC. In PCA including all 5 metabolic markers and CRP along with VS-vmPFC FC, the first principle component (eigenvalues contributing 29.68% of the overall variance) was comprised of VS-vmPFC FC, insulin, resistin, and CRP (Table 2). Using all markers with dcP-vmPFC FC, the first principle component (eigenvalues contributing 31.36% of the overall variance) similarly included dCP-vmPFC FC, insulin, resistin, and CRP (Table 2).

3.4 Inflammatory and metabolic gene expression pathways are associated with functional connectivity in reward and motor circuits: Exploratory analyses

To explore whether both inflammatory and metabolic signaling pathways in immune cells were related to deficits in reward and motor-related pathways, targeted corticostrital FC described above was used to identify transcriptional pathways in peripheral blood immune cells that were negatively associated with VS and dcP to vmPFC FC in all 48 patients. In linear models controlling for clinical covariates (age, race, sex and BMI), 266 probes were identified to be negatively correlated with VS-vmPFC FC, whereas 836 were negatively associated with dcP-vmPFC FC ($R_{partial}$ >−0.3, p<0.05) (SData 1). Of the 12 significant pathways (FDR p<0.05) enriched in the 266 transcripts that negatively correlated with VS-vmPFC (SData 2), 7 were related to the immune system or inflammation (e.g. JAK/STAT, DPP4, and TNF signaling) and two were related to metabolism (HIF-1 and AKT signaling) (Figure 3). Similarly, of the top 20 of 87 pathways enriched in the 836 transcripts negatively correlated with dcP-vmPFC FC (SData 2), 11 were related to the immune system or inflammation (e.g. M-CSF, NFkB, and TLR signaling) and one to metabolism (proinsulin signaling) (Figure 3). Networks of canonical pathways built from the genes identified from the top ten pathways enriched in transcripts negatively correlated with VS-vmPFC FC and dcP-vmPFC FC (network objects; n=29 for VS and n=47 dcP) were also primarily related to inflammatory and metabolic processes (Figure S2). Of the 16 pathways enriched in the 191

genes positively associated with VS-vmPFC FC, 6 were related to immune responses (e.g. interleukin (IL) 2/3/6 and M-CSF/G-CSF signaling) but were primarily driven by a single network object, PI3K cat class IA (SData 2) representing the transcript PIK3CD from the dataset (ILMN_1766275); of the only 9 pathways significantly enriched in 992 genes positively correlated with dcP-vmPFC, the top 5 were interestingly related to interferon and IL-4 signaling (see SData 1 and 2).

4. Discussion

This study demonstrated that peripheral biomarkers of metabolic disturbance relevant to glucose metabolism are associated *with low FC in reward* and motor circuits in MD. Moreover, a significant interaction was observed between the glucose composite score and level of CRP concentration whereby significant correlations between the glucose-related metabolic markers and FC was found only in patients with high plasma CRP (>3 mg/L). Interestingly, insulin and resistin were the individual markers most predictive of VS-vmPFC and dcP-mPFC FC, respectively, and insulin, resistin and CRP clustered together and in association with both LV-vmPFC and dcP-vmPFC in PCA. Together, the interaction and PCA results provide support for the idea that plasma inflammatory and metabolic markers contributed jointly to decreased FC in corticostriatal reward and motor pathways in depression. These findings were mirrored in exploratory gene expression analyses indicating that both inflammatory and metabolic pathways in peripheral blood immune cells were negatively correlated with VS-vmPFC and dcP-vmPFC FC. While relationships between metabolism and inflammation are complex, this study provides some preliminary findings regarding their potential roles in circuit deficits that contribute to MD, which can inform future longitudinal and interventional studies.

The composite scores for the five glucose-related plasma markers were negatively correlated with VS-vmPFC and dcP-vmPFC FC Z-scores (which were associated with symptoms of anhedonia and motor slowing in our previous work), whereby MD patients with higher levels of plasma markers had lower levels of FC. An interaction was also uncovered indicating that the relationship between glucose score and FC depended on the level of plasma CRP, and patients with both high glucose scores and high CRP exhibited low levels of FC. Despite a strong relationship between the glucose score and BMI, these relationships, including a metabolism by inflammation interaction, remained significant when controlling for BMI and other covariates. This finding suggests that connectivity in reward and motor circuits may be sensitive to metabolic disturbances involving glucose metabolism that are independent of variability in BMI and adiposity. With regard to individual markers that contributed to the relationship between the glucose score and FC, insulin was the strongest metabolic marker associated with VS-vmPFC FC and resistin was the most significant metabolic predictor of dcP-vmPFC FC. Interestingly, both insulin and resistin in conjunction with CRP were clustered together with FC in PCA.

The above findings are interesting when further considering the relationships between both resistin and insulin, and resistin and inflammation. The role of resistin as a factor produced in adipose tissue that contributes to insulin resistance (Tilg and Moschen, 2006) is now thought to be true primarily in rodents (Steppan et al., 2001). While the primary action of

resistin in humans is controversial, initially pointing to metabolic disturbances in obesity (Ukkola, 2002), recent evidence shows that it is produced in tissues other than adipocytes and supports a second role of resistin (as well as other adipokines) in inflammation (Kusminski et al., 2005). Indeed, resistin has been shown to induce production of inflammatory cytokines (Beier et al., 2008; Silswal et al., 2005), and may serve as a key intermediate link between disturbances in glucose metabolism/insulin resistance and inflammatory processes that jointly impact the brain and behavior in depression. Our results interpreted in this light illuminate a scenario whereby adiposity and metabolic disturbances cause rising insulin levels that increase inflammatory mediators such as adipokines and CRP, which may together drive activation of immune cells and inflammatory cytokines on the brain and behavior (Capuron et al., 2017; Luppino et al., 2010), while feeding back on adipose cells and other tissues to promote further metabolic dysfunction.

Another area relevant to the intersection of metabolism and inflammation, and which may have treatment implications for depression, is the intracellular shift in metabolism required to sustain immune cell activation, i.e. “immunometabolism” (O’Neill et al., 2016; Treadway et al., 2019). In states of chronic low-grade inflammation, such as in depression, increased energy demands and activation of inflammatory pathways may cause immune cells to shift from more energy-efficient oxidative phosphorylation to glycolysis, a less-efficient but more rapid producer of energy (Lacourt et al., 2018; O’Neill et al., 2016). This shift in metabolic pathways may be evidenced in gene expression findings from our previous studies demonstrating that glucose-related genes predict treatment response to inflximab in individuals with high inflammation (Bekhbat et al., 2018; Mehta et al., 2013), and that both high CRP and anhedonia were required to observe shifts in immunometabolic transcripts in peripheral blood immune cells in patients with depression (Bekhbat et al., 2020). These studies are complemented by the gene expression findings herein supporting associations between inflammatory and metabolic gene transcripts and low FC in reward and motor circuits.

Gene pathways related to both inflammation and metabolism were among the top of those that were enriched in gene transcripts negatively correlated with both vs-vmPFC and dcP-vmPFC FC. For example, NFkB signaling pathways have consistently been shown to be related to immune and inflammatory responses (Liu et al., 2017; Mitchell et al., 2016), though may also play a role in energy homeostasis, regulation of glycolysis, and maintenance of inflammation in metabolic disorders (Hasegawa et al., 2012; Moretti et al., 2012). We have previously shown that TNF signaling pathways are predictive of the antidepressant response to infliximab, a TNF antagonist, in addition to a number of metabolic transcripts (Mehta et al., 2013), and TNF is a primary driver of insulin resistance (Shi et al., 2006). DPP4 is widely expressed in immune cells and in the setting of obesity, may promote inflammation and insulin resistance (Ghorpade et al., 2018). DPP4 inhibitors may also be of potential benefit in metabolic disorders such as diabetes (Barnett, 2006). HIF-1 and AKT pathways may contribute to the Warburg effect whereby cells rapidly shift glucose metabolism from the slow, energy-maximizing oxidative phosphorylation to the rapid but less energy-yielding glycolysis (Courtnay et al., 2015; Nagao et al., 2019), and both have been implicated in the pathophysiology and treatment of depression (Li et al., 2019a; Matsuda et al., 2019; Shibata et al., 2013). Of note, although 6 immune pathways

