Atypical Antipsychotics Rapidly and Inappropriately Switch Peripheral Fuel Utilization to Lipids, Impairing Metabolic Flexibility in Rodents

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Patients taking atypical antipsychotics are frequented by serious metabolic (eg, hyperglycemia, obesity, and diabetes) and cardiac effects. Surprisingly, chronic treatment also appears to lower free fatty acids (FFAs). This finding is paradoxical because insulin resistance is typically associated with elevated not lower FFAs. How atypical antipsychotics bring about these converse changes in plasma glucose and FFAs is unknown. Chronic treatment with olanzapine, a prototypical, side effect prone atypical antipsychotic, lowered FFA in Sprague–Dawley rats. Olanzapine also lowered plasma FFA acutely, concomitantly impairing in vivo lipolysis and robustly elevating whole-body lipid oxidation. Increased lipid oxidation was evident from accelerated losses of triglycerides after food deprivation or lipid challenge, elevated FFA uptake into most peripheral tissues (~2-fold) except heart, rises in long-chain 3-hydroxylated acyl-carnitines observed in diabetes, and rapid suppression of the respiratory exchange ratio (RER) during the dark cycle. Normal rises in RER following refeeding, a sign of metabolic flexibility, were severely blunted by olanzapine. Increased lipid oxidation in muscle could be explained by ~50% lower concentrations of the negative cytoplasmic regulator of carnitine palmitoyltransferase I, malonyl-CoA. This was associated with loss of anaplerotic metabolites and citric acid cycle precursors of malonyl-CoA synthesis rather than adenosine monophosphate-activated kinase activation or direct ACC1/2 inhibition. The ability of antipsychotics to lower dark cycle RER in mice corresponded to their proclivities to cause metabolic side effects. Our studies indicate that lipocentric mechanisms or altered intermediary metabolism could underlie the FFA lowering and hyperglycemia (Randle cycle) as well as some of the other side effects of atypical antipsychotics, thereby suggesting strategies for alleviating them.

Key words: antipsychotics/fatty acid oxidation/metabolomics/side effects/malonyl-CoA/anaplerosis/obesity/diabetes/metabolic flexibility

Introduction

Second-generation antipsychotic drugs (atypical antipsychotics), including olanzapine, are a chemically diverse class of medications prescribed for a variety of mental illnesses that lack the sedative and movement side effects that plagued first-generation compounds.1,2 While their clinical utility continues to rise, patients taking atypical antipsychotics are frequented by unique metabolic side effects, including body weight gain/obesity, type 2 diabetes, dyslipidemia, and hypertension, eg.3–9 An early side effect is development of insulin resistance independent of increased body weight, hyperphagia, or adiposity.10–12 Research on this side effect has been mainly glucocentric, although to date no cell signaling mechanisms have been found at relevant doses to explain how atypical antipsychotics exert their rapid and body weight-independent effects on glucose disposal.

Despite its ability to cause insulin resistance, obesity and type 2 diabetes, olanzapine but not haloperidol, a typical antipsychotic, has been reported to lower free fatty acid (FFA) after chronic treatment of healthy subjects or patients.13,14 This chronic effect on reducing FFA is unexpected because insulin resistance, adiposity, and diabetes are typically associated with elevated not lowered FFAs, eg.15 The FFA lowering effect is also interesting because FFAs are the heart’s preferred fuel,16 and atypical antipsychotics have recently been implicated in sudden cardiac death.17 They can cause a specific type of ventricular tachycardia that is frequently associated
with malnourishment and metabolic disorders wherein energy production is affected.

In this article, we focused on how atypical antipsychotics lower FFAs. This effect was observed in a high-fidelity animal model even after acute treatment and is due to both impaired in vivo lipolysis along with a rapid and inappropriate acceleration of lipid oxidation. The drug also acutely altered metabolic fuel preference reflected in the respiratory exchange ratio (RER) and prevented normal changes in RER seen during fed and fasted state transitions. Increased FFA oxidation appears to involve decreased anapleurosis needed to synthesize the endogenous negative regulator of fat metabolism, malonyl-CoA. Our findings suggest a potential mechanism that could explain the opposing changes in glucose and FFA (Randle cycle) with potential implications for the apparent insulin resistance, hunger, and cardiovascular side effects that can occur in those who take these drugs. A noninvasive screen for the impaired metabolic flexibility side effect in mice is suggested along with a potential strategy for reversing the initiating metabolic disturbance.

**Experimental Procedures**

**Animals**

The animal facilities and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine. Male and female Sprague–Dawley rats (~200–225 g) were purchased from Charles River Laboratories (Cambridge, MA). Male C57Bl/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were maintained on a 12:12 h light-dark cycle with food (Harlan-Teklad Rodent Chow, no. 2018) and water provided ad libitum.

**Chronic Dosing**

Olanzapine was administered with ramped dosing as previously described.18 An exception was that on the morning of day 28, after a 14-h fast, a half dose of olanzapine, 6 mg/kg, was given to animals by oral gavage 2 h before blood sampling for measurement of FFA and glycerol or insulin tolerance testing.

**Acute Dosing**

In acute studies, endpoints were measured after 2 doses of vehicle (water, pH 5.5) or olanzapine (10 mg/kg). The first dose was administered before the start of the dark cycle at approximately 1800 h. The following morning a second dose of vehicle or olanzapine was administered typically between 0700 and 0800 h 2 h prior to the beginning of any experiment or blood/tissue sampling. This regimen is based on our previous study18, and the maximum dose of olanzapine (10 mg/kg) was chosen because it produced a consistent elevation of blood glucose in pilot studies.

In the calorimetry time course studies, drug was provided at the time indicated in the figure in fed or food-deprived animals as indicated.

**GIR during Hyperinsulinemic Euglycemic Clamp**

Glucose infusion rate (GIR) was measured as previously described19 using a dose of insulin that approximated fed insulin concentrations. During the basal period \((t = -120\) to 0 min), saline was infused through the venous catheter and a tracer amount of tritiated glucose \((3-[^3]H\text{-glucose};\text{Perkin-Elmer, Waltham, MA})\) was infused as a primed-continuous infusion \((10 \mu\text{Ci bolus, 0.2 }\mu\text{Ci/min})\) through the hybrid venous catheter for measurement of basal hepatic glucose output. At time zero \((t = 0)\), a primed-continuous insulin infusion \((75 \mu\text{U/kg bolus, 1 }\mu\text{U/kg/min, 0.3% bovine serum albumin})\) was started and glucose \((20\% \text{ dextrose})\) was infused to maintain euglycemia \((\sim 100 \text{ mg/dl})\).

**ITTs**

Insulin tolerance tests (ITTs) were performed after either 5 h (acute) or 14 h (chronic) of food deprivation18 following intraperitoneal injection of 0.75 U/kg, Humulin-R (Lilly; Indianapolis, IN).

**Indirect Calorimetry**

RER in rats and mice after acclimation was calculated by indirect calorimetry (Oxymax, Columbus Instruments, Columbus, OH) as previously described.19,20 Both the Columbus Instruments (CI) rat and mouse calorimeters we used for these studies utilize 2 gas standards. One is a mixture of CO2 and O2 at concentrations recommended by CI. The second is certified pure N2 for a zero standard. These are produced, analyzed, and certified by Airgas (Harrisburg, PA). However, since we have, in the past, obtained incorrectly calibrated standards from this and other suppliers, we verify these concentrations in our laboratory. For this purpose, we purchased 3 additional standards spanning above and below the CO2 and O2 concentrations recommended by CI for the CO2 and O2 standard used by their instrument. These other reference gases were used to validate CI instrument linearity and the concentrations of the CI reference gas. In the later case, we utilized separate O2 and CO2 detectors and develop a linear standard curve based on a voltage read out from the sensor of that equipment and verified that the CO2 and O2 standard used for the CI calorimeter was calibrated accurately based on the standard line formed by these other reference gases.

**Oral Lipid Tolerance Test**

Weight-matched animals were food restricted for 14 h. After collection of baseline blood samples, olive oil was administered by oral gavage (6 ml/kg). Tail blood samples were collected at the indicated times and
centrifuged at 1800g for 10 min at 4°C; plasma was collected and frozen at −80°C for further analysis.

**Hepatic VLDL-Triglyceride Secretion**

To measure the rate of hepatic very low density lipoprotein (VLDL)-triglyceride secretion, tyloxapol (600 mg/kg) was given intravenously in 14 h food-restricted animals as a 20% (w/v) solution. This dose of tyloxapol inhibits lipoprotein lipase for at least 2 h. Baseline blood samples were collected for measurement of triglycerides and at subsequent indicated times to measure plasma triglycerides. Rates of hepatic triglyceride secretion were calculated as previously described assuming a plasma volume of 3.5%.22,23

**Tissue FFA Uptake**

Tissue-specific FFA uptake was measured using the non-metabolizable fatty acid analog, [125I]-BMIPP, as previously described and validated.24 Four days after catheter implantation, rats were dosed with olanzapine or vehicle according to the acute dosing protocol. At approximately 0700 h, a tracer amount of [125I]-BMIPP (10 μCi) was given as an intravenous (i.v.) bolus (t = 0 min). Serial blood samples (∼250 μl) were collected (t = 2, 5, 10, 20, 30, 40) to determine the areas under the curves (AUC) for plasma [125I]-BMIPP during the in vivo labeling period. After final blood samples were collected (t = 40 min), animals were euthanized and tissues (ie, gastrocnemius, soleus, heart, liver, kidney, gut, skin, brain) were freeze clamped in liquid nitrogen. Total [125I]-BMIPP activity was measured in plasma (25 μl) and whole tissue (∼100 mg) for calculation of FFA metabolic rate (Rf).

**Analytical Procedures and Clinical Chemistry Measurements**

Blood glucose measurements were made with an Ascensia Contour blood glucometers (Bayer Healthcare LLC; Mishawaka, IN). Insulin concentrations were determined using a commercial ELISA kit for rat insulin (Merckodia; Uppsala, Sweden). The following plasma metabolite concentrations were made using colorimetric assays: plasma glucose (Thermo Scientific, Waltham, MA), FFAs (Wako Diagnostics, Richmond, VA), glycerol (Cayman Chemical, Ann Arbor, MI), and beta-hydroxybutyrate (Stanbio Laboratory, Boerne, TX). Plasma triacylglycerides and lactate were measured using the DT60II module of the Vitros Clinical System (Ortho-Clinical Diagnostics, Rochester, NY). Branched-chain amino acids and plasma alanine concentrations were measured using enzyme-linked reactions.25 Tissue Co-A species were measured by high performance liquid chromatography as previously described.26 Metabolomic profiling of tissue amino acids,27 organic acids,28 and acyl-carnitines29 were determined as previously described.

**Calculations**

The FFA metabolic rate, Rf, was calculated by taking the total tissue counts of [125I]-BMIPP and dividing by the AUC of [125I]-BMIPP during the 40 min in vivo labeling period and multiplying by the mean plasma FFA concentration.29 Cardiac minute work (dyne-cm/min) was calculated as the product of pulse (beats/min), stroke volume (cm³), and arterial pressure (mmHg) using the conversion factor of 1 mmHg = 1332 dyne/cm². An index of cardiac energy expenditure, the “double-product,” was calculated as the product of pulse (beats/min) and arterial pressure (mmHg).

**Statistical Analyses**

For all results, data are expressed as the mean ± SE. In the text, increases relative to control are presented as % change from control, in which no change would be 0%. To calculate statistical significance (P < .05), Student’s t-test or one-way ANOVA with Bonferroni’s multiple comparison posttest was used when appropriate. Sample size for each group is presented in the respective figure legends. Asterisks indicate at least a certain P value as follows: *P < .05; **P < .01; ***P < .001. All statistical analyses and data manipulations were made using GraphPad Prism and InStat computer programs (GraphPad Software, San Diego, CA).

**Results**

**Effect of Olanzapine on Plasma FFA and Insulin Sensitivity**

We and others have recently described the hyperglycemia and adiposity inducing effects of chronic olanzapine treatment in male rats30,31, chronic administration for 28 days increased adiposity without a change in body weight as measured by 1H-NMR and impaired insulin tolerance.31 The fasting hyperglycemia and impaired insulin tolerance were also observed acutely implying these effects are adiposity independent.31 Chronic treatment also paradoxically lowered plasma FFA in 14 h food-deprived rats (table 1). To further investigate the mechanism underlying the converse effects on plasma FFA and glucose, we conducted acute studies on male rats using an overnight dosing protocol from our previous study on female rats.18 In one of these experiments, a euglycemic-hyperinsulinemic clamp study was performed in which insulin was clamped to fed conditions (fed plasma insulin concentrations are not affected by olanzapine, figure 1). Consistent with previous studies,10,32 acute olanzapine decreased the GIR (whole-body glucose uptake, Rg) during the euglycemic-hyperinsulinemic clamp by ~50%, suggesting strongly impaired whole-body glucose disposal. However, olanzapine did not affect the Ser473 phosphorylation of protein kinase B (AKT in fed rats (Supplementary figure S1). Phosphorylation at
that site is frequently associated with increased AKT activity. Ser473 phosphorylation was also not affected by olanzapine in muscle tissues obtained from 14 h food-restricted rats or tissues from a euglycemic insulin clamp (data not shown) despite the fact that 14C-deoxyglucose uptake into gastrocnemius was impaired under clamp conditions as previously shown.31

In addition to lowering FFAs after chronic treatment, olanzapine also acutely lowered fasting plasma FFA. These findings were initially surprising and confusing to us, so we repeated them in several cohorts of rats (table 1; figure 1).31 Thus despite the presence of insulin resistance, olanzapine acutely lowered plasma FFA concentrations in food-deprived male rats by $\approx 40\%$ (table 1).

Table 1. Plasma FFA, Glycerol, Insulin Tolerance Test Areas Under the Curve (ITT-AUC), and Euglycemic Clamp Glucose Infusion Rate (GIR) After Chronic or Acute Olanzapine Treatment in Male, Sprague–Dawley Rats

<table>
<thead>
<tr>
<th>Treatment Day</th>
<th>Dose, mg/kg/day</th>
<th>Food Restriction</th>
<th>Endpoint</th>
<th>Condition</th>
</tr>
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<tbody>
<tr>
<td>28 (chronic)</td>
<td>Ramp, 4–12$^b$</td>
<td>14 h</td>
<td>FFA</td>
<td>Control$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycerol</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycerol</td>
<td>1.79 ± 0.13</td>
</tr>
<tr>
<td>2 (acute)$^c$</td>
<td>4</td>
<td>5 h</td>
<td>FFA</td>
<td>0.63 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycerol</td>
<td>1.95 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GIR</td>
<td>81.4 ± 5.3</td>
</tr>
</tbody>
</table>

Note: FFA, free fatty acid.

Values are means ± SE; n = 10–12. Asterisks indicate statistical difference from control group (*P < .05, **P < .01, ***P < .001). Parameters reported in SI units: FFA, mM; glycerol, mM, GIR, μmol/kg/min.

$^b$‘‘Ramp, 4–12’’ indicates ramp in dosage from 4 to 12 mg/kg day as follows: the dose of olanzapine was 4 mg/kg once per day during days 0–6. On day 7, the dose was increased to 8 mg/kg per day. On day 14, olanzapine was provided at 12 mg/kg/day and this continued through the end of the study as previously described.31

$^c$‘‘Acute’’ indicates that the endpoints were measured after 2 doses of vehicle (water, pH 5.5) or olanzapine (10 mg/kg). The first dose was administered before the start of the dark cycle at approximately 1800 h, and the second was administered typically between 0700 and 0800 h 2 h prior to plasma sampling.

Fig. 1. Circulating Nutrient Concentrations in Fed and Food-Restricted Rats. Blood samples were collected from different cohorts of animals in the fed state (time 0) or after 5 h or 14 h of food restriction. All animals received either olanzapine (10 mg/kg) or vehicle via oral gavage 2 h prior to blood sampling, as described in Experimental Procedures. (A) Glucose was measured in whole blood while (B) insulin, (C) lactate, (D) free fatty acids, (E) alanine, and (F) triglycerides were measured in plasma. Data represent the mean ± SE (n = 10–20); asterisks indicate significant differences (***P < .001, **P < .01, *P < .05) compared with time-matched control values.

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The effects of olanzapine on the plasma concentrations of lipid fuels (ie, triglycerides and FFA) were different depending on the metabolic state. Plasma triglycerides in the fed state tended to be lower after olanzapine, with the olanzapine group being significantly lower than control in some but not all of 3 separate studies (figure 1F and data not shown). Triglycerides (figure 1F) were consistently lower after 5 h of food restriction, with olanzapine-treated animals reaching 14 h fasting levels more rapidly than control animals, suggesting that the rats receiving olanzapine were using triglyceride more rapidly. FFA (figure 1D) were not affected by olanzapine in the fed state. However, the expected rise in FFA following food restriction was blunted by olanzapine, in agreement with a separate cohort of rats (table 1). Plasma FFA were significantly lower in the olanzapine group after 5 h and 14 h of food restriction. Collectively, olanzapine appears to accelerate disposal of plasma triglycerides and FFA, while glucose remains elevated even in the presence of persistent hyperinsulinemia.

**Triglyceride Clearance, Hepatic Secretion, Tissue-Specific FFA Uptake**

We addressed the hypothesis that olanzapine inappropriately increased lipid utilization in addition to simultaneously inhibiting in vivo lipolysis. Consistent with this idea, olanzapine improved oral lipid tolerance following an oral lipid challenge. The peak concentration (figure 2A) and AUC for plasma triglycerides during the lipid tolerance test was decreased by olanzapine (figure 2B). As plasma triglycerides are also derived from the liver, the effect of olanzapine on the hepatic secretion of VLDL-triglyceride particles was measured. As seen in figure 2C, the increased triglyceride concentration following tyloxapol injection is near linear and was not different between control and olanzapine-treated groups; calculated VLDL-secretion rates also did not differ (control, 29.4 ± 3.4 vs olanzapine, 33.7 ± 3.7 mg/kg/h). Collectively, our data suggest that the lower plasma triglyceride concentrations following acute olanzapine treatment are due to increased plasma clearance rather than decreased hepatic secretion.

The above findings imply that olanzapine increases whole-body lipid fuel disposal, but it is unclear whether this is a tissue-specific phenomenon or a generalized effect on all or most peripheral tissues. To address this question, we measured tissue fatty acid uptake in vivo in fed rats using an intravenously-infused, radiolabeled, nonmetabolizable fatty acid analog, [125I]-BMIPP (figures 2D–F), as previously described. Olanzapine increased FFA uptake by 120% in gastrocnemius and soleus, which are representative fast- and slow-twitch muscle, respectively (figure 2D). Liver and kidney, 2 other tissues with normally high rates of fatty acid oxidation also showed ~100%–120% increases in FFA uptake after acute olanzapine treatment (figure 2E).
However the heart, which has the highest rate of fatty acid oxidation per gram, showed no significant difference in FFA uptake (figure 2D) despite elevated heart rate (Supplementary figure S2) and cardiac work (calculated in Supplementary data) but not blood pressure (Supplementary figure S2). FFA uptake was also increased 80%–120% in the proximal duodenum and terminal ileum as well as skin (figure 2F). In addition, we recently reported that olanzapine increases FFA uptake into all adipose tissue depots tested.31 As expected, brain FFA uptake was too low (\(<0.1\) nmol/g tissue/min) to determine any affect of olanzapine reliably.

**Effect of Olanzapine on Metabolic Fuel Preference**

The above data support the conclusion that olanzapine acutely increases lipid disposal. To determine whether it also increases lipid fuel oxidation, we examined the immediate and acute (24–36 h after initial dose) effects of olanzapine on RER. Indirect calorimetry was used to examine the effects of olanzapine on RER in 3 separate experiments, during the fed tofasted transition (figure 3A), in ad libitum fed rats (figure 3B), and in fasted and refed rats (figures 3C and 3D). Animals were allowed to acclimatize to the calorimetry cages for at least 24 h. In figure 3A, they were removed from the cages briefly for gavage of olanzapine or vehicle and then replaced in calorimetric cages without food. Automated data collection was then initiated (the system only collects data from one cage at a time, and the olanzapine group cages were sampled last in each round of RER measurements). RER declined with food deprivation in control animals as expected. However, in the olanzapine group, RER was already significantly lower by the time the first measurement was made of the olanzapine group’s chambers and RER remained lower than control values for 2.5 h. After that period, there was no further difference between the groups. These studies show that the drug effects on RER are much faster than occurs by food deprivation. By the end of the dark cycle, both experimental groups had been food restricted for \(\sim12\) h and the RER in each group was \(\sim0.7\) indicating that animals were predominately using fat for fuel. Upon repeat gavage at the beginning of the light cycle and continued food restriction, there was no further decline in...
Fig. 3. Effect of Olanzapine on Respiratory Exchange Ratio (RER). RER (A–C) was calculated based on expired CO₂ and O₂ consumed (RER = VCO₂/VO₂). Each cage was measured for 1 min every 15 min. All data represent the mean ± SE. Time intervals that are statistically different are described in “Results.” Gray background shading or lack thereof indicates the dark and light cycles, respectively. Arrows indicate the time of olanzapine (10 mg/kg) or vehicle gavage. (A) After acclimatization period, ad libitum fed animals were briefly removed from the metabolic chambers for olanzapine (10 mg/kg) or vehicle gavage and then were replaced into the chambers without food. Automated calorimetry measurements were then initiated for a 24-h period. Using this approach, the RER was already significantly different by the time of the first measurement. Therefore, in (B), calorimetry measurements were recorded during the acclimatization period, and olanzapine or vehicle gavage was staggered in order to provide treatment a few minutes before readings from each cage were automatically collected by the calorimeter. After gavage, rats were replaced in the metabolic chambers but in this case (B) they retained ad libitum access to food and water. A second gavage was provided after 12 h as indicated (n = 12/group). A subset of animals remained in the cages for up to 36 h for additional measurements. (C and D) After acclimatization to the chambers, rats were 14 h food restricted and then gavaged with olanzapine 45 min prior to the start of the dark cycle. Refeeding began approximately 30 min after drug dosing. (C) RER and (D) food intake (2 and 12 h) were measured during the experiment (n = 6/group).

RER increased during the dark cycle (figure 3B), which is normal and considered a result of increased dark cycle intake of the rat chow, which has a low 5%–6% fat content. Oral olanzapine administration (indicated by arrows) rapidly decreased RER and such a change was absent in vehicle-treated controls. The olanzapine-mediated decline in the RER slowly returned to control levels over the course of the dark cycle with a time course consistent with olanzapine’s ~2 h half-life of olanzapine in rodents33 cf ~35 h in humans. When olanzapine was readministered shortly after the beginning of the light cycle, it again decreased the RER. The 24-h food intake did not differ between control and olanzapine-treated animals (27.2 ± 1.0 vs 26.3 ± 0.9 g) during this 24-h period. Experiments continued for 36 h in a subset of the animals to determine if the robust RER decrease was a short-lived phenomenon, but, as seen in figure 3B, a third dose of olanzapine at 25 h was again able to elicit a decrease in RER comparable with that observed after the first dose. On average, RER in both the dark and light cycles was lower in olanzapine animals compared with control values as a result of the robust decreases following olanzapine. Thus, drug-treated rats appear to shift their whole-body substrate metabolism from mostly carbohydrate to one in which energy expenditure is derived predominately from fat oxidation.

In a third experiment, the effect of olanzapine was assessed in refeed rats after food deprivation. Animals were food restricted for 14 h and refeed approximately 30 min after oral olanzapine or vehicle. RER in the 14 h food-deprived (ie, fasted) rats (figure 3C) was approximatively 0.7. Olanzapine blunted the rate of increase in RER with refeeding (figure 3C), even though food intake in the first 2 h was the same between groups (figure 2D). The ability to quickly switch to carbohydrate oxidation following food deprivation is termed metabolic flexibility.34 Thus, olanzapine rapidly activates fatty acid oxidation and impairs metabolic flexibility. Consistent with previous studies, 12-h food intake, like 24-h food intake, did not differ between control and drug-treated animals (figure 3D).

Muscle Metabolomics and Mechanistic Studies

The rate-limiting step for fatty acid oxidation is the activity of carnitine palmitoyltransferase I (CPT1). The activity of CPT1 is regulated by changes in the cellular concentration of malonyl-CoA. We found that gastrocnemius malonyl-CoA concentrations were reduced by ~56% following acute olanzapine treatment (table 2). Such differences in malonyl-CoA are similar to those observed in comparisons of starved and refeed rats, which have comparable changes in RER.35 Olanzapine’s effect on the malonyl-CoA concentration could be due to a direct inhibition of acetyl CoA carboxylase (ACC) or alteration in adenosine monophosphate-activated kinase (AMPK) pathway activity (fig. 3A). Thus, the magnitude of the drug-induced change in RER is similar to that observed with extended food deprivation; however, the decline is much faster in the presence of olanzapine.

Alerted to the rapidity of olanzapine’s effects, we started the RER measurements during the pretreatment period and dosed each rat with vehicle or olanzapine just before the gas from each chamber was scheduled to be automatically measured. In fed rats, the pretreatment
Table 2. Effect of Acute Olanzapine on Intermediate Metabolites in Fed Rat Gastrocnemius

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control</th>
<th>Olanzapine</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>nmol/g protein</td>
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<tr>
<td>Free Co-A</td>
<td>176 ± 40</td>
<td>177 ± 29</td>
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<tr>
<td>Acetyl-CoA</td>
<td>525 ± 51</td>
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<tr>
<td>Malonyl-CoA</td>
<td>36 ± 6.3</td>
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<tr>
<td>Citrate</td>
<td>643 ± 36</td>
<td>533 ± 28</td>
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<tr>
<td>α-Ketoglutarate</td>
<td>6622 ± 290</td>
<td>4953 ± 278</td>
<td>.0007</td>
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<tr>
<td>Succinyl-CoA</td>
<td>47 ± 8.6</td>
<td>23 ± 5.1</td>
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<tr>
<td>Succinate</td>
<td>573 ± 91</td>
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<tr>
<td>Fumarate</td>
<td>2535 ± 129</td>
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<td>Malate</td>
<td>4954 ± 133</td>
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μmol/g protein

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<th>Metabolite</th>
<th>Control</th>
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<th>P Value</th>
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<td>Pyruvate</td>
<td>29 ± 3</td>
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<td>Lactate</td>
<td>167 ± 11</td>
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<td>Ala</td>
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<td>Gly</td>
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<tr>
<td>Orn</td>
<td>0.75 ± 0.040</td>
<td>0.53 ± 0.027</td>
<td>.003</td>
</tr>
<tr>
<td>Phe</td>
<td>1.0 ± 0.05</td>
<td>1.2 ± 0.05</td>
<td>.012</td>
</tr>
<tr>
<td>Pro</td>
<td>4.3 ± 0.2</td>
<td>2.9 ± 0.2</td>
<td>.001</td>
</tr>
<tr>
<td>Tyr</td>
<td>1.6 ± 0.01</td>
<td>1.3 ± 0.5</td>
<td>.024</td>
</tr>
<tr>
<td>Val</td>
<td>1.37 ± 0.057</td>
<td>1.58 ± 0.056</td>
<td>.011</td>
</tr>
</tbody>
</table>

Note: All data represent the mean ± SE. P values < .1 are shown.

*Gastrocnemius samples from figure 4 (n = 8/group) were analyzed for Coenzyme-A (Co-A) species, and other metabolites were measured from a separate cohort of rats not infused with radioactivity (n = 10/group).

activity. Because ACC inhibitors vary widely in their structures, we examined the ability of olanzapine to directly inhibit recombinant human ACC1 and ACC2 along with 2 known inhibitors, Soraphen A and CP-640186 (Supplementary figure S3). While Soraphen A and CP-640186 inhibited ACC1 and ACC2 in vitro with expected IC50s, olanzapine had no appreciable effect over relevant and even toxic concentrations. We also detected no change in fed gastrocnemius AMPK activity due to acute olanzapine treatment based on phospho-specific pT172 AMPK immunoreactivity (Supplementary figure S1B).

Alternatively ACC is regulated by substrate availability, ie, cellular citrate arising from anapleurosis to the citric acid (TCA) cycle. Gastrocnemius citrate concentrations, measured in a separate cohort of animals (table 2), were significantly depressed by olanzapine, as were other measured TCA intermediates in the first span of the TCA cycle including α-KG (−26% decrease) and succinyl-CoA (51% decrease). Surprisingly, olanzapine had no effect on intermediates in the next span of the TCA cycle: succinate, fumarate, and malate (table 2, oxaloacetate, cis-aconitate and isocitrate, could not be detected). Olanzapine also did not acutely affect free CoA, acetyl-CoA, pyruvate, or lactate concentrations in muscle. Consistent with fed plasma alanine concentrations (figure 1), fed gastrocnemius alanine was not different after olanzapine. Anapleurotic amino acids were also affected by acute olanzapine. Concentrations of amino acids related metabolically to α-KG declined, including Glx (−20%), Pro (−30%), Orn (−30%), and Cit (30%). The decrease in muscle succinyl-CoA was associated with elevated Val but not Leu/Ile concentrations; however, BCAA metabolic intermediates accumulated (see below). Other amino acids, Arg, Asx, Gly, His, and Met, were not significantly affected. However, Phe was increased, whereas its metabolite Tyr was significantly decreased by olanzapine compared with control values.

Acyl-carnitines serve multiple functions including as buffers for corresponding acyl-CoAs. Buffering activity may help preserve CoA levels for fatty acid oxidation and energy production. Thus, a rise in these acyl-carnitines may reflect increases in their corresponding acyl-CoAs due to either accelerated or impaired metabolism. Acyl-carnitines from fed gastrocnemius of control and olanzapine-treated rats were also measured (table 3). No change in acetyl-carnitine (C2) was observed consistent with the lack of effect on acetyl-CoA concentrations. However, a significant increase or trend for increase in several other short-chain acyl-carnitines in response to olanzapine treatment was detectable. For example, propionyl-carnitine (C3) was increased ~28%. C3 can be derived from several amino acids including BCAAs,36,37 gut propionate, and odd-chain fatty acids, and the corresponding propionyl-CoA can yield succinyl-CoA. Several species associated with BCAA to succinyl-CoA pathways36,37 were elevated: C4/Ci4 (includes isobutyryl-carnitine from Val) and C5s (includes isovaleryl-carnitine from Leu, tