Triglyceride Level is Predicted by Reduced Functional Connectivity Between Nodes of Salience and Default Networks

A Thesis

SUBMITTED TO THE FACULTY OF THE

UNIVERSITY OF MINNESOTA BY

Matthew Rogers

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF ARTS

Advisor: Colin DeYoung

April 2024

Abstract

Metabolic disorders like insulin resistance and dyslipidemia can indirectly affect brain activity and have separately been found to be associated with depressive symptoms. In addition, previous research has shown that Depression Disorder (MDD) is associated with specific differences in cross-network connectivity. Yet, no research has analyzed how metabolic variables are associated with the differences in network-level brain connectivity in general, and whether they map onto the patterns characteristic of MDD. Using data from patients in the Leipzig study for Mind-Body-Emotion Interactions (LEMON) (N = 193), we investigated whether metabolic variables (concentration of triglycerides, HDL, the ratio of triglycerides to HDL (TRIG/HDL), hemoglobin a1c) predict the level of internetwork connectivity between the so-called triple networks. HDL and hemoglobin a1c did not significantly predict connectivity between any networks, while TRIG/HDL was significantly predicted by reduced SN-FPCN connectivity, and triglyceride levels was significantly predicted by reduced SN-DN and reduced SN-FPCN connectivity. In a more fine-grained analysis, we found that these metabolic variables were specifically predicted by connections between the DN lateral parietal areas and SN nodes (predicting triglycerides), and connections between the LPFC area and the SN Anterior Insula nodes (predicting triglycerides and TRIG/HDL). This finding shows no resemblance to the connectivity patterns observed in depression, either in our sample or in previous depression research, and therefore serves as a reminder that the link between peripheral metabolism and depression is highly indirect, and metabolic theories will face challenges when expected to operate at the same level of analysis that is used to describe the mechanisms underlying depression.

Table of Contents

List of Tables

List of Figures

Introduction

Metabolic psychiatry is a recent movement in psychiatric research that studies the ways that metabolic abnormalities such as oxidative stress, insulin resistance, and inflammation may contribute to psychopathology (Palmer, 2022). A search on Web of Science yielded 332 articles in 2003 mentioning "metabolic psychiatry" and 1801 articles in 2023, suggesting that a metabolic perspective on psychopathology is gaining currency.

"Metabolic syndrome," an increasingly common medical problem, is a convenient topic of study because the body's systems for regulating levels of glucose, insulin, and lipids have clear dependencies on one another such that extreme concentrations of these variables tend to cluster together. When blood glucose concentration is outside the normal range, peripheral metabolic homeostasis is maintained via a negative feedback loop in which insulin secretion from pancreatic beta cells trigger fat and muscle cells to increase their absorption of glucose until blood sugar returns to the normal range. Insulin resistance occurs when persistently high exposure to insulin (resulting from persistently high blood sugar) causes cells to become desensitized to its increase. Type II Diabetes occurs when cells have become so insulin resistant that the pancreas cannot secrete enough insulin to reduce blood glucose levels to the normal range. Hyperinsulinemia is also associated with dyslipidemia, or abnormal levels of triglycerides, LDL, and HDL. Increased insulin affects liver and adipose tissue by inhibiting the breakdown of lipids into fatty acids, while stimulating the conversion of excess glucose into fatty acids and then triglycerides, the main form of stored fat in the body (i.e., insulin decreases lipolysis and increases lipogenesis). Persistent insulin also stimulates production of VLDL in the liver, which decreases HDL (Jamkhande, et al., 2014). Due to the clear effect of insulin on lipid metabolism,

triglyceride level and the ratio of triglycerides to HDL are often used as proxies for insulin resistance (McLaughlin, et al., 2005).

The bi-directional association between metabolic syndrome (as a clustering of extreme metabolic variables) and Major Depressive Disorder (MDD) is one of the most frequently cited findings in metabolic psychiatry. In animal studies, depression-like behavior can be reversed by treating insulin resistance, and insulin resistance is associated with factors related to depression such as decreased neurogenesis, neuroinflammation, dysregulation of mitochondria, and depression-like behaviors (Lyra e Silva, et al., 2019). One longitudinal study asked whether depression increases the risk of metabolic syndrome (thus establishing causal directionality), finding that depression appears to predict higher risk of developing metabolic syndrome in women but not in men (Raikkonen, et al., 2002).

The strongest evidence that metabolic syndrome may contribute to depression (and not only vice versa) comes from longitudinal studies showing that metabolic syndrome predicts onset of major depression. In the Whitehall II study (Marmot, et al., 1991), 5,232 middle aged participants were tested for depressive symptoms and metabolic syndrome and then tested again six years later for depressive symptoms. Later analysis found that metabolic syndrome (defined as scoring high on a cluster of metabolic risk factors) predicted increased risk for onset of symptoms of depression with an odds ratio of 1.38. The individual variables that significantly predicted depression were components of dyslipidemia: central adiposity, high triglyceride levels, and low HDL cholesterol levels (Akbaraly, et al., 2009). In another study, Watson et al. (2021) tested whether insulin resistance, rather than metabolic syndrome as a whole, may predict depression. The study used three proxy measures of insulin resistance (high triglyceride-HDL

ratio, fasting plasma glucose level, and waist circumference) in a group of participants with no history of depression or anxiety to see whether high scores on the measures were associated with higher rate of onset of MDD over a 9-year follow-up period. They found that each of the measures predicted incident MDD, with TRIG/HDL ratio predicting it the most highly (hazard ratio = 1.89) followed by fasting plasma glucose level (1.37) and high waist circumference (1.11).

Despite the evidence that metabolic abnormalities may contribute to depression, there is very little understanding of the neural mechanisms that mediate this relationship. Some research has studied the effects of peripheral metabolic variables on resting state brain activity, but it has proved inconclusive. For example, one metanalysis of 16 fMRI studies (total sample size, N = 825) found that patients with Type II diabetes mellitus showed resting-state hypoactivity in the right medial superior frontal gyrus, right superior temporal gyrus, and left lingual gyrus, and hyperactivity in the right cerebellum (Liu, et al., 2021). Another meta-analysis of 8 fMRI studies (total sample size, N = 640) found that type II diabetes mellitus was associated with reduced resting-state activity in the bilateral lingual gyrus, left postcentral gyrus, right inferior temporal gyrus, right cerebellar culmen, right insula and right posterior cingulate cortex (Xia, et al., 2017). The results from these two meta-analyses show little overlap and are far from easily interpretable, and therefore they provide little help when searching for the neural mechanisms underlying the link between metabolic syndrome and MDD.

Though metabolic disorder predicts onset depression, no studies have used fMRI data to investigate the association between metabolic syndrome and depression. If the functional connectivity differences associated with concentration of metabolic variables resemble the

connectivity differences we see in MDD, then this would indicate that some metabolic variables might somehow contribute to MDD via indirect effects on large-scale brain networks and their interaction with each other. Testing this hypothesis in full would require a mediation analysis, and to be sufficiently powered such an analysis would require a large sample size with a large fraction of depressed participants. Unfortunately, the dataset used in the present study had only a small number of depressed participants (see Participants section, below). Nevertheless, the sample provided a good opportunity to explore the relationship between metabolic variables and resting state functional connectivity. We hypothesized that metabolic variables would be predicted by patterns of functional connectivity which resemble those connectivity patterns associated with MDD.

What are the major differences in functional connectivity associated with MDD? Research on the relationship between MDD and large-scale brain networks has often studied how depression is associated with altered connectivity within and between the so-called “triple networks": the Default Network (DN), the Salience Network (SN), and the Fronto-Parietal Control Network (FPCN). A meta-analysis found that MDD was associated with increased connectivity between FPCN and regions of the DN (Kaiser, et al., 2015). Also associated with MDD was increased connectivity within the DN (putatively reflecting habits of self-referential thought) and decreased connectivity within FPCN. One study found disrupted activity in the MPFC hub of the DN, and other studies found disruption in the ability of the MPFC/ACC hub to mediate internetwork interactions (Sheline et al., 2010, Treadway and Pizzagalli, 2014). A study by Manoliu (2014) found that major depressive disorder was associated with increased FC

between the SN and DN, and found that decreased FC within the right anterior insula (SN) was correlated with symptom severity.

Given the extensive body of work studying the associations between depression and disrupted connectivity between and within the triple networks, we decided to focus on analyzing how connectivity within and between nodes of these major networks could be used to predict metabolic variables. Another reason for focusing on the triple networks is that they are located at the top of the cortical hierarchy and are highly involved in the most integrated and complex functions for regulating attention, emotion, and self-referential thinking -- processes often disrupted in people with depression (Chand, et al., 2016).

The hypothesis can now be phrased more precisely: can metabolic variables be predicted by decreased connectivity within FPCN, increased connectivity within DN, increased DN-FPCN connectivity, or increased SN-DN connectivity? To test these hypotheses, we used a dataset containing resting-state fMRI data and a set of metabolic parameters. We constructed models to investigate relationships between connectivity and metabolic variables at three levels of analysis: first, we tested whether metabolic variables were predicted by gross averages of all within-network and between-network ROI-to-ROI connections in the triple networks; second, we tested if metabolic variables were predicted by average correlations between individual pairs of networks; and finally, we tested whether metabolic variables could be predicted by specific ROI-to-ROI correlations.

Method

Participants

We used the Leipzig Study for Mind-Body-Emotion Interactions (LEMON) dataset, containing 227 healthy participants (Babayan, et al., 2019). Exclusion criteria included: hypertension, cardiovascular disease, positive drug anamnesis, and any history of malignant disease, neurological disorders, or hospitalization from psychiatric disease. Participants were separated by age into a young group (N = 153, 20-35 years, 45 female) and an elderly group (N=74, range 59-77 years, 37 female). The assessments were administered on two separate days: the first included a cognitive test battery, resting state MRI scanning, blood pressure, physiological measurements, and a blood sample; the second included resting-state EEG and a follow-up emotion and personality test battery. 34 participants were excluded for reasons described below, leaving 193 for analyses.

Measures

Depressive symptoms were measured using the Hamilton Depression Scale (HAM-D; Hamilton, 1960). Blood samples were collected after the first fMRI scan and sent to the Institute for Laboratory Medicine at the University of Leipzig for analysis. The metabolic panel included measures of triglycerides, LDL, HDL, hemoglobin a1c, and CRP. Blood glucose level was recorded, but discarded in this study because it was not taken after fasting. Age was recorded as a binary variable (indicating the young vs. old group) and in a 5-year age range (e.g., '20 - 25'). The following analyses control for age by using a continuous variable that was created by taking the midpoints of the age range for each participant.

fMRI Data Acquisition

High-resolution T1-weighted MPRAGE structural images were acquired for anatomical surface registration with the following parameters: Sagittal acquisition orientation; one 3D

volume with 176 slices; TR=5000 ms; TE=2.92 ms; TI1=700 ms; TI2=2500 ms; FA1=4°; FA2=5°; pre-scan normalization; echo spacing=6.9 ms; bandwidth=240 Hz/pixel; FOV=256 mm; voxel size= 1 mm isotropic; GRAPPA acceleration factor 3; slice order=interleaved; duration=8 min 22 s (Babayan, et al., 2019).

Additionally, a T2-weighted gradient echo echo planar imaging (EPI) multiband BOLD rs-fMRI scan was acquired to enable functional connectivity analyses. Participants were told to stay awake and lie still with their eyes open while looking at a low-contrast fixation cross. To enable correction for geometric distortions in EPI images from rs-fMRI, a gradient echo fieldmap scan and two pairs of spin echo EPI images with reversed phase encoding direction were acquired. The scanning parameters were specified as follows: Axial acquisition orientation; phase encoding=A ≫ P; voxel size=2.3 mm isotropic; FOV=202 mm; imaging matrix=88×88; 64 slices with 2.3 mm thickness; TR=1400 ms; TE=30 ms; flip angle=69°; echo spacing=0.67 ms; bandwidth=1776 Hz/pixel; partial fourier 7/8; no pre-scan normalization; multiband acceleration factor=4; 657 volumes; slice order=interleaved; duration=15 min 30 s (Babayan, et al., 2019).

fMRI Preprocessing

Using the CONN toolbox, the anatomical data were normalized into standard MNI space, segmented into grey matter, white matter, and CSF tissue classes, and resampled to 1 mm isotropic voxels using the SPM unified segmentation and normalization algorithm (Ashburner & Friston, 2005; Ashburner, 2007) with the default IXI-549 tissue probability map template. We used functional data from the T2 resting scan preprocessed by Babayan et al. (2019) using Nipype. Their preprocessing steps included: 1) discarding the first five EPI volumes to allow for signal equilibration and steady state, 2) 3D motion correction (FSL MCFLIRT; Jenkinson, et al.,

2002), 3) distortion correction (FSL FUGUE; Jenkinson, et al., 2012), 4) rigid-body coregistration of unwarped temporal mean image to the individual's anatomical image, using FreeSurfer bbregister (Greve, et al., 2009), 5) denoising with Nipype rapidart and aCompCor (Behzadi, et al., 2017), 6) band-pass filtering between 0.01-0.1 Hz (FSL), mean-centering, and variance normalization of the resulting time series (Nitime; Rokem, et al., 2009), 7) spatial normalization to MNI152 2 mm standard space via transformation parameters that were derived from structural preprocessing (ANTs SyN; Avants, et al., 2011). The ROI locations were defined using CONN's default atlas of network seeds, which contained 31 ROIs belonging to 8 networks (4 ROIs were included in DN, 7 in SN, and 4 in FPCN). See Figure 1 for the ROIs across all networks contained in the atlas. After preprocessing, the structural and functional images were in MNI-space so that they were coregistered with the ROI files, which were already in MNI-space.

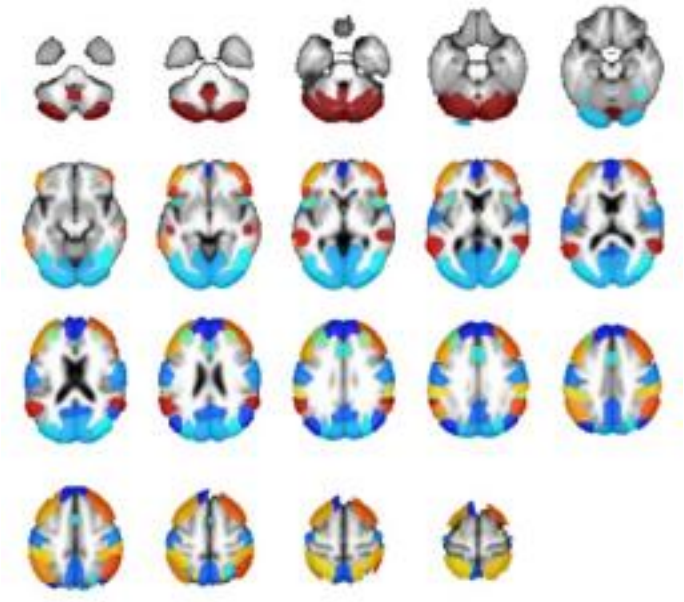

Figure 1: ROIs from CONN's Network Atlas, including 31 ROIs across 8 networks. (Analyses only used ROIs from DN (royal blue), SN (light green), and FPCN (gold)).

Note: picture from Nieto-Castanon, A. (2020).

In the course of preprocessing, the images were visually inspected for proper alignment and segmentation, and scans were discarded for several participants (N = 3) due to being improperly aligned. Other scans were excluded because they did not contain both a structural and a functional image (N = 6). Also, the study originally intended to analyze EEG variables along with fMRI, and some scans (N = 25) were omitted because they did not have data from each scan. These scans were not re-added later due to time constraints, but it is reasonable to assume that they were randomly distributed and therefore their absence will not systematically bias the results.

Additional denoising was performed on the preprocessed functional data using a standard denoising pipeline (Nieto-Castanon, 2020) including the regression of potential confounding effects characterized by white matter timeseries (5 CompCor noise components), CSF timeseries (5 CompCor noise components), session effects and their first order derivatives (2 factors), grey matter timeseries (1 components) (Behzadi, et al., 2007), and linear trends (2 factors) within each functional run, followed by bandpass frequency filtering of the BOLD timeseries (Hallquist, et al., 2013) between 0.008 Hz and 0.09 Hz. CompCor (Behzadi, et al., 2007; Chai, et al., 2012) noise components within white matter and CSF were estimated by computing the average BOLD signal as well as the largest principal components orthogonal to the BOLD average within each subject's eroded segmentation masks. From the number of noise terms included in this denoising strategy, the effective degrees of freedom of the BOLD signal after denoising were estimated to be 146.3 across all subjects (Nieto-Castanon, 2022).

Statistical Analysis

The timeseries among the voxels in each ROI were averaged to calculate a timeseries for each ROI. We calculated ROI-to-ROI correlations for each subject and transformed each using Fisher's r to z transformation. To summarize internetwork and intranetwork covariance, we created a variable representing the average connectivity values for all within-network ROI-to-ROI connections for each subject, and another variable for the average connectivity values for all between-network ROI-to-ROI connections. Before testing the metabolic variables, we explored whether the LEMON sample showed associations between functional connectivity and depression. First, intra- and inter-network means were used as predictors of depression in a multiple regression model, controlling for age and gender. Second, we computed the mean connectivity between pairs of the three triple networks (DN-SN, SN-FPCN, FPCN-DN) by averaging the connectivity values for all ROI-to-ROI connections that were associated with a given pair of networks. We then used these mean internetwork connectivity values as predictors of depression in a multiple regression model, controlling for age and gender. Next, we used a set of multiple regressions with each individual ROI-to-ROI connection as a predictor of depression.

The hypothesis that internetwork and intranetwork connectivity would predict metabolic variables was tested with a multiple regression model that used the internetwork and intranetwork means as simultaneous predictors of metabolic variables, controlling for age and gender. Using the metabolic measures as the criterion variables provided the advantage of controlling for the shared variance of internetwork and intranetwork means. The results of these analyses motivated more detailed analyses of the internetwork connections. Multiple regressions were used to investigate which inter-network connections and, later, which ROI-to-ROI connections significantly predicted TRIG. Results included in this manuscript come from

analyses performed using CONN (Whitfield-Gabrieli, et al., 2012) release 22.a (Nieto-Castanon, et al., 2022) and SPM release 12.7771 (Penny, et al., 2011).

Results

Descriptive statistics for depression and metabolic variables are presented in Table 1, and zero-order correlations among the same variables are presented in Table 2.