(interleukin (IL) 2/3/6 and M-CSF/G-CSF signaling) were enriched in the transcripts positively associated with VS-vmPFC FC, they were represented by genes that were repeated across these and the other pathways, all of which contained PIK3CD. Unlike other class IA PI3Ks, PIK3CD is expressed primarily in leukocytes and involved in regulation of acquired immune responses (Clayton et al., 2002; Rolf et al., 2010). This was consistent with significant pathways for acquired immune cytokines (IL-4, interferon-gamma) enriched in genes positively correlated with dcP-vmPFC FC.

Activated inflammatory and metabolic intracellular signaling pathways may reflect processes that sustain low-grade peripheral inflammation in patients with depression who exhibit high CRP and metabolic disturbances, thus leading to cytokine release that impacts dopamine signaling in the brain to drive symptoms relevant to motivational deficits and psychomotor slowing (Capuron et al., 2012; Felger et al., 2016b; Felger and Treadway, 2017; Goldsmith et al., 2016a). Furthermore, evidence exists that dopaminergic neurons may be sensitive to metabolic signals that may relate important information about metabolic states in the periphery to guide reward-related decision making (Treadway et al., 2019). For example, there may be bidirectional communication between peripheral insulin and striatal dopamine that guide food choices and energy utilization (Stouffer et al., 2015; Ter Horst et al., 2018), which may, in part, explain why insulin was the strongest predictor of vs-vmPFC FC herein.

One strength of this study was the use of an *a priori*, targeted method of examining corticostriatal FC using seeds and a ROI from the literature (which we had previously shown to be related to anhedonia and motor slowing)(Felger et al., 2016b), allowing examination of relationships with metabolic and gene markers without bias of ROIs derived by variables within the dataset. Data also support our previous findings demonstrating relationships between immune and metabolic markers/pathways with depression and antidepressant response (Bekhbat et al., 2018; Mehta et al., 2013). Primary limitations of this study were the cross-sectional nature and limited sample size, which did not permit higher level modeling such as a formal path or mediation analysis to disentangle complex causal relationships among highly related metabolic and inflammatory variables, or examination of sex differences. A healthy control group was also not studied, however patients with low glucose composite scores (and low CRP) had similar positive FC in the assessed corticostriatal circuits as frequently observed in controls groups in numerous studies (Barnes et al., 2010; Di Martino et al., 2008; Kelly et al., 2009), and glucose-related markers were consistently elevated in patients with high glucose composite scores and in association with high plasma CRP. Another limitation is that the blood samples were not collected under fasting conditions so that basal measures of insulin resistance (e.g. HOMA-IR) could not be calculated and hemoglobin A1c was not measured. However, it should be noted that conditions were standardized across participants; blood was collected ~3 ±1 hours from the last meal and at the same time as the rfMRI scan (conducted ~24 hours apart). Therefore, the study captured approximately “normal” circulating levels of insulin, leptin, adiponectin and resistin for these subjects on a given day, but may have led to glucose levels not contributing to the association with FC due to increased variability. Future work involving fasting sample collection in longitudinal and interventional studies is necessary to isolate causal relationships between inflammation, metabolism, and corticostriatal FC in depression.

Strategies that employ metabolic and inflammatory challenge paradigms may help elucidate these complex relationships. Whether these relationships are present in other psychiatric populations that exhibit motivation and motor-related symptoms remains unknown. Nevertheless, findings suggest that both inflammation and metabolic disturbances represent potential novel treatment targets to improve FC in circuits that underlie motivational deficits and psychomotor slowing, providing a framework for future investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Metabolism and inflammation may interact to contribute to depression (MD)
- Inflammation has predicted low reward circuit functional connectivity (FC) in MD
- Glucose-related metabolic markers also negatively correlated with FC in MD
- Relationship between metabolic markers and FC depended on level of CRP concentration
- Inflammatory and metabolic gene pathways were also associated with FC

