

Atypical Antipsychotic Exposure May Not Differentiate Metabolic Phenotypes of Patients with Schizophrenia

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STUDY OBJECTIVE Patients with schizophrenia are known to have higher rates of metabolic disease than the general population. Contributing factors likely include lifestyle and atypical antipsychotic (AAP) use, but the underlying mechanisms are unknown. The objective of this study was to identify metabolomic variability in adult patients with schizophrenia who were taking AAPs and grouped by fasting insulin concentration, our surrogate marker for metabolic risk.

DESIGN Metabolomics analysis

PARTICIPANTS Ninety-four adult patients with schizophrenia who were taking an AAP for at least 6 months, with no changes in their antipsychotic regimen for the previous 8 weeks, and who did not require treatment with insulin, participated in the study. Twenty age- and sex-matched nonobese (10 subjects) and obese (10 subjects) controls without cardiovascular disease or mental health diagnoses were used to match the body mass index (BMI) range of the patients with schizophrenia to account for metabolite concentration differences attributable to BMI.

MEASUREMENTS AND MAIN RESULTS Existing serum samples were used to identify aqueous metabolites (to differentiate fasting insulin concentration quartiles) and fatty acids with quantitative nuclear magnetic resonance and gas chromatography methods, respectively. To exclude metabolites from our pathway mapping analysis that were due to variability in weight, we also subjected serum samples from the non-obese and obese controls to the same analyses. Patients with schizophrenia had a median age of 47.0 years (interquartile range 41.0–52.0 years). Using a false discovery rate threshold of less than 25%, 10 metabolites, not attributable to weight, differentiated insulin concentration quartiles in patients with schizophrenia and identified variability in one-carbon metabolism between groups. Patients with higher fasting insulin concentrations (quartiles 3 and 4) also trended toward higher levels of saturated fatty acids compared with patients with lower fasting insulin concentrations (quartiles 1 and 2).

CONCLUSION Our results illustrate the utility of metabolomics to identify pathways underlying variable fasting insulin concentration in patients with schizophrenia. Importantly, no significant difference in AAP exposure was observed among groups, suggesting that current antipsychotic use may not be a primary factor that differentiates middle-aged adult patients with schizophrenia by fasting insulin concentration.

KEY WORDS adverse drug reactions, Insulin, antipsychotics.
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Cardiovascular disease (CVD) is a major cause of premature mortality in patients with schizophrenia.^{1, 2} It is not fully understood why these patients experience more CVD than the general population, but medication is likely an important contributing factor. Atypical antipsychotics (AAPs), in particular, have been identified as culprits because of their tendency to cause adverse metabolic effects such as weight gain, dyslipidemias, and diabetes mellitus. These adverse events are consistent with components of metabolic syndrome (MetS),³ a well-known risk factor for CVD. In 2004, the American Psychiatric Association collaborated with the American Diabetes Association and others to describe monitoring recommendations for the development of MetS in AAP users.⁴ Unfortunately, these recommendations are not often followed in practice,⁵ stressing a need for improved understanding of the underlying mechanisms of MetS to develop more precise approaches for monitoring and preventing adverse metabolic side effects.

An alternative option for assessing CVD risk in AAP users is fasting insulin concentration associated with insulin resistance and future development of prediabetes and diabetes.^{6, 7} The development of insulin resistance was also observed before weight gain in response to AAPs,^{8, 9} and it is considered an essential component of the definition of MetS.¹⁰ Therefore, we reasoned that a hypothesis-generating metabolomics study would identify unique metabolite signatures associated with variable fasting insulin concentrations in AAP users with schizophrenia and provide added insight on the biological pathways differentiating patients with schizophrenia who have variable CVD risk. We also expected to see greater exposure to AAPs in general and increased use of more obesogenic AAPs (such as clozapine and olanzapine) in participants with relatively higher fasting insulin concentrations. We therefore conducted a metabolomics analysis of existing serum samples from adult patients with schizophrenia, grouped by log-transformed fasting insulin concentration quartiles, using quantitative ID-¹H-nuclear magnetic resonance (NMR) spectroscopy and a gas chromatography assay for the detection of fatty acids (FAs). Because the median body mass indexes (BMIs) of patients in each fasting insulin quartile were different, we also assayed existing serum samples from obese (BMI 30 kg/m² or higher) and nonobese (BMI lower than 30 kg/m²) subjects without CVD or mental health diagnoses who were matched to the schizophrenia cohort by sex, age, and BMI range to account for metabolite concentration differences attributable to BMI. AAP use was compared across quartiles with two approaches: normalizing exposure using chlorpromazine equivalents; and comparing percentages of patients using AAPs divided into three groups based on their propensity to cause weight gain.

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Methods

Study Design, Patients, and Data Collection

For this metabolomics pilot study, existing serum samples from patients with schizophrenia were selected from a large ongoing cross-sectional study on CVD risk in schizophrenia. Inclusion criteria for the parent study were 18–

90 years of age; *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*,¹¹ diagnosis of schizophrenia, schizophreniform disorder, schizoaffective disorder, or psychotic disorder not otherwise specified; and taking an antipsychotic for at least 6 months with no changes in antipsychotic regimen for the previous 8 weeks. Exclusion criteria were inability to give informed consent, presence of diabetes mellitus type 2 diagnosis before initiation of antipsychotic therapy, and an active substance abuse diagnosis. For inclusion in this pilot study, patients had to be taking an AAP and not require treatment with insulin.