Table 1: Descriptive statistics for measures of metabolic variables and depression

Measure	Mean	Standard Deviation
HAM-D (0 - 23)	2.48	2.64
TRIG (mmol/l)	1.35	0.79
HDL (mmol/l)	1.65	0.45
TRIG/HDL	0.95	0.83
HBA1C (mmol/mol)	34.03	3.73

Table 2. Correlation Matrix Among Depression, Metabolic Variables, and Intra- and Inter-network Connectivity Means

	HAM-D	TRIG	HDL	TRIG /HDL	HBA1 C	Intra-network mean	Inter-network mean

HAM-D	--	-0.08	-0.05	-0.06	-0.01	0.07	0.05
TRIG	0.29	--	**-0.42**	**0.94**	**0.20**	0.05	**-0.26**
HDL	0.48	<0.001	--	**-0.58**	-0.04	-0.06	0.09
TRIG/HDL	0.38	<0.001	<0.001	--	**0.21**	0.05	**-0.21**
HBA1C	0.93	0.01	0.62	<0.001	--	0.01	-0.12
Intra-network mean	0.37	0.53	0.44	0.53	0.93	--	**-0.30**
Inter-network mean	0.46	<0.001	0.21	<0.001	0.09	<.001	--

Note: The top half presents Pearson correlations between each of the major measurements (significant results in bold), including Hamilton's depression scale, metabolic variables, and the intra-network/inter-network means. The bottom half presents the corresponding p-values.

Initial tests revealed that depression score was not related to any other variables. Table 1 shows that depression score was not significantly correlated to any of the other metabolic variables. Table 2 shows the results of a multiple regression in which neither intra- nor inter-network mean connectivity were significant predictors of depression.

For a more granular view, we used a multiple regression to test whether the HAM-D score could be predicted by mean connectivity within and between pairs of the triple networks, controlling for age and gender. The results are presented in Table 3. Depression was significantly

predicted by SN-FPCN mean connectivity (**β** = 0.15; p = 0.047) and FPCN-DN mean connectivity (**β** = 0.20; p = 0.02).

Table 3: Multiple Regression with Depression Being Predicted by Mean Connections Within and Between Triple Networks

Criterion	**Predictor**	**β**	***t***	***p***	***N***	**df**	***F***	***R^2***
HAM-D					190	181	2.63	0.104
	DN-SN	-0.01	-0.08	0.94				
	SN-FPCN	0.15	2.00	0.047				
	FPCN-DN	0.20	2.30	0.02				
	DN-DN	0.07	0.90	0.37				
	SN-SN	0.05	0.73	0.47				
	FPCN-FPCN	-0.01	-0.16	0.87				

Next, we investigated whether any specific ROI-to-ROI connections predicted depressive symptoms, and if so, whether these connections were related to the connectivity differences believed to be associated with MDD. To this end, a set of 105 linear regressions (one for each connection between 15 ROIs) tested whether depression scores could be predicted by individual ROI-to-ROI correlations between nodes of the triple networks, controlling for age and gender. 14

connections were nominally significant predictors before correction for multiple testing. After correction using the Benjamini and Hochberg (2000) method, depressive symptoms were not significantly predicted by any of the connections.

Subsequent analysis left out the variable of depressive symptoms and tested our hypothesis by looking for associations between brain activity and metabolic variables. Table 4 presents the results of the multiple regression with intranetwork and internetwork means simultaneously predicting the metabolic variables. HDL, hemoglobin a1c, and the ratio of triglycerides to HDL were not significantly predicted by either mean. Triglyceride level was not significantly predicted by mean intra-network connectivity ($p = 0.38$), but was significantly predicted by mean inter-network connectivity ($\beta = -0.26$; $p < 0.001$).

Table 4: Linear Regressions with Average Within- and Between-Network Correlations Predicting Metabolic Variables and Depression Score

Criterion	**Predictors**	**β**	***t***	***p***	***N***	**df**	***F***	***R^2***
HAM-D					190	185	2.46	0.05
	Within-network mean	0.11	1.46	0.15				
	Between-network mean	0.08	1.01	0.31				
TRIG					186	181	8.85	0.16
	Within-network mean	-0.06	-0.87	0.38				

	Between-network mean	-0.26	-3.71	<.001				
HDL					186	181	16	0.26
	Within-network mean	0.02	0.31	0.76				
	Between-network mean	0.06	0.92	0.36				
TRIG/HDL					186	181	7.32	0.14
	Within-network mean	-0.05	-0.70	0.48				
	Between-network mean	-0.20	-2.80	0.01				
HBA1C					184	179	33.7	0.43
	Within-network mean	-0.06	-0.94	0.35				
	Between-network mean	-0.11	-1.74	0.08				

Because correlation between ROIs in the same network is higher than the correlation of ROIs between networks, it seemed possible that this finding could be the result of an artifact: perhaps the greater ROI-to-ROI connectivity within networks would make the within-network ROI-to-ROI correlations more consistent across participants, and there would not be enough variance for TRIG to be a good predictor of the within-network correlations. To investigate this

possibility, we calculated the variance of each ROI-to-ROI connection. The mean variance of all within-network connections was 0.060 and the mean variance of all between-network correlations was 0.056, indicating that their different associations with TRIG are not a result of insufficient variance.

The significant association between the inter-network mean and two metabolic variables (triglyceride levels and TRIG/HDL) justified a network-level analysis of these two variables. A multiple regression was used to test whether triglyceride level could be predicted by mean connectivity between pairs of each of the three networks, controlling for age and gender. Another multiple regression used the same intra- and internetwork connectivity means to predict TRIG/HDL. The results are presented in Table 5. Mean connectivity between DN and SN negatively predicted triglyceride level (**β** = -0.26; p = 0.004), as did mean connectivity between SN and FPCN (**β** = -0.14; p = 0.047). No other predictors showed significance. When predicting TRIG/HDL, only mean connectivity between SN and FPCN (**β** = -0.14; p = 0.04) was a significant predictor.

Table 5: Multiple Regressions with Triglycerides and TRIG/HDL Being Predicted by Mean Connections Within and Between Triple Networks

Criterion	**Predictor**	**β**	***t***	***p***	***N***	**df**	***F***	***R^2***
Triglycerides					186	177	4.55	0.171
	DN-SN	-0.26	-2.88	0.004				

	SN-FPCN	-0.14	-2.00	0.047				
	FPCN-DN	-0.09	-0.99	0.32				
	DN-DN	-0.02	-0.32	0.75				
	SN-SN	-0.06	-0.82	0.42				
	FPCN-FPCN	-0.04	-0.62	0.54				
TRIG/HDL					186	177	4.29	0.16
	DN-SN	-0.16	-1.74	0.08				
	SN-FPCN	-0.14	-2.03	0.04				
	FPCN-DN	0.01	0.10	0.92				
	DN-DN	-0.02	-0.24	0.81				
	SN-SN	-0.02	-0.32	0.75				
	FPCN-FPCN	-0.10	-1.46	0.15				

To investigate these results in more detail, we used a set of multiple regressions to test the ability of each between-network ROI-to-ROI connection to predict triglyceride levels while controlling for age and gender. The significance values were corrected for multiple testing (BHST method) only of the between-network effects. In a separate set of tests, we performed the same analyses with the within-network ROI-to-ROI connections, correcting the significance

values for the within-network tests. Figure 2 presents the combined results from both sets of regressions as a matrix of p-values. The connections which significantly predict triglyceride level after the FDR correction are marked with a bold outline in Figure 2, and their associated statistics are displayed in Table 6. The same set of tests were performed using TRIG/HDL as the criterion, and only two connections showed significance as predictors (see Table 6 for regression statistics).

Figure 2 shows that ten connections showed significance as predictors of triglycerides. Interestingly, eight of these significant results were connections between the right/left Lateral Parietal area (DN) and nodes of the SN. Connections between the right LPFC (FPCN) and right and left Anterior Insula (SN) were the other two significant predictors of triglycerides. The latter two connections were the only significant predictors of TRIG/HDL. All connections negatively predicted the criterion, matching the finding in Table 5 showing that triglycerides were negatively predicted by DN-SN connectivity, and both triglycerides and TRIG/HDL were negatively predicted by SN-FPCN connectivity.

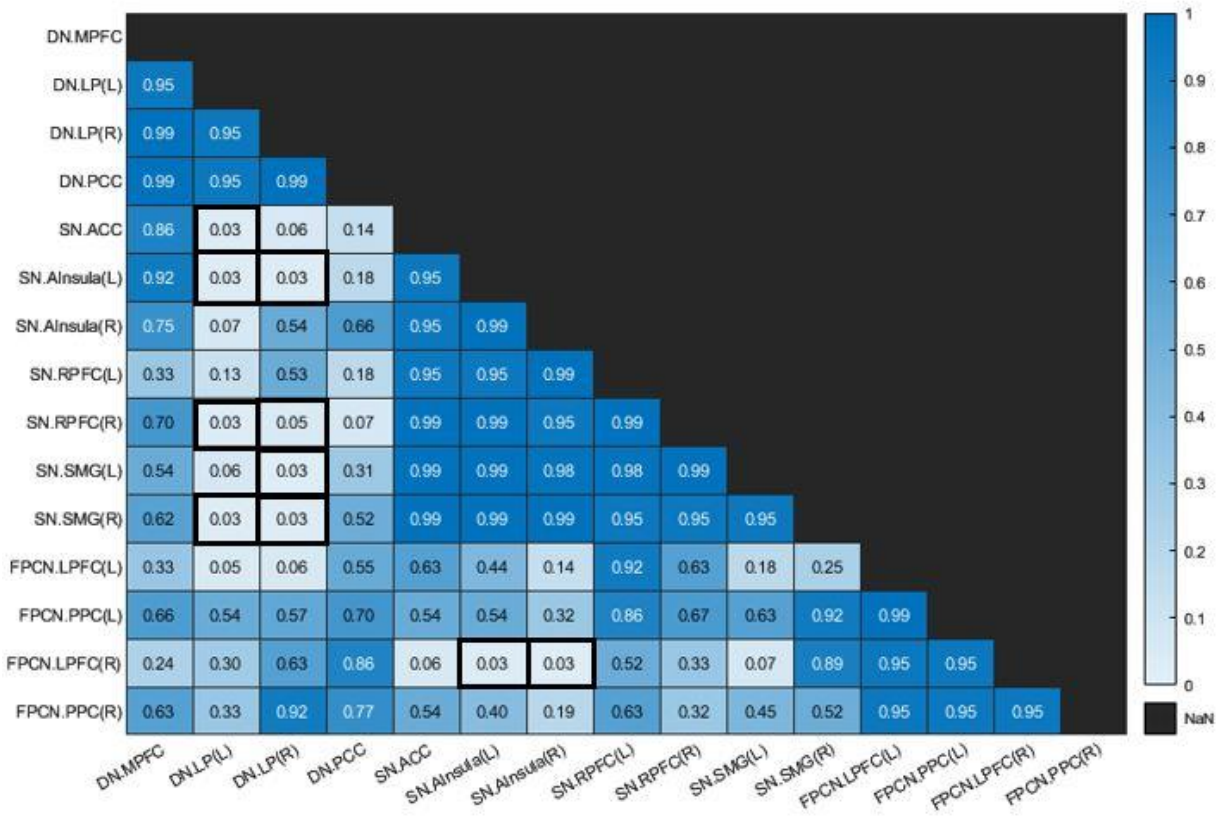

Figure 2: ROI-ROI connectivity statistics (FDR-corrected p-values) for a model that predicts triglyceride levels from connectivity values (corrected for age and gender). Bold outline indicates $p < 0.05$.