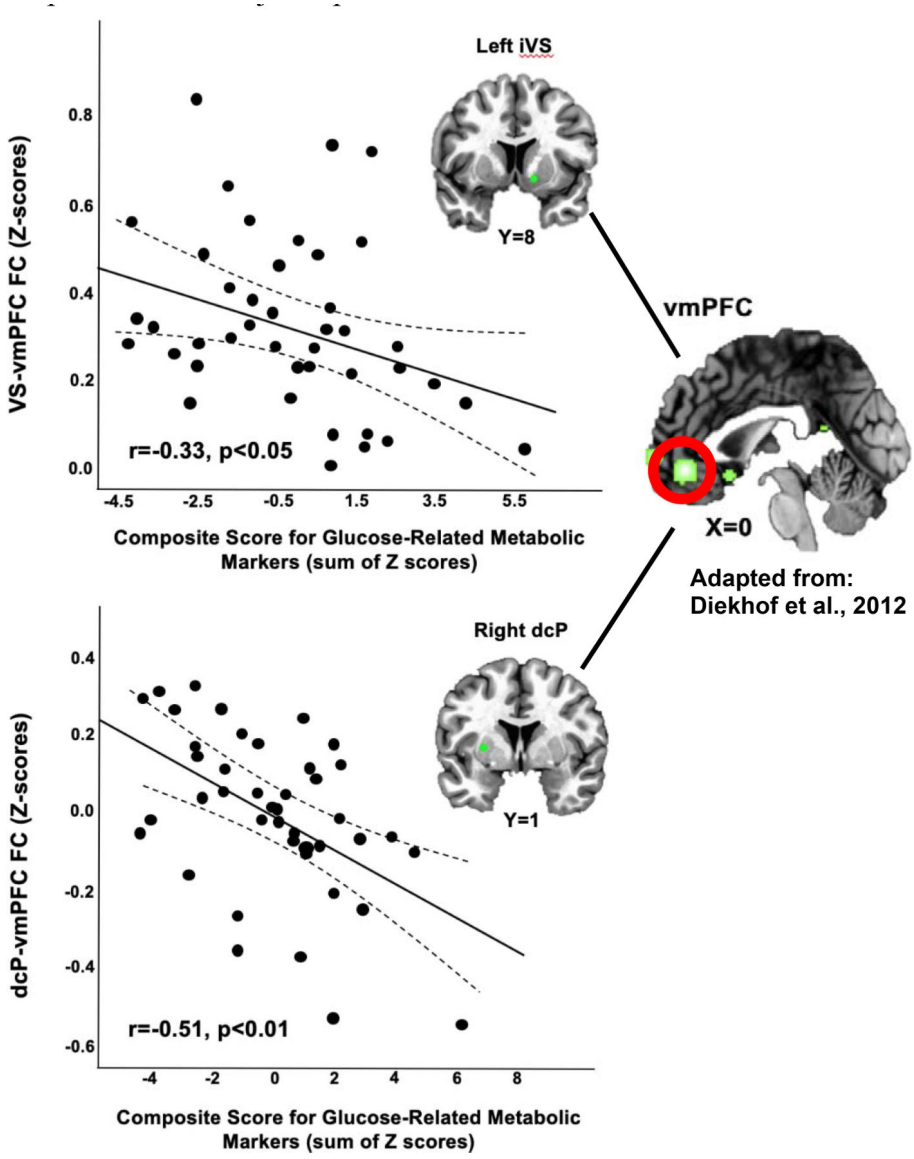

Figure 1. Glucose composite score was negatively associated with ventral striatum (VS)-ventromedial prefrontal cortex (vmPFC) and dorsal caudal putamen (dcP)-vmPFC functional connectivity (FC).

A composite score for glucose-related markers, with increasing values reflective of greater concentrations of these markers (see Figure S1), was created as the sum of Z-scores for non-fasting plasma concentrations of glucose, insulin, leptin, adiponectin and resistin. Glucose composite score was negatively associated with functional connectivity between left VS and the vmPFC [image reprinted from Diekhof et al. with permission from Elsevier, copyright 2012] and right dcP and the vmPFC, in 42 medically-stable, medication free outpatients with major depression.