For the parent study, anthropometric measurements and venipuncture were performed at the University of Michigan Clinical Research Unit (MCRU; <http://www.michr.umich.edu/services/mcru>). A dietician collected information on dietary recall from all participants, and information on past and current medication use was collected by the study team. The parent study protocol was approved by the University of Michigan institutional review board (IRBMED HUM00017774) and the IRBs of the Washtenaw County Health Organization, the Detroit-Wayne County Community Mental Health Agency, and the Ann Arbor Veterans Affairs Medical Center. The study was performed in accordance with the ethical standards of the Helsinki Declaration of 1975 (as revised in 1983).

AAP Exposure Standardization and Risk Categories

Atypical antipsychotic exposure was standardized between groups using formulas previously described¹² to calculate chlorpromazine equivalents for aripiprazole, clozapine, olanzapine, quetiapine, risperidone, and ziprasidone. The equations for calculating chlorpromazine equivalents for paliperidone, iloperidone, and lurasidone were obtained from a previous study.¹³ Because AAPs are known to have a varying propensity to cause adverse metabolic side effects, medication use was further described by risk group, as follows: high (olanzapine or clozapine), moderate (quetiapine, risperidone, iloperidone, or paliperidone), and low (aripiprazole, ziprasidone, and lurasidone).^{14–17} When participants were taking more than one AAP, the medication with the highest risk was used to group the participant into a medication risk category. Antipsychotic polypharmacy was also described

as the use of two to three antipsychotics (typical or atypical).

Controls

Existing serum samples from a cohort of patients who were enrolled in the University of Michigan's Weight Management Program (NCT02043457)^{18, 19} and technical replicate serum samples from healthy subjects who were recruited from the Claude D. Pepper Older Americans Independence Center Research Participant Program at the University of Michigan's Geriatric Center^{18–20} were used as controls. To be included in the weight management program, participants were required to meet a minimum BMI of 32 kg/m² with at least one comorbidity or a BMI of at least 35 kg/m².^{18, 19} For this pilot study, these additional exclusion criteria were applied: present CVD or diabetes, psychiatric disorder, and use of weight-potentiating medication such as steroids. From these cohorts, two control groups (without CVD or mental health diagnoses) were formed to match the range of BMI of the patients with schizophrenia. The first group was selected to have a mean BMI lower than 30 kg/m² (nonobese) and the second higher than 30 kg/m² (obese). All were matched to the schizophrenia cohort for sex and age range. The protocols under which the control data were acquired were also approved by IRBMED (obese controls: HUM00030088, non-obese controls: HUM00038122), and all participants in the schizophrenia and BMI control groups provided informed consent.

Serum Sample Preparation and Fasting Insulin Assay

Whole blood from fasting patients with schizophrenia was collected by direct venipuncture into additive-free collection tubes, allowed to coagulate for at least 30 minutes and then centrifuged (2500 × g for 10 min) to generate serum. Serum was aliquoted into vials for storage (–80°C) until time of assay except for exposure to one freeze-thaw cycle for an analysis related to the parent study. The blood collections from obese and nonobese participants were similar and were previously described.^{18–20} For this pilot study, an additional aliquot (minimum of 125 µL) of frozen serum from participants with schizophrenia and negative controls was sent to the Michigan Diabetes Research Center

to assess fasting insulin concentration by radioimmunoassay.

Before the time of assay, serum samples were randomized into batches of 10 for extraction and NMR analysis. At the time of assay, samples were subjected to a water (H₂O)/methanol (MeOH):chloroform (CHCl₃) extraction as previously described.^{21, 22} The dried CHCl₃ fraction was transported to the Michigan Comprehensive Regional Metabolomics Core (MRC2) for FA analysis.

Quantitative 1D-¹H-NMR Metabolomics and Gas Chromatography to Detect Free FA

The 1D-¹H-NMR spectra of the H₂O/MeOH fraction of each sample were acquired at the University of Michigan's NMR Biochemical Core Laboratory on a Varian (now Agilent Inc., Santa Clara, CA, USA) 11.74-T (500 MHz) NMR spectrometer. This process was previously described in detail,²³ and further description of the methods are provided in the supplementary information in addition to a representative NMR spectrum (Figure S1). The CHCl₃ fractions containing the lipids of the serum samples were assayed for FA content, using an Agilent 5890 gas chromatograph with an Agilent HP 88 column, by the MRC2 using a modified technique previously described.²⁴ A representative gas chromatogram can also be found in the supporting information (Figure S2).

Statistical and Pathway Analysis

For the patients with schizophrenia, groups were created by separating their log-transformed fasting insulin concentrations into quartiles. The quantified metabolomics data sets were transformed and scaled in MetaboAnalyst v.3.0 (<http://www.metaboanalyst.ca/>) to achieve normal distribution in preparation for parametric statistical analyses.^{25, 26} The FA data were analyzed as relative percentage of total to account for different initial sample volumes. Normalized data were analyzed by analysis of variance (ANOVA) with a Tukey-Kramer post hoc test, and demographic and clinical data were compared with Wilcoxon, ANOVA, or χ^2 tests, as appropriate in JMP Pro v.11 (SAS Institute, Inc., Cary, NC, USA). For the control participants, metabolite mean concentrations were compared between BMI groups with an unpaired Student *t* test. For all normalized mean metabolite comparisons, Welch's corrections were applied when

variance between groups was not similar. Daily AAP exposure was calculated by translating total AAP medication dose into chlorpromazine equivalents.^{12, 13} To control for multiple comparisons, a false discovery rate (FDR) of the resulting post hoc *p* values was calculated for each metabolite using the method previously described.²⁷ Metabolites with an FDR lower than 25% were used in pathway analysis by Metscape (<http://metscape.ncibi.org/>),²⁸ a plug-in for Cytoscape (<http://www.cytoscape.org/>). Figures were constructed using R (<https://www.r-project.org/>).²⁹

Results

Participant Characteristics

Patients with Schizophrenia

Ninety-four patients with schizophrenia were included in this study. Patients were grouped by log-transformed insulin concentration quartile that was different across all four quartiles (Figure 1A). Of note, several metabolic parameters were significantly different among schizophrenia groups (*p*<0.05) including BMI (Figure 1C) and MetS diagnosis (Table 1). Cumulative average AAP dose exposure was available for 96.8% (91/94 patients) and was not significantly different among groups, and neither was the variability among quartiles when examining AAP risk groups. With respect to polypharmacy, the rates were not significantly different among quartiles (Table 1). A total of six patients were using a typical and atypical antipsychotic medication combination regimen: five were using fluphenazine and one was using haloperidol, in addition to an AAP. The participant taking haloperidol was also using olanzapine, and four participants taking fluphenazine were using moderate-risk AAPs; one was taking ziprasidone. Risk category was not modified for participants based on typical antipsychotic use. Time since schizophrenia diagnosis was assessed by group as a surrogate marker for approximate length of exposure to antipsychotics. This measure was also not significantly different between groups.

Obese and Nonobese Control Subjects

The median age of the 10 obese control participants was 46.5 years (interquartile range [IQR] 45.0–49.0 years) and 46.5 years (IQR 41.8–50.3 years) for the 10 nonobese

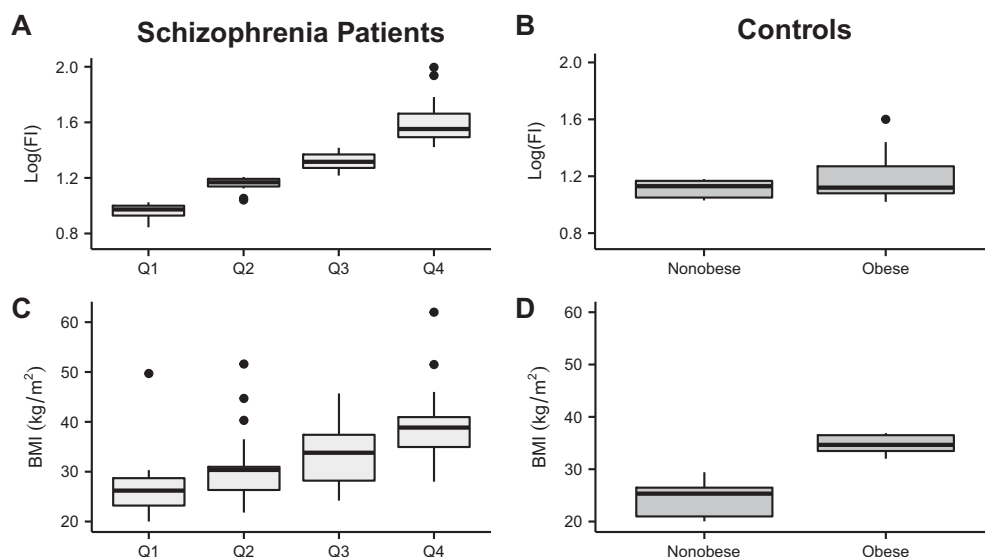


Figure 1. Box and whisker plots of log-transformed fasting insulin concentration (Log(FI)), measured as $\mu\text{U/ml}$, and body mass index (BMI) by quartile for the patients with schizophrenia (A and C, respectively) and by BMI group for the controls (B and D, respectively). The crossbar of the box indicates the median, the box spans the interquartile range, and the whiskers indicate the entire data range, with outliers identified as individual points. The schizophrenia quartiles (Q1–4) are designated by log(FI). BMI was significantly different among all of the schizophrenia groups ($p < 0.01$) with the exceptions of Q2 and Q3. Log(FI) was significantly different among all schizophrenia groups ($p < 0.05$). BMI was significantly different between the two obese and nonobese control groups ($p < 0.001$). The BMI of the obese control group was significantly greater than the BMI of schizophrenia quartiles Q1 ($p < 0.001$) and Q2 ($p = 0.003$). The BMI of the nonobese controls was significantly less than that of Q2 ($p = 0.003$), Q3 ($p < 0.001$), and Q4 ($p < 0.001$). Log(FI) was significantly different between the BMI control groups and all the schizophrenia quartiles, with the exception of the obese controls and Q2. Log(FI) was not significantly different between the nonobese and obese controls. Wilcoxon tests were used to compare BMI and fasting insulin concentrations among groups, and post hoc Wilcoxon each pair analyses were completed to identify differences within groups. For example, a Wilcoxon test noted that fasting insulin was significantly different between schizophrenia quartiles, and the post hoc tests identified that all quartiles varied significantly from each other by fasting insulin concentration.

participants ($p = 0.704$). Median log-transformed fasting insulin values were $1.13 \mu\text{U/ml}$ (IQR $1.05\text{--}1.17 \mu\text{U/ml}$) and $1.12 \mu\text{U/ml}$ (IQR $1.02\text{--}1.32 \mu\text{U/ml}$) for the nonobese and obese controls, respectively ($p = 0.500$) (Figure 1B). The median BMI for the nonobese and obese controls was 25.3 kg/m^2 (IQR $20.9\text{--}27.0 \text{ kg/m}^2$) and 34.7 kg/m^2 (IQR $33.4\text{--}36.7 \text{ kg/m}^2$), respectively ($p < 0.001$) (Figure 1D).

Quantitative ¹H-NMR Metabolomics and Pathway Analysis

Thirty-six metabolites were identified and quantified by ¹H-NMR in all serum samples. Of these, 13 ¹H-NMR detected metabolites differentiated (FDR less than 25%) the insulin quartiles based on the ANOVA p value (Table 2). To identify associated metabolic pathways, these compounds were entered into MetScape (<http://metscape.ncibi.org/>), an open-source bioinformatics platform that permits the visualization and identification of associated metabolic pathways.²⁸ Among resulting pathways, one-carbon

metabolism, and glycine, serine, alanine, and threonine metabolism were represented by at least three metabolites.

If metabolites were different (FDR less than 25%) between obese and nonobese controls, they were considered to be attributable to BMI and were removed from the MetScape analysis. However, if differentiating metabolites between the obese and nonobese controls were also represented as a differentiating metabolite in the schizophrenia analysis, but their concentrations trended in the opposite direction as weight (e.g., glycine), they were not removed from the MetScape analysis (Table 2). After this adjustment, we determined that differences in the concentrations of the remaining seven metabolites were influenced by insulin concentration (Figures 2A–N). MetScape mapping of these compounds generated a one-carbon metabolism pathway that involved three of these metabolites: serine, glycine, and taurine (Figure 3). Supporting information includes all differentiating metabolites (FDR less than 25%) from the control analyses (Table S1) and all pathways that

Table 1. Demographic and Clinical Characteristics of the Patients with Schizophrenia

Characteristic	Insulin concentration quartile ^a				p value ^b
	Q1 (n=25)	Q2 (n=22)	Q3 (n=25)	Q4 (n=22)	
BMI, kg/m ²	26.2 (23.2–29)	30.4 (26.2–31.2)	33.8 (28.0–37.9)	38.9 (34.3–41.6)	<0.001
Age, yrs	45.0 (34.5–50.5)	46.5 (41.0–50.5)	49.0 (42–53)	47.5 (40.8–53)	0.486
Metabolic syndrome diagnosis	6 (24.0)	13 (59.1)	14 (56.0)	19 (86.4)	0.002
Use of antidiabetic medication	3 (12.0)	5 (22.7)	2 (8.0)	4 (18.2)	0.502
Female sex	11 (44.0)	8 (36.4)	12 (48.0)	9 (40.9)	0.875
Average daily calorie intake, kcal, mean ± SD	1986.0 ± 652.3	1873.2 ± 563.9	1882.3 ± 628.2	1933.5 ± 499.5	0.890
White race	16 (64.0)	15 (68.2)	14 (56.0)	13 (59.1)	0.837
Smoker	15 (60.0)	12 (54.6)	9 (36.0)	11 (50.0)	0.370
Log-transformed fasting insulin concentration	0.973 (0.922–1.002)	1.169 (1.136–1.197)	1.316 (1.272–1.369)	1.552 (1.485–1.674)	<0.001
Time since schizophrenia diagnosis, yrs	17.0 (9.0–30.5)	20.0 (14.3–27.8)	26.0 (15.5–32.5)	16.0 (9.5–21.8)	0.342
Daily exposure to AAPs in chlorpromazine equivalents, mg	516.0 (247.2–795.8)	456.6 (334.7–669.6)	692.1 (379.5–1069.0)	516.8 (352.0–639.4)	0.092
AAP risk group ^c					
High	6 (24.0)	11 (50.0)	11 (44.0)	7 (31.8)	0.420
Moderate	16 (64.0)	7 (31.8)	10 (40.0)	12 (54.6)	
Low	3 (12.0)	4 (18.2)	4 (16.0)	3 (13.6)	
Antipsychotic polypharmacy ^d	8 (32.0)	2 (9.1)	10 (40.0)	6 (27.3)	0.115

Data are median (interquartile range) or no. (%) of patients unless otherwise specified.

AAP = atypical antipsychotic; BMI = body mass index.

^aInsulin concentration quartiles were based on log-transformed fasting insulin values, with Q1 corresponding to the lowest quartile and Q4 corresponding to the highest quartile.

^bThe χ^2 or analysis of variance p value as appropriate.

^cAAP risk groups were defined as follows: high = olanzapine or clozapine; moderate = risperidone, quetiapine, paliperidone, or iloperidone; low = aripiprazole, lurasidone, or ziprasidone.

^dAntipsychotic polypharmacy was defined as use of two to three total antipsychotics including first-generation (typical) and second-generation (atypical) antipsychotic medications.

Table 2. Serum Metabolites and Fatty Acids Identified and Quantified

	KEGG ID	ANOVA p value	False discovery rate (%)	Post hoc results ^a
Metabolites				
<i>Glutamate</i>	C00302	0.0001	0.36	Q3 > Q1; Q3 > Q2; Q4 > Q1; Q4 > Q2
Acetate	C00033	0.0057	10.26	Q2 > Q4; Q3 > Q4; Q1 > Q4
<i>Tyrosine</i>	C00082	0.0129	15.48	Q4 > Q1
3-hydroxybutyrate	C01089	0.0269	16.87	Q1 > Q4
Carnitine	C00487	0.0269	16.87	Q2 > Q4
Glycine	C00037	0.0286	16.87	Q1 > Q4
<i>Alanine</i>	C00133	0.0328	16.87	Q3 > Q1
Serine	C00065	0.0536	22.76	Q3 > Q4
Creatine phosphate	C02305	0.0690	23.84	Q3 > Q4
<i>Valine</i>	C00183	0.0842	23.84	
Taurine	C00245	0.0862	23.84	
<i>Proline</i>	C00148	0.0891	23.84	
<i>Leucine</i>	C00123	0.0927	23.84	
Fatty acids				
14:0	C06424	<0.0001	0.02	Q3 > Q1; Q4 > Q1; Q2 > Q1
16:1	C08362	<0.0001	0.02	Q3 > Q1; Q4 > Q1; Q3 > Q2; Q4 > Q2
18:2	C01595	0.0008	0.61	Q1 > Q4; Q1 > Q3; Q2 > Q4
16:0	C00249	0.0025	1.44	Q4 > Q1
24:0	C08320	0.0349	16.05	

Serum metabolites detected by ¹H-nuclear magnetic resonance and fatty acids detected with gas chromatography were identified as differentiating patients with schizophrenia across insulin concentration quartiles (false discovery rate less than 25%). Metabolites in italics and bold-face were those determined to be associated with body mass index (see text).

ANOVA = analysis of variance; Q = quartile.

^aPost hoc results were determined to be significant when Tukey-Kramer p values were less than 0.05.

were mapped with Metscape (Table S2). The ¹H-NMR and FA data sets are freely accessible via the Metabolomics Workbench (<http://www.metabolomicsworkbench.org/>).

Quantitative Fatty Acid Analysis

A total of 23 serum FAs were identified and quantified. In the schizophrenia cohort, significant differences were apparent in several saturated FAs, such as 16:0 (palmitic acid), 14:0 (myristic acid), 24:0 (lignoceric acid), and the unsaturated essential FA 18:2 (linoleic acid). Three FAs, 16:0, 18:2, and 24:0, were not associated with BMI and differentiated the four insulin quartiles of the schizophrenia cohort (Table 2 and Figures 4A–F).

Discussion

We conducted a metabolomics analysis of serum samples from patients with schizophrenia who were taking AAPs and analyzed the results based on their fasting insulin concentration quartiles. This approach permitted the detection of unique metabolic signatures associated with variable fasting insulin concentrations and demonstrated the utility of fasting insulin concentration as a biochemical anchor for the metabolic phenotyping of this population. We

discuss the importance of perturbed one-carbon metabolism as a contributing factor to cardiovascular risk in patients with schizophrenia. We suggest that analyzing CVD risk by fasting insulin concentration in this population provides insight into metabolic variances between AAP users because no significant differences were found in average daily AAP exposure or higher metabolic risk AAP use between the insulin quartiles. Furthermore, these findings were not solely attributable to differences in BMI.

Among the affected pathways identified from differentiating metabolites by Metscape, the folate cycle and methionine and cysteine metabolism intersect through their involvement in one-carbon metabolism that is essential for DNA methylation, purine synthesis, and homocysteine metabolism (Figure 3). In our cohort, concentrations of the methyl donors glycine and serine trended higher in the lower insulin quartiles. This suggests increased availability of one-carbon groups available for the folate cycle. In addition, serine is used in the formation of cystathionine from homocysteine, ultimately leading to production of the endogenous antioxidant glutathione. This is important because elevations in homocysteine have repeatedly been linked to CVD through mechanisms that are not yet understood.^{30, 31}

