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

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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Abstract

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. 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 range of the patients with schizophrenia to account for metabolite concentration differences attributable to body mass index.

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 (NMR) and gas chromatography (GC) methods, respectively. To exclude metabolites from our pathway mapping analysis that were due to variability in weight, we also subjected serum samples from the nonobese and obese controls to the same analyses. Patients with schizophrenia had a median age of 47.0 (interquartile range 41.0-52.0) years. Using a false discovery rate threshold of <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 having higher levels of saturated fatty acids compared with patients with lower fasting insulin concentrations (quartiles 1 and 2).

Conclusion—These 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.

Keywords

Adverse drug reactions; insulin; antipsychotics

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] which stresses a need for improved understanding of the underlying mechanisms of MetS in order to develop more precise mechanisms for monitoring and preventing adverse metabolic side effects.

An alternative option for assessing CVD risk in AAP users is fasting insulin concentration, which has been associated with insulin resistance and future development of prediabetes and diabetes.[6,7] The development of insulin resistance has also been observed prior to 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. Therefore, we conducted a metabolomics analysis of existing serum samples from adult patients with schizophrenia, grouped by log-transformed fasting insulin concentration quartiles, using quantitative 1D-^{1}H-nuclear magnetic resonance (NMR) spectroscopy, and a gas chromatography (GC) assay for the detection of fatty acids (FA). Since 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/m^2) and nonobese (BMI <30 kg/m^2) subjects without CVD or mental health diagnoses that 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: (1) normalizing exposure using chlorpromazine equivalents, and (2) comparing percentages of patients using AAPs divided into three groups based on their propensity to cause weight gain.

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 are age 18-90 years old; *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 prior to 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's 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's Institutional Review Board (IRBMED HUM00017774) and the IRBs of the Washtenaw County Health Organization (WCHO), the Detroit-Wayne County Community Mental Health Agency (DWCCMHA), 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

AAP exposure was standardized between groups using the formulas described by Andreasen et al.[12] 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 Woods.[13] As 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 medication risk category. Antipsychotic polypharmacy was also described as use of 2-3 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 (OAIC) 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/m^2 with at least one comorbidity, or a BMI of at least 35 kg/m^2.[18,19] For this pilot study, the additional exclusion criteria were applied: present cardiovascular disease 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 <30 kg/m^2 (nonobese) and the second >30 kg/m^2 (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, nonobese 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 have previously been 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.

Prior to 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 (H_2O)/methanol (MeOH):chloroform ($CHCl_3$) extraction as previously described.[21,22] The dried $CHCl_3$ fraction was transported to the Michigan Comprehensive Regional Metabolomics Core (MRC)[2] for FA analysis.

Quantitative 1D-^{1}H-NMR Metabolomics and GC to Detect Free FA

The 1D-^{1}H-NMR spectra of the H_2O/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 Tesla (500 MHz) NMR spectrometer. This process has been previously described in detail,[23] and further description of the methods are provided in the supplementary information in addition to a representative NMR spectrum (Supplementary Figure 1). The $CHCl_3$ fractions containing the lipids of the serum samples were assayed for FA content, utilizing an Agilent 5890 gas chromatograph with an Agilent HP 88 column, by the MRC2 using a modified technique previously described by Das and Hajra.[24] A representative gas chromatogram can also be found in the supporting information (Supplementary Figure 2).

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 3.0 (http://www.metaboanalyst.ca/) to achieve normal distribution in preparation for parametric statistical analyses.[27,28] 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 11 (SAS Institute, Inc. Cary, NC). 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 described by Storey et al.[29] Metabolites with an FDR <25% were used in pathway analysis by Metscape (http://metscape.ncibi.org/)[30], a plugin for Cytoscape (http://www.cytoscape.org/). Figures were constructed using "R" (https://www.r-project.org/).[31]

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, which 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 the participants taking fluphenazine were using moderate-risk AAPs (n=4) or ziprasidone (n=1). 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 (IQR 45.0-49.0) years and 46.5 (IQR 41.8-50.3) years for the 10 nonobese participants ($p=0.704$). Median log-transformed fasting insulin values were 1.13 (IQR 1.05-1.17) μU/mL and 1.12 (IQR 1.02-1.32) μU/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 (IQR 20.9-27.0) kg/m^2, and 34.7 (IQR 33.4-36.7) kg/m^2, respectively ($p<0.001$) (Figure 1D).

Quantitative ^{1}H-NMR Metabolomics and Pathway Analysis

Thirty-six metabolites were identified and quantified by ^{1}H-NMR in all serum samples. Of these, 13 ^{1}H-NMR detected metabolites differentiated (FDR < 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.[30] 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 < 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 < 25%) from the control analyses (Supplementary Table 1) and all pathways that were mapped with Metscape (Supplementary Table 2). The ^{1}H-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 FA were identified and quantified. In the schizophrenia cohort, significant differences were apparent in several saturated FA, such as 16:0 (palmitic acid), 14:0 (myristic acid), 24:0 (lignoceric acid), and the unsaturated essential FA 18:2 (linoleic acid). Three FA—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 there were no significant differences 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, which 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. Additionally, 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 been repeatedly linked to CVD through mechanisms that are not yet understood.[32,33]

Perturbed one-carbon metabolism has also been implicated in CVD risk in patients with schizophrenia.[34] Our group has observed decreased methylation capacity and worsening endothelial function in schizophrenia patients with genetic polymorphisms in folate cycle enzymes associated with hyperhomocysteinemia.[35,36] We also found that supplemental folate administration improved methylation capability and endothelial function in schizophrenia patients taking AAPs.[37] Additionally, the folate cycle is intimately tied to FA metabolism, as it is needed to contribute methyl groups for carnitine methylation and FA chain elongation and desaturation.[38] Assies et al [39] recently proposed that the complex relationship between FA 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 transsulfation compared to 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 FA were elevated in schizophrenia patients with higher fasting insulin concentrations (e.g., Q3-4), whereas patients with lower fasting insulin concentrations (e.g., Q1-2) had higher concentrations of methyl-donating amino acids. This pattern of elevation in saturated FA has also been observed in settings of CVD[40] in patients without mental health disorders.

As insulin concentration is known to be associated with weight, this complicated our analysis because 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 FA, palmitic acid (16:0), linoleic acid (18:2), and lignoceric acid (24:0) remained uniquely altered in schizophrenia patients. Palmitic acid trended upward in Q3-4, whereas linoleic and lignoceric acids trended lower. Of these FA, 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 that were performed for the BMI controls without psychiatric diagnoses or cardiovascular disease. The table of differentiating metabolites from this analysis is provided in the supplement (Supplementary Table 3). There were no differentiating ^{1}H-NMR metabolites that 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 that was 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 interpretation of these results less straightforward. Although not confirmatory, these results indicate that the metabolite variability of the schizophrenia cohort are 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: (1) comparing the proportion of patients using medications at three different levels of metabolic risk, and 2) normalizing average daily AAP exposure using chlorpromazine equivalents. This was done because AAPs have variable propensity to cause metabolic adverse effects, and no AAP is likely free from this risk.[41] 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 be reflective of previous exposure to a variety of antipsychotic treatment trials. Since 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 study by McEvoy et al[42] noted significant changes in lipid profiles following only 2 weeks of drug therapy in patients experiencing their first episode of schizophrenia, and Suvitival et al[43] found metabolites associated with future weight gain in patients with first-episode psychosis followed over one 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.[44] 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, as recent research shows this to be an important factor in determining changes in folate cycle metabolites following antipsychotic administration.[45]

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. This prevents us from making conclusions about AAP-induced changes on the metabolome. Additionally, the use of NMR-based metabolomics results in significantly fewer metabolites than approaches using mass spectrometry–based detection methods. It is possible that we 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 <25%, which is consistent with exploratory genetics analyses,[46] but increases the likelihood of type 1 error.