Criterion	**Predictors**	**β**	***t***	***p***	***p-FDR***	***N***	**df**	***F***	***R^2***
TRIG									
	DN.LateralParietal (L) – SN.ACC	-.20	-2.93	0.004	.03	186	182	9.9	0.14
	DN.LateralParietal (L) - SN.AInsula (L)	-.20	-3.00	0.003	.03	186	182	10.8	0.15

	DN.LateralParietal (L) – SN.RPFC (R)	-.22	-3.21	0.002	.03	186	182	10.5	0.15
	DN.LateralParietal (L) – SN.SMG (R)	-.21	-3.04	0.003	.03	186	182	10.1	0.14
	DN.LateralParietal (R) – SN.Ainsula (L)	-.20	-2.88	0.004	.03	186	182	9.79	0.14
	DN.LateralParietal (R) – SN.RPFC (R)	-.19	-2.77	0.006	.05	186	182	9.55	0.14
	DN.LateralParietal (R) – SN.SMG (L)	-.24	-3.43	0.001	.03	186	182	11.1	0.14
	DN.LateralParietal (R) – SN.SMG (R)	-.21	-3.09	0.002	.03	186	182	10.3	0.15
	SN.Insula (L) – FPCN.LPFC (R)	-.19	-2.82	0.005	.03	186	182	9.7	0.14
	SN_Insula (R) – FPCN.LPFC (R)	-.24	-3.53	0.001	.03	186	182	11.3	0.16
TRIG/ HDL									
	SN.Insula (L) – FPCN.LPFC (R)	-.23	-3.43	0.001	.03	186	182	11.3	0.16
	SN.Insula (R) – FPCN.LPFC (R)	-.25	-3.72	<.001	.02	186	182	12	0.17

Table 6: ROI-to-ROI connections that significantly predict triglyceride level after FDR correction

Discussion

The results showed that depressive symptoms were predicted by increased SN-FPCN connectivity and FPCN-DN connectivity. The former finding is novel, while the latter was

consistent with the meta-analysis by Kaiser et al. (2015), which found that MDD was associated with increased connectivity between FPCN and regions of DN. Our results did not replicate within-network effects from the meta-analysis, however, such as the association between increased connectivity within DN and decreased connectivity within FPCN (Kaiser, et al., 2015).

The more central hypothesis that metabolic variables would be associated with the connectivity patterns associated with depression was motivated by evidence for the linkage between metabolic syndrome and depression. The results reported here fail to support this hypothesis. Within-network connectivity did not significantly predict any metabolic variables at any level of analysis. The fact that triglycerides and TRIG/HDL were predicted by reduced FPCN-SN connectivity is orthogonal to the between-network connectivity differences associated with depression . In one respect, patterns of between-network connectivity that predicted triglycerides were the opposite of what we expected: the finding that lower DN-SN connectivity predicts triglyceride level is in direct tension with Manoliu's (2014) finding that *higher* DN-SN FC is associated with depression.

Though unpredicted, our results nonetheless constitute a significant finding indicating that triglyceride level is associated with differences in cross-network connectivity. There are no clear theoretical reasons for predicting that triglycerides would be associated with the left lateral parietal node's connection with SN nodes or LPFC connections with the anterior insula, in particular. What follows is a brief summary of possible pathways which may be contributing (non-exclusively) to the reported effect and should be considered highly speculative.

Triglyceride level and TRIG/HDL are often used as a proxy for peripheral insulin resistance (McLaughlin, et al., 2015), and there are several lines of evidence showing that

peripheral insulin resistance and the accompanying excess of insulin may contribute to altered ROI-to-ROI connectivity. Acute peripheral hyperinsulinemia does not necessarily translate to excess levels of brain insulin because the insulin receptors on the blood brain barrier (BBB) are saturable, but evidence shows that some regions of the BBB might still allow transport of excess insulin (Banks, et al., 2012). Once crossing the BBB, excess insulin can affect brain activity in a number of ways. First, insulin receptors are widespread throughout the brain, and evidence suggests that when activated they play a role in regulating neuroplasticity and offer neuroprotective effects (Scherer, et al., 2021). Second, insulin has been shown in an animal studies to have effects on dopaminergic (DA) and serotoninergic neurons. Martin et al. (2022) found that insulin inhibits the activity of serotonergic neurons in the dorsal raphe nucleus by acting on 5-HT1A autoreceptors. Meanwhile, Gruber et al. (2023) report that insulin modulates dopamine release differently in different areas, increasing DA when supplied to the striatum and inhibiting DA when supplied to the ventral tegmental area (Krupa, et al., 2024). Third, although glucose uptake in the brain is almost always insulin-independent (Banks & Lim, 2006), some studies show some neurons in hypothalamus, hippocampus, and cerebellum have insulin-sensitive glucose receptors (Schulingkamp, et al., 2000), leaving open the possibility that insulin can affect neural firing in these regions by affecting neural metabolism. As mentioned previously, none of these pathways would be predicted to affect the DN-SN ROIs in particular, but indirect effects leading to these areas are at least conceivable.

Moreover, some evidence shows that triglycerides can affect brain activity directly. Triglycerides are known to pass through the BBB and block the ability of leptin and insulin to activate their respective receptors in the hypothalamus of mice (Banks et al., 2018). One study

showed that ingestion of polyunsaturated lipids decreased the BOLD response during a motor task and suggested that this was a result of blood lipids affecting cerebral vasculature (Noseworthy, et al., 2003), but a follow up study showed blood total triglyceride content after a meal did not directly alter the hemodynamic BOLD response to neural activity (Slade, et al., 2009). Another study showed that triglycerides being metabolized within the mesocorticolimbic (MCL) system can affect the quantity of lipoproteinlipase (LPL) which can gate the activity of dopamine receptor subtype 2 (Berland, et al., 2020). Triglycerides can also activate the insulin receptor on brain endothelial cells, which allows more insulin transport across the blood-brain barrier and can induce central insulin resistance (Urayama & Banks, 2008).

An alternative explanation is that, rather than insulin or triglycerides affecting DN-SN connectivity, the latter may be associated with traits relating to dietary habits and therefore triglyceride levels. For example, low Conscientiousness is associated with higher triglyceride levels (Sutin, et al., 2010). A study by Sassenberg et al. (2023) showed in three samples that Conscientiousness is associated with resting-state connectivity in the salience network, which has been hypothesized to enable the prioritization of some goals over others and the suppression of disruptive impulses, functions consistent with Conscientiousness. Sassenberg et al. did not investigate connectivity between networks, but given that Conscientiousness is associated with SN connectivity, it might well also be associated with SN-FPCN or DN-SN connectivity. In that case, one explanation of the findings above might be that the connections which significantly predict triglyceride levels might be associated with low Conscientiousness, which would contribute to higher triglyceride levels because it would be associated with an inability to resist cravings and maintain a healthy diet. Indeed, since low Conscientiousness is associated with

depression (Hakulinen et al., 2015; Kotov et al., 2010), it is reasonable to speculate that low Conscientiousness might serve as a common underlying factor contributing to both metabolic issues and depression, and hence their association.

Limitations

This study was limited by the nature of the LEMON dataset. As mentioned in the introduction, a relatively small sample size and a lack of depressed participants prevented us from testing whether functional connectivity differences mediate the relationship between triglycerides and depression. A sample including more people with more severe problems, such as diagnosed MDD or metabolic syndrome, may have provided clearer results.

Another limitation was the method we used to identify brain networks. The location of each ROI and its network membership was pre-specified by the atlas, and network-network connectivity was calculated simply by averaging the connectivity values of each of the node-to-node correlations across the two networks. A method like Group Prior Individualized Parcellation (GPIP) would have allowed us to adjust the boundaries of the ROIs in a cortical atlas to fit each subject's unique pattern of functional connectivity, creating custom cortical maps that more closely approximate the location of network nodes for each participant (Sassenberg et al., 2023). This method would be more effective at identifying voxels that covary due to neural activity rather than artifacts, and would be useful for constructing networks with minimal a priori assumptions about their structure.

Conclusion

The results of these analyses provide no evidence that metabolic variables contribute to the differences in large-scale network interaction that are associated with depression. Though

previous longitudinal studies suggest that metabolic syndrome can contribute to onset of depression, specifying the underlying neural mechanism will likely be challenging because metabolic and psychopathological research study phenomena that operate on very different levels of analysis. Effects of metabolism on brain function have mostly been studied on the small scale of specific chemical pathways and neural receptors that show sensitivity to metabolic compounds. Meanwhile, depression is a type of persistent emotional dysregulation that operates at the scale of the whole person, and is therefore more conducive to analysis at the level of large-scale brain networks. The result serves as a reminder to fields like metabolic psychiatry to be cautious when theorizing about the specific mechanisms by which metabolic variables may contribute to psychopathology, and warns that the bridge between the two is long, indirect, and complicated.

Bibliography

Akbaraly, T. N., KIVIMaki, M. I. K. A., Brunner, E. J., Chandola, T., Marmot, M. G., Singh-Manoux, A., & Ferrie, J. E. (2009). Association between metabolic syndrome and depressive symptoms in middle-aged adults: results from the Whitehall II study. *Diabetes care*, *32*(3), 499-504.

Ashburner, J., & Friston, K. J. (2005). Unified segmentation. Neuroimage, 26(3), 839-851.

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *Neuroimage*, *38*(1), 95-113.

Avants, B. B. et al. (2011). A reproducible evaluation of ANTs similarity metric performance in brain image registration. *NeuroImage*, *54*, 2033–2044.

Babayan, A., Erbey, M., Kumral, D., Reinelt, J. D., Reiter, A. M., Röbbig, J., ... & Villringer, A. (2019). A mind-brain-body dataset of MRI, EEG, cognition, emotion, and peripheral physiology in young and old adults. *Scientific data*, *6*(1), 1-21.

Banks, W. A., & Lim, W. S. (2006). Insulin and the Blood-Brain Barrier. *Blood-Brain Barriers: From Ontogeny to Artificial Interfaces*, *1*, 265-285.

Banks, W. A., Owen, J. B., & Erickson, M. A. (2012). Insulin in the brain: there and back again. *Pharmacology & therapeutics*, *136*(1), 82-93.

Banks, W. A., Farr, S. A., Salameh, T. S., Niehoff, M. L., Rhea, E. M., Morley, J. E., ... & Craft, S. (2018). Triglycerides cross the blood–brain barrier and induce central leptin and insulin receptor resistance. *International journal of obesity*, *42*(3), 391-397.

Behzadi, Y., Restom, K., Liau, J. & Liu, T. T. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *NeuroImage 37*, 90–101 (2007).

Benjamini, Y., & Hochberg, Y. (2000). On the adaptive control of the false discovery rate in multiple testing with independent statistics. Journal of educational and Behavioral Statistics, 25(1), 60-83.

Berland, C., Montalban, E., Perrin, E., Di Miceli, M., Nakamura, Y., Martinat, M., ... & Luquet, S. H. (2020). Circulating triglycerides gate dopamine-associated behaviors through DRD2-expressing neurons. *Cell metabolism*, *31*(4), 773-790.

Chai, X. J., Nieto-Castanon, A., Ongur, D., & Whitfield-Gabrieli, S. (2012). Anticorrelations in resting state networks without global signal regression. *Neuroimage*, *59*(2), 1420-1428.

Chand GB, Dhamala M. Interactions Among the Brain Default-Mode, Salience, and Central-Executive Networks During Perceptual Decision-Making of Moving Dots. (2016). *Brain Connect,* Apr, *6*(3): 249-54. doi: 10.1089/brain.2015.0379. Epub 2016 Jan 27. PMID: 26694702.

Greve, D. N. & Fischl, B. (2009). Accurate and robust brain image alignment using boundary-based registration. *NeuroImage 48*, 63–72.

Gruber, J., Hanssen, R., Qubad, M., Bouzouina, A., Schack, V., Sochor, H., ... & Thanarajah, S. E. (2023). Impact of insulin and insulin resistance on brain dopamine signalling and reward processing–an underexplored mechanism in the pathophysiology of depression?. *Neuroscience & Biobehavioral Reviews*, 105179.

Hallquist, M. N., Hwang, K., & Luna, B. (2013). The nuisance of nuisance regression: spectral misspecification in a common approach to resting-state fMRI preprocessing reintroduces noise and obscures functional connectivity. *Neuroimage, 82,* 208-225.

Hamilton, M. (1960). A rating scale for depression. J. Neurol Neurosurg Psychiatry 23, 56–62.

Jamkhande, P. G., Chandak, P. G., Dhawale, S. C., Barde, S. R., Tidke, P. S., & Sakhare, R. S. (2014). Therapeutic approaches to drug targets in atherosclerosis. *Saudi Pharmaceutical Journal*, *22*(3), 179-190.

Jenkinson, M., Bannister, P., Brady, M. & Smith, S. (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage 17*, 825–841.

Jenkinson, M., Beckmann, C. F., Behrens, T. E., Woolrich, M. W. & Smith, S. M. (2012). FSL. *NeuroImage 62*, 782–790.