Perturbed one-carbon metabolism was also implicated in CVD risk in patients with

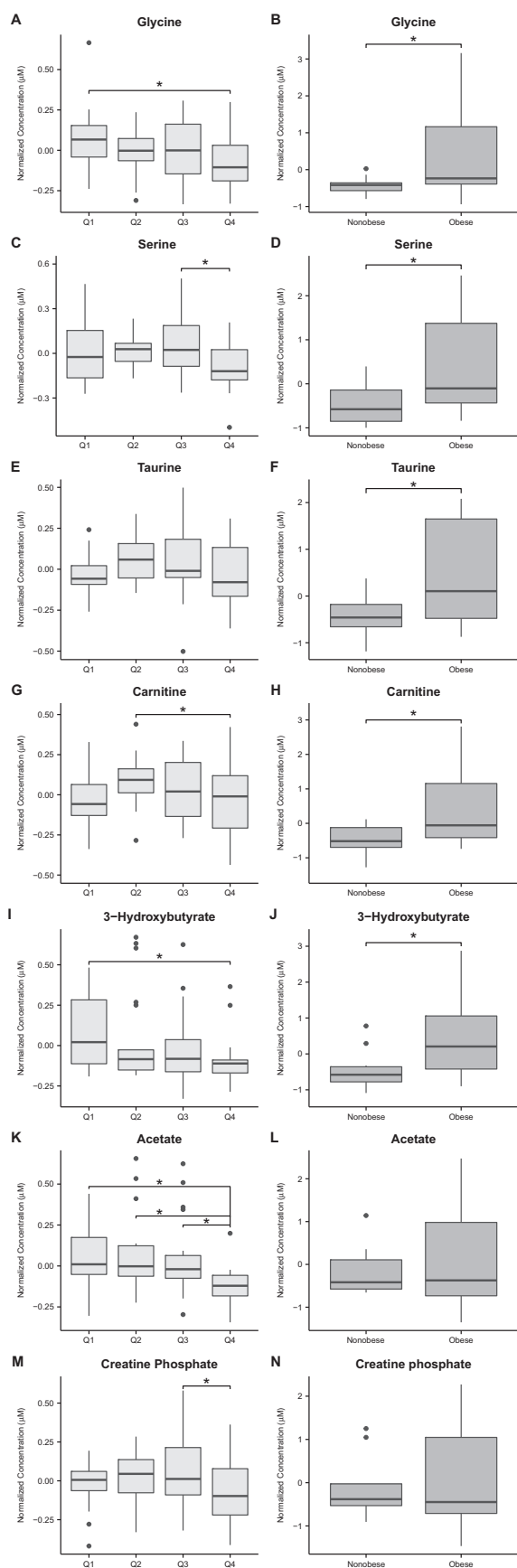


Figure 2. Box and whisker plots of normalized serum metabolite concentrations detected and quantified by ^1H -nuclear magnetic resonance (A–N) in the schizophrenia quartiles (A, C, E, G, I, K, and M) and body mass index (BMI) controls (B, D, F, H, J, L, and N). Metabolites shown in the schizophrenia column are those that differentiated insulin quartiles (analysis of variance false discovery rate $< 25\%$) that were determined to be independent of BMI (see text). This was determined if the Student t test results comparing the same normalized metabolite concentrations between the BMI control groups was not significant or the metabolite concentrations trended in opposite directions with weight in the BMI controls and schizophrenia groups (e.g., glycine). In the patients with schizophrenia, glycine (A) concentration trended lower but was increased in obese BMI controls compared with nonobese BMI controls (B). Serine (C and D), taurine (E and F), carnitine (G and H), and 3-hydroxybutyrate (I and J) concentrations followed the same trends. Acetate and creatine phosphate concentrations trended lower in patients with schizophrenia (K and M) but were not significantly different between nonobese and obese controls (L and N). Asterisks indicate metabolite concentrations in the schizophrenia group that were significantly different between quartiles (Tukey-Kramer post hoc $p < 0.05$) or between BMI controls (Student t test $p < 0.05$).

schizophrenia.³² Our group has observed decreased methylation capacity and worsening endothelial function in schizophrenia patients with genetic polymorphisms in folate cycle enzymes associated with hyperhomocysteinemia.^{33, 34} We also found that supplemental folate administration improved methylation capability and endothelial function in schizophrenia patients taking AAPs.³⁵ In addition, the folate cycle is intimately tied to FA metabolism because it is needed to contribute methyl groups for carnitine methylation and FA chain elongation and desaturation.³⁶ It was proposed in 2014³² that the complex relationship between FAs and the folate cycle in CVD and psychiatric disease could be described as oxidative stress leading to the one-carbon cycle spending proportionally more time on transsulfuration compared with methylation. This could decrease available methyl groups, resulting in less FA chain elongation and desaturation. We observed metabolite concentration changes that may agree with this hypothesis: saturated FAs were elevated in schizophrenia patients with higher fasting insulin concentrations (e.g., Q3 and 4), whereas patients with lower fasting insulin concentrations (e.g., Q1 and 2) had higher concentrations of methyl-donating amino acids. This pattern of elevation in saturated FAs was also observed in settings of CVD³⁷ in patients without mental health disorders.

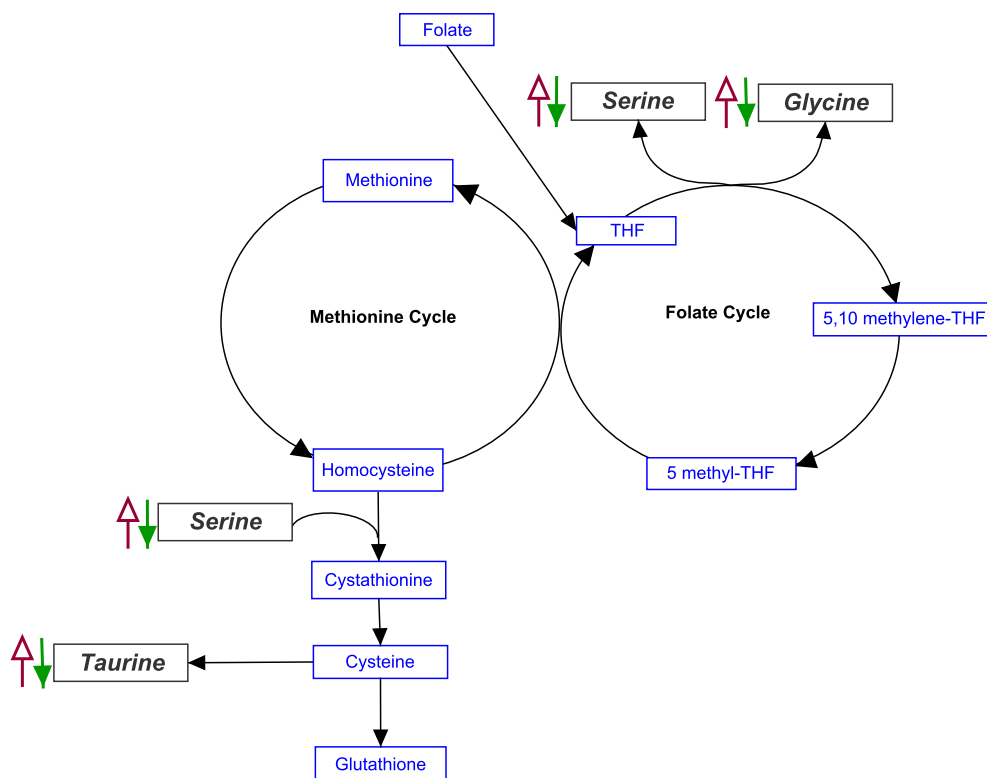


Figure 3. Simplified schematic diagrams of one-carbon metabolism. Arrows depict trend of metabolite concentration with increasing body mass index (BMI). Solid (green) arrows indicate differentiating metabolites whose concentrations trended inversely with weight among the schizophrenia quartiles. Open (red) arrows indicate differentiating metabolites whose concentrations increased with weight among the BMI controls. The inverse relationship between the concentration of these metabolites with BMI indicates that variability in one-carbon metabolism is not solely due to weight. THF = tetrahydrofolate. [Color figure can be viewed at wileyonlinelibrary.com]

Insulin concentration is known to be associated with weight, thus complicating our analysis. Both weight and fasting insulin concentration were significantly different between quartiles. To account for weight variability, we used two groups of control subjects, obese and nonobese participants without CVD or any psychiatric diagnosis, and completed the same metabolomics assays for this cohort. Importantly, fasting insulin concentration was not significantly different between groups. As such, we attributed differences in metabolite and FA concentrations between the BMI control groups as those that were primarily due to differences in weight. We used these results to interpret the metabolomics data from the schizophrenia cohort's insulin concentration quartiles. With respect to FAs, palmitic acid (16:0), linoleic acid (18:2), and lignoceric acid (24:0) remained uniquely altered in schizophrenia patients. Palmitic acid trended upward in Q3 and 4, whereas linoleic and lignoceric acids trended lower. Of these FAs, linoleic acid (18:2) is an essential FA and reflects variable diet. This suggests that linoleic acid intake

trended inversely with fasting insulin in the schizophrenia cohort.

To test the rigor of our analysis, we separated the schizophrenia participants into two groups (obese and nonobese) to act as their own controls for BMI variability and completed the same analyses performed for the BMI controls without psychiatric diagnoses or CVD. The table of differentiating metabolites from this analysis is provided in the supplement (Table S3). No differentiating $^1\text{H-NMR}$ metabolites were common to both the fasting insulin quartile analysis and the BMI analysis that were not already identified as associated with BMI by the control analysis (and indicated in Table 2). After a similar comparison of the FA differentiating the schizophrenia insulin quartiles and BMI groups, palmitic acid was the only remaining FA associated with fasting insulin concentration and not BMI. Unlike the BMI controls used for the primary analysis, log-transformed fasting insulin concentration differentiated the schizophrenia groups separated by obese or nonobese BMI classification (Wilcoxon test $p < 0.001$), making

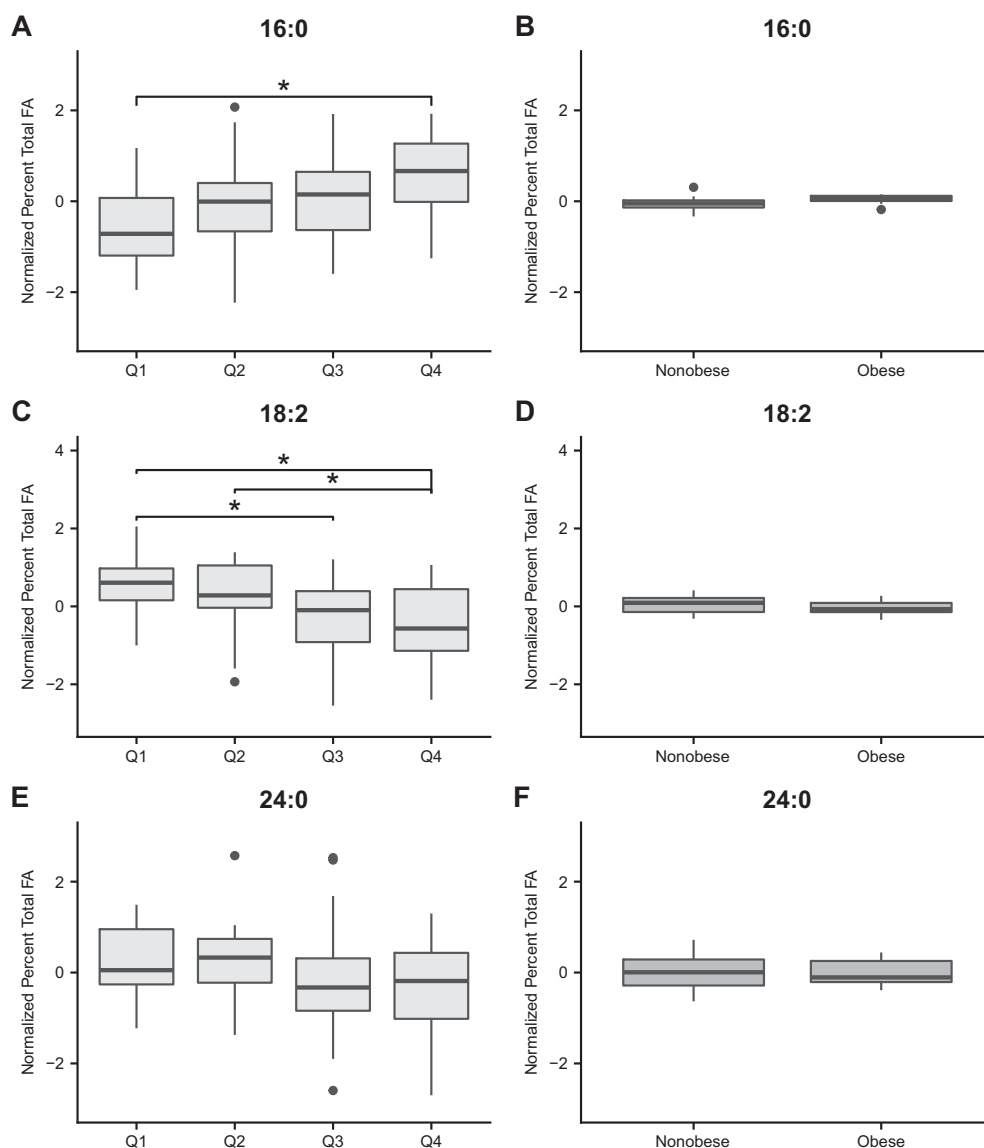


Figure 4. Box and whisker plots of normalized concentrations of individual fatty acids (FAs) by percentage total that differentiated insulin quartiles in patients with schizophrenia (analysis of variance false discovery rate < 25%; A, C, and E) but not body mass index (BMI) (B, D, and F). This was determined if the Student *t* test *p* values comparing the same normalized FA concentrations between the BMI control groups were not significant or if the FA concentrations were not associated with weight in the BMI schizophrenia groups. Asterisks indicate FAs that were significantly different (Tukey-Kramer post hoc $p < 0.05$) between quartiles. Q = quartile.

interpretation of these results less straightforward. Although not confirmatory, these results indicate that the metabolite variability of the schizophrenia cohort is neither entirely due to differences in BMI nor to AAP exposure between the insulin quartiles.

We chose to describe AAP use among insulin quartiles in the schizophrenia cohort with two different approaches: comparing the proportion of patients using medications at three different levels of metabolic risk, and normalizing average daily AAP exposure using chlorpromazine

equivalents. This was done because AAPs have a variable propensity to cause metabolic adverse effects, and no AAP is likely free from this risk.³⁸ Neither measure was significantly different among schizophrenia quartiles (Table 1). This was an interesting finding because it suggests that current AAP use may not impact fasting insulin concentration in this population. The lack of difference in AAP exposure among quartiles may be due to the similar length of time since schizophrenia spectrum diagnosis (Table 1). This could reflect previous exposure

to a variety of antipsychotic treatment trials. Because many patients could not recall an in-depth account of past antipsychotic exposure, it was not possible to assess the impact of the duration of treatment on the metabolome. Previous research suggests that duration of exposure to AAP influences the metabolome early in schizophrenia treatment, but it is still unclear how drug use influences the metabolome and metabolic risk after several antipsychotic trials. For example, a lipidomics study³⁹ noted significant changes in lipid profiles following only 2 weeks of drug therapy in patients experiencing their first episode of schizophrenia, and another study⁴⁰ found metabolites associated with future weight gain in patients with first-episode psychosis followed over 1 year.

The lack of a relationship between fasting insulin concentration and current AAP exposure is interesting, particularly given the association between AAP use and the development of adverse metabolic events.⁴¹ This leaves unanswered an important question regarding how essential the timing and duration of AAP exposure is to encouraging the disruption of glucose-insulin homeostasis. In the future, it will be important to capture changes in the metabolome before and for an extended period of time after AAP initiation and medication switches. It will also be necessary to have studies adequately powered to allow for further group separation by sex because research in 2014 showed this to be an important factor in determining changes in folate cycle metabolites following antipsychotic administration.⁴²

Despite these intriguing findings, we acknowledge that this was an exploratory study with several limitations. First, this was a retrospective investigation that used existing blood samples from a relatively small sample size, preventing us from drawing conclusions about AAP-induced changes on the metabolome. In addition, the use of NMR-based metabolomics results in significantly fewer metabolites than approaches using detection methods based on mass spectrometry. We possibly could have identified additional biochemical pathways associated with variability in fasting insulin concentration with the use of a more sensitive platform like liquid chromatography–mass spectrometry. However, for this preliminary study, the advantages of quantitative NMR metabolomics outweighed this option because NMR is routinely quantitative, highly reproducible, and nondestructive to the sample. Use of this

approach will also direct the selection of more sensitive targeted assays for future studies. Another limitation of the study stems from the liberal FDR of less than 25% that is consistent with exploratory genetics analyses⁴³ but increases the likelihood of type 1 error.

Conclusion

Our metabolomics analyses identified differences in concentrations of metabolites associated with variable one-carbon metabolism (glycine, serine, and taurine) among fasting insulin concentration quartiles of a schizophrenic cohort: specifically, that fasting insulin concentration trended inversely with increased methylation capability. We also found that patients with schizophrenia who had higher insulin concentrations (e.g., Q3 and 4) had higher levels of the saturated FA 16:0 (palmitic acid). These findings appeared to be independent of current AAP use and demonstrated the utility of anchoring metabolomics studies to fasting insulin concentration as a measure of metabolic risk in this population, as compared with MetS diagnosis that is observable after fasting insulin concentration elevations. In aggregate, these results have implications for furthering knowledge about the mechanisms involved in the development of CVD in schizophrenic patients because loss of, or a decline in, methylation capability may be associated with endothelial dysfunction. Future studies will need to include a targeted prospective metabolomics approach with a more stringent FDR. Ultimately, understanding the metabolic consequences of AAPs across all phases of schizophrenia and how AAPs impact variable baseline risk for CVD will allow for safer medication use through improved metabolic monitoring and the design of more precise treatment regimens.

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Supporting Information

The following supporting information is available in the online version of this paper:

Appendix S1. Metabolomics Analyses.

Figure S1. Representative nuclear magnetic resonance spectra.

Figure S2. Representative gas chromatography spectrum.

Table S1. Metabolites Differentiating Body Mass Index Control Groups

Table S2. Metscape Pathway Mapping Results.

Table S3. Metabolites Differentiating Schizophrenia Participants Grouped by Obesity Status