Conclusion

Our metabolomics analyses identified differences in concentrations of metabolites that are 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., Q3-4) 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 to 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 since 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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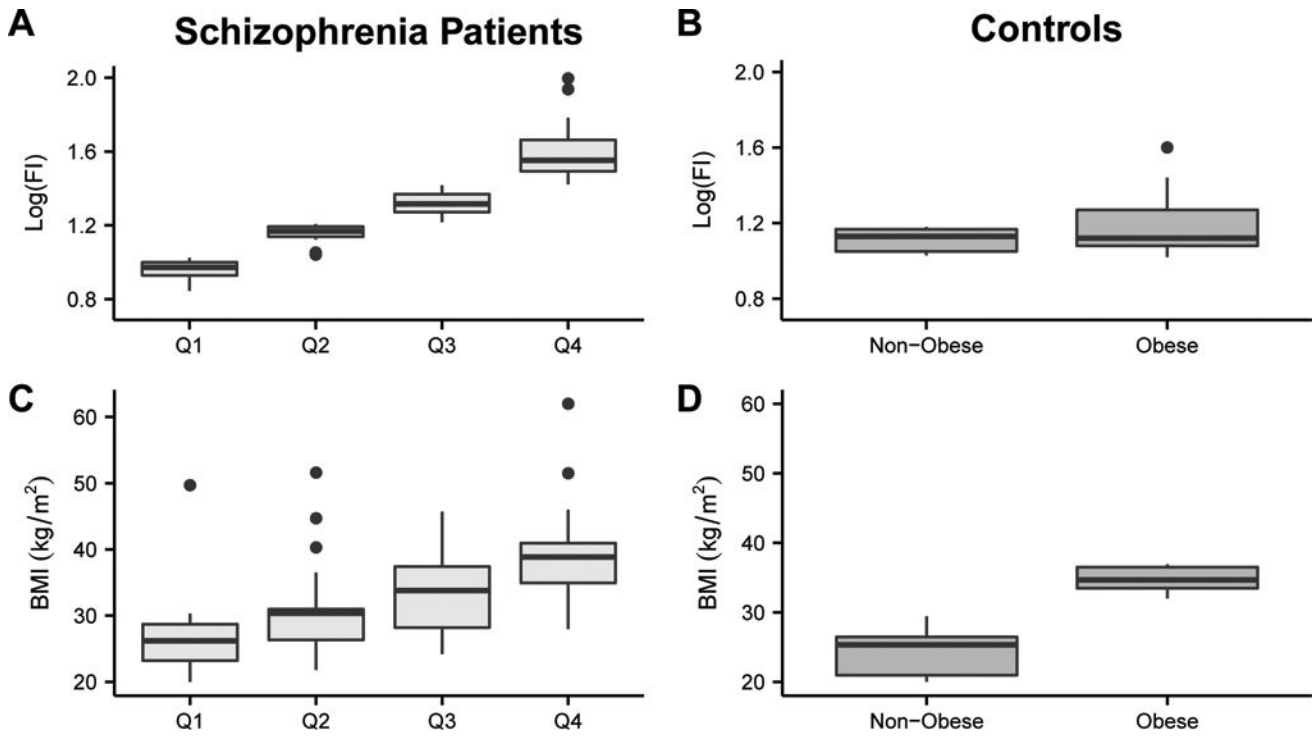

Figure 1.

Box and whisker plots of log-transformed fasting insulin concentration (Log(FI), measured as mU/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 amongst 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.