Kaiser, R. H., Andrews-Hanna, J. R., Wager, T. D., & Pizzagalli, D. A. (2015). Large-scale network dysfunction in major depressive disorder: a meta-analysis of resting-state functional connectivity. *JAMA psychiatry, 72*(6), 603-611.

Kotov R, Gamez W, Schmidt F, Watson D. (2010). Linking "big" personality traits to anxiety, depressive, and substance use disorders: a meta-analysis. *Psychol Bull.* Sep 136(5):768-821. doi: 10.1037/a0020327. PMID: 20804236

Kullmann, S., Heni, M., Veit, R., Ketterer, C., Schick, F., Häring, H. U., ... & Preissl, H. (2012). The obese brain: association of body mass index and insulin sensitivity with resting state network functional connectivity. *Human brain mapping, 33*(5), 1052-1061.

Liu, J., Li, Y., Yang, X., Xu, H., Ren, J., & Zhou, P. (2021). Regional spontaneous neural activity alterations in type 2 diabetes mellitus: a meta-analysis of resting-state functional MRI studies. *Frontiers in Aging Neuroscience*, *13*, 678359.

Lyra e Silva, N. D. M., Lam, M. P., Soares, C. N., Milev, R., & De Felice, F. G. (2019). Insulin resistance as a shared pathogenic mechanism between depression and type 2 diabetes. *Frontiers in psychiatry*, *10*, 420372.

Manoliu, A., Meng, C., Brandl, F., Doll, A., Tahmasian, M., Scherr, M., ... & Sorg, C. (2014). Insular dysfunction within the salience network is associated with severity of symptoms and aberrant inter-network connectivity in major depressive disorder. *Frontiers in human neuroscience*, *7*, 930.

Marmot, M., Davey Smith, G., Stansfeld, S. (1991). Health inequalities among British civil servants: the Whitehall II study. *Lancet*; *337* : 1387-93.

Martin, H., Bullich, S., Martinat, M., Chataigner, M., Di Miceli, M., Simon, V., ... & Fioramonti, X. (2022). Insulin modulates emotional behavior through a serotonin-dependent mechanism. *Molecular psychiatry*, 1-10.

McLaughlin, T., Reaven, G., Abbasi, F., Lamendola, C., Saad, M., Waters, D., ... & Krauss, R. M. (2005). Is there a simple way to identify insulin-resistant individuals at increased risk of cardiovascular disease? *The American journal of cardiology*, *96*(3), 399-404.

Nieto-Castanon, A. & Whitfield-Gabrieli, S. (2022). CONN functional connectivity toolbox: RRID SCR_009550, release 22. doi:10.56441/hilbertpress.2246.5840.

Nieto-Castanon, A. (2020). FMRI denoising pipeline. In Handbook of functional connectivity Magnetic Resonance Imaging methods in CONN (pp. 17-25). Hilbert Press.

Nieto-Castanon, A. (2020). Handbook of functional connectivity Magnetic Resonance Imaging methods in CONN. *Boston, MA: Hilbert Press.*

Nieto-Castanon, A. (2022). Preparing fMRI Data for Statistical Analysis. In M. Filippi (Ed.). fMRI techniques and protocols. Springer. doi:10.48550/arXiv.2210.13564

Noseworthy MD, Alfonsi J, Bells S (2003): Attenuation of brainBOLD response following lipid ingestion. *Hum Brain Mapp 20*:116–121.

Palmer, C. M. (2022). *Brain Energy: A Revolutionary Breakthrough in Understanding Mental Health--and Improving Treatment for Anxiety, Depression, OCD, PTSD, and More*. BenBella Books.

Penny, W. D., Friston, K. J., Ashburner, J. T., Kiebel, S. J., & Nichols, T. E. (Eds.). (2011). Statistical parametric mapping: the analysis of functional brain images. Elsevier.

Räikkönen K, Matthews KA, Kuller LH. (2002). The relationship between psy-chological risk attributes and the metabolic syndrome in healthy women:antecedent or consequence? *Metabolism, 51*, 1573–1577

Rokem, A., Trumpis, M. & Perez, F. (2009). Nitime: time-series analysis for neuroimaging data. *Proceedings. of the 8th Python in Science Conference, 2*, 68–75.

Rueter, A. R., Abram, S. V., MacDonald III, A. W., Rustichini, A., & DeYoung, C. G. (2018). The goal priority network as a neural substrate of Conscientiousness. *Human Brain Mapping*, *39*(9), 3574-3585.

Scherer, T., Sakamoto, K., & Buettner, C. (2021). Brain insulin signalling in metabolic homeostasis and disease. *Nature Reviews Endocrinology*, *17*(8), 468-483.

Schulingkamp, R. J., Pagano, T. C., Hung, D., & Raffa, R. B. (2000). Insulin receptors and insulin action in the brain: review and clinical implications. *Neuroscience & Biobehavioral Reviews*, *24*(8), 855-872.

Sheline, Y. I., Price, J. L., Yan, Z., & Mintun, M. A. (2010). Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. *Proceedings of the National Academy of Sciences*, *107*(24), 11020-11025.

Sutin, A. R., Terracciano, A., Deiana, B., Uda, M., Schlessinger, D., Lakatta, E. G., & Costa Jr, P. T. (2010). Cholesterol, triglycerides, and the Five-Factor Model of personality. *Biological psychology*, *84*(2), 186-191.

Treadway, M. T., & Pizzagalli, D. A. (2014). Imaging the pathophysiology of major depressive disorder-from localist models to circuit-based analysis. *Biology of mood & anxiety disorders*, *4*, 1-13.

Urayama, A., & Banks, W. A. (2008). Starvation and triglycerides reverse the obesity-induced impairment of insulin transport at the blood-brain barrier. *Endocrinology*, *149*(7), 3592-3597.

Watson, K. T., Simard, J. F., Henderson, V. W., Nutkiewicz, L., Lamers, F., Nasca, C., ... & Penninx, B. W. (2021). Incident major depressive disorder predicted by three measures of insulin resistance: a Dutch cohort study. *American Journal of Psychiatry*, *178*(10), 914-920.

Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain connectivity*, *2*(3), 125-141.

Xia, W., Chen, Y. C., & Ma, J. (2017). Resting-state brain anomalies in type 2 diabetes: a meta-analysis. *Frontiers in Aging Neuroscience*, *9*, 14.