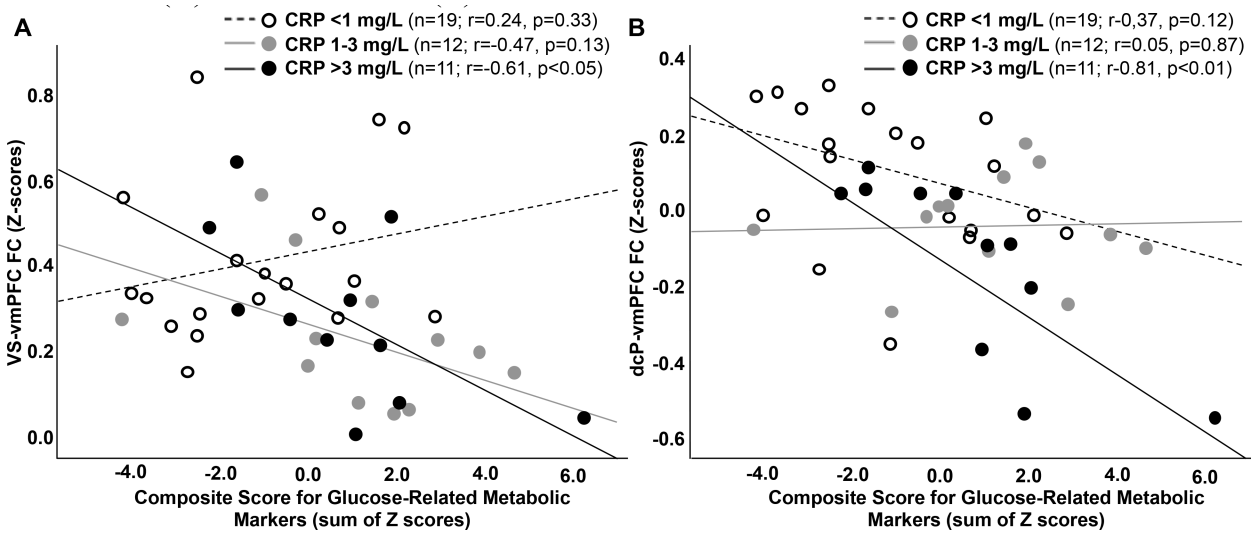

Figure 2. Relationships between the glucose composite score and ventral striatum (VS)-ventromedial prefrontal cortex (vmPFC) and dorsal caudal putamen (dcP)-vmPFC functional connectivity (FC) depended on plasma levels of C-reactive protein (CRP).

Specifically, an interaction between the glucose-related metabolic markers and level of plasma CRP concentration was observed whereby significant correlations between the glucose composite score and FC was found only in patients with high plasma CRP (>3 mg/L) for both VS-vmPFC (**A**) and dcP-vmPFC (**B**) FC.

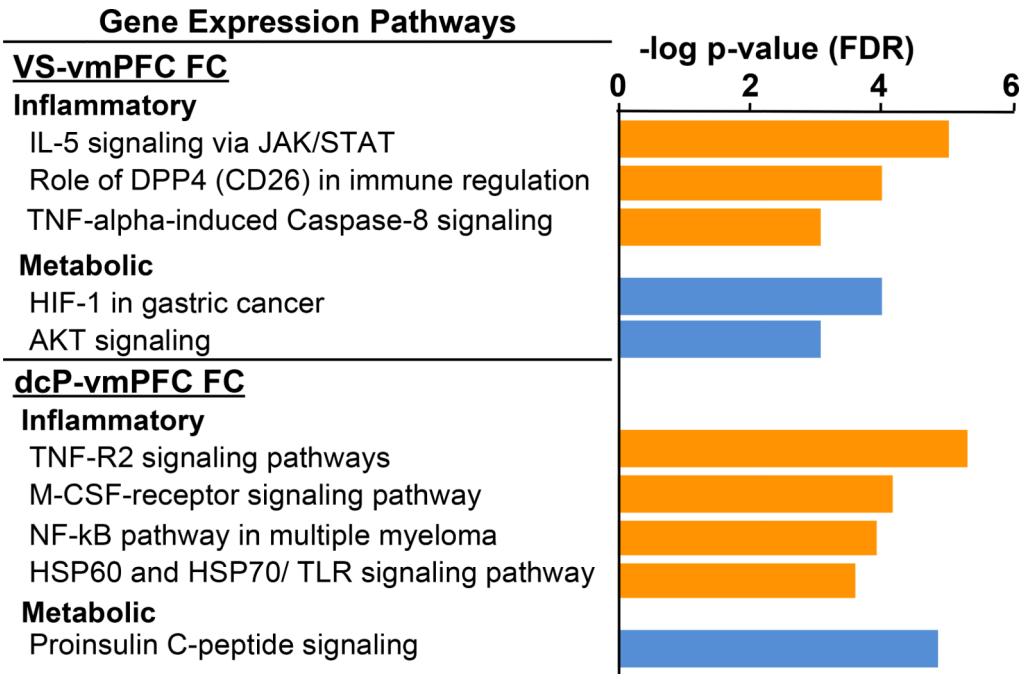

Figure 3. Gene expression pathways enriched in genes negatively associated with ventral striatum (VS)-ventromedial prefrontal cortex (vmPFC) and dorsal caudal putamen (dcP)-vmPFC functional connectivity (FC).

Pathways related to both inflammation and metabolism were in the top pathways significantly enriched (FDR $p<0.05$) in the genes negatively correlated with VS-vmPFC and dcP-vmPFC FC in 48 medically-stable, medication free outpatients with major depression. Orange = inflammatory and blue = metabolic-related pathways.

Table 1.

Demographic and clinical variables of the study sample, and their relationship to the glucose-related composite score and peripheral inflammation as measured by plasma CRP.

Variable	Mean (s.d.) (n=48)	Correlation with Glucose Composite Score, r (p)	Correlation with CRP, r (p)
Demographic and CRP			
Age (years)	38.3 (10.9)	0.314 (0.043)	−0.086 (0.561)
Sex, Male (*n*, %)	14 (29.2)	0.164 (0.301)	0.129 (0.382)
Race			
Caucasian (n, %)	18 (37.5)	−0.287 (0.065)	−0.071 (0.629)
African American (n, %)	30 (62.5)		
BMI (kg m^{-2})	31.3 (7.6)	0.426. (0.005)	0.619 (<0.001)
CRP (mg/L)	1.5 (1.6)	0.328 (0.034)	-
Metabolic marker (n=42)			
Glucose Composite Score	0.0 (2.43)	-	0.328 (0.034)
Glucose (mg/dL)	110.8 (19.6)	0.330 (0.033)	−0.198 (0.208)
Insulin (U/mL)	54.3 (34.4)	0.427 (0.005)	0.454 (0.003)
Leptin (pg/mL)	13957.5 (5227.0)	0.521 (<0.001)	0.532 (<0.001)
Adiponectin (ng/mL)	1272.7 (430.7)	0.554 (<0.001)	−0.022 (0.891)
Resistin (pg/mL)	2379.9 (2319.7)	0.652 (<0.001)	0.391 (0.01)

Abbreviations: BMI - Body Mass Index; CRP - C-reactive protein

Table 2.

Loading factors for Principle Component Analysis of FC, CRP, and metabolic markers

	VS-vmPFC Factor			dcP-vmPFC Factor		
Marker	**1**	**2**	**3**	**1**	**2**	**3**
FC	−0.535			−0.764		
CRP	0.785			0.756		
Leptin		−0.725			0.740	
Insulin	0.780			0.698		
Glucose		0.908			−0.901	
Adiponectin			0.920			0.907
Resistin	0.505		0.504	0.593		0.531

Data reflect factors after Varimax rotation with Kaiser Normalization and variable contributions CRP - C-reactive protein; dcP- dorsal caudal putamen; FC - functional connectivity; vmPFC - ventromedial prefrontal cortex; VS - ventral striatum