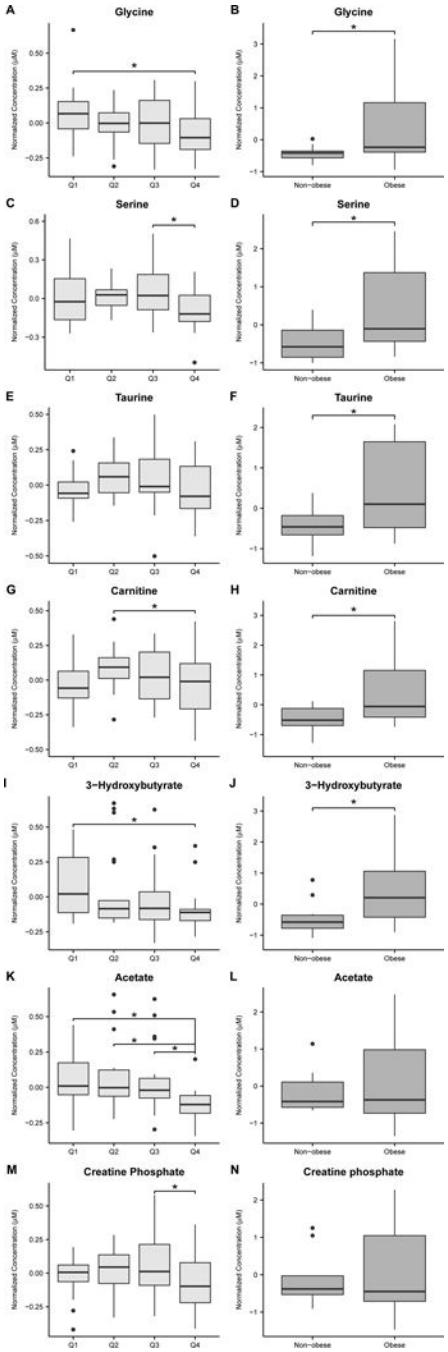

Figure 2.

Box and whisker plots of normalized serum metabolite concentrations detected and quantified by ^{1}H-nuclear magnetic resonance (A-N) in the schizophrenia quartiles (A, C, E, G, I, K, and M) and BMI controls (B, D, F, H, J, L, and N). Metabolites shown in the schizophrenia column are those that differentiated insulin quartiles (ANOVA FDR <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 $P<0.05$).

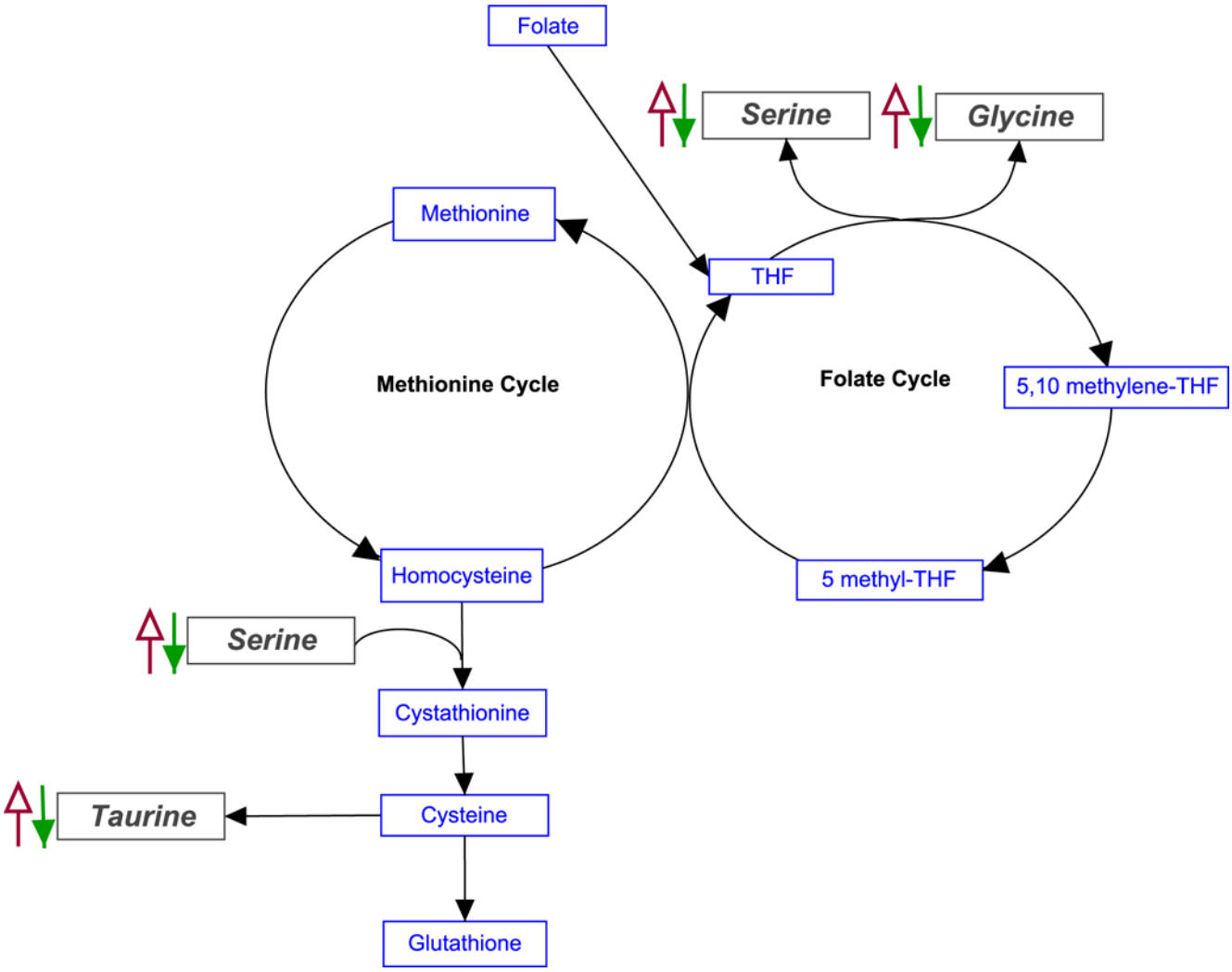

Figure 3.

Simplified schematic diagrams of one-carbon metabolism. Arrows depict trend of metabolite concentration with increasing 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.

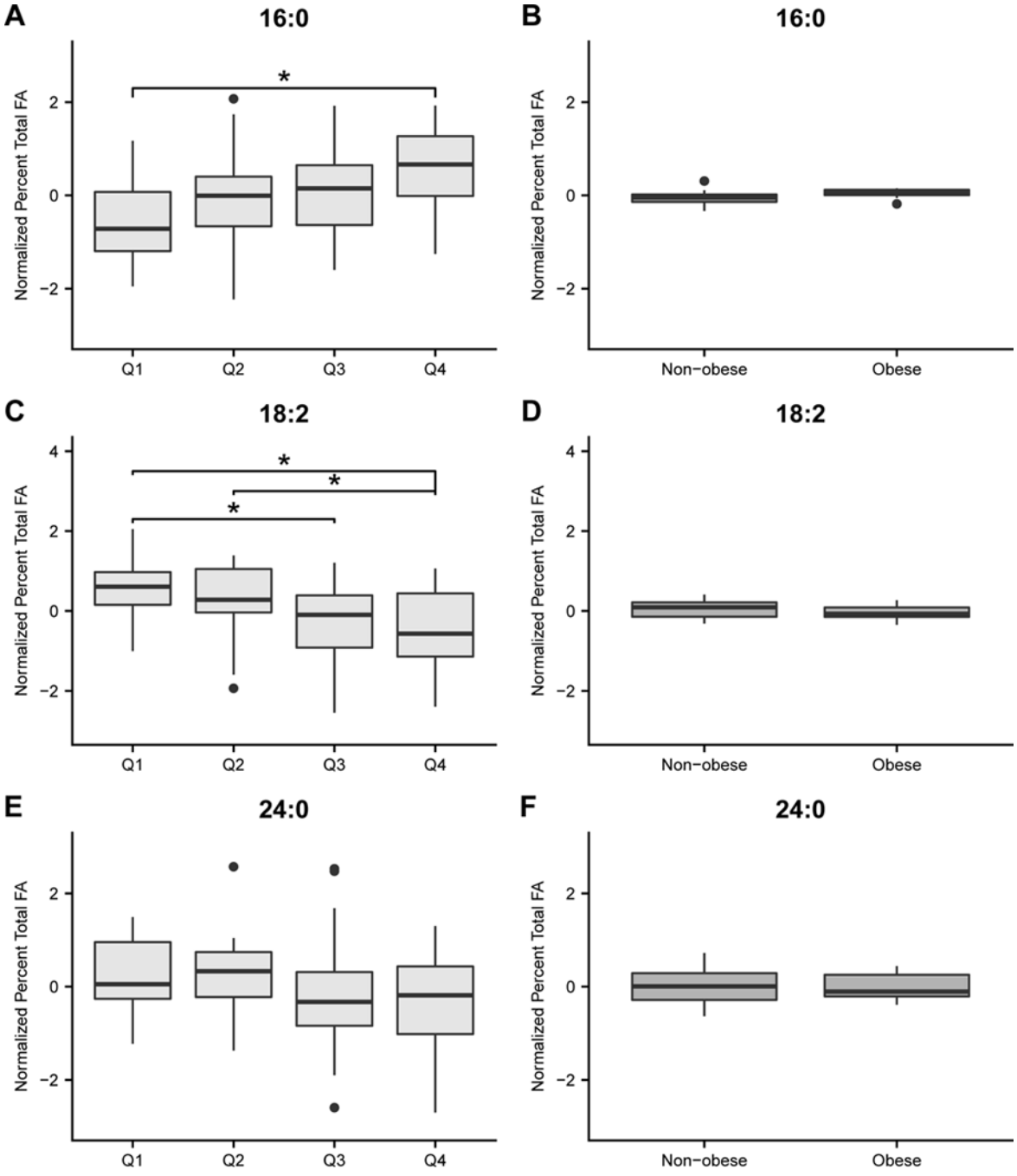

Figure 4.

Box and whisker plots of normalized concentrations of individual fatty acids (FA) by percent total that differentiated insulin quartiles in patients wih schizophrenia (ANOVA FDR <25%; A, C, and E) but not 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 FA that were significantly different (Tukey Kramer post hoc $P<0.05$) between quartiles.

Table 1

Demographic and Clinical Characteristics of the Patients with Schizophrenia

	Insulin Concentration Quartile[a]				
Characteristic	**Q1 (n=25)**	**Q2 (n=22)**	**Q3 (n=25)**	**Q4 (n=22)**	**p Value[b]**
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
Caucasian 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)	
Moderate	16 (64.0)	7 (31.8)	10 (40.0)	12 (54.6)	0.420
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.

BMI = body mass index; AAP = atypical antipsychotic.

[a] Insulin concentration quartiles were based on log-transformed fasting insulin values, with Q1 corresponding to the lowest quartile and Q4 corresponding to the highest quartile.

[b] χ^2 or analysis of variance p value as appropriate.

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

[d] Antipsychotic polypharmacy was defined as use of 2-3 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				
Glutamate	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
Tyrosine	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
Alanine	C00133	0.0328	16.87	Q3>Q1
Serine	C00065	0.0536	22.76	Q3>Q4
Creatine phosphate	C02305	0.0690	23.84	Q3>Q4
Valine	C00183	0.0842	23.84	
Taurine	C00245	0.0862	23.84	
Proline	C00148	0.0891	23.84	
Leucine	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; CQ3>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 ^{1}H-nuclear magnetic resonance and fatty acids detected with gas chromatography were identified as differentiating patients with schizophrenia across insulin concentration quartiles (false discovery rate of <25%). Metabolites in italics and boldface were those that were determined to be associated with body mass index (see text).

ANOVA = analysis of variance; Q = quartile.

[a]Post hoc results were determined to be significant when Tukey-Kramer p values were <0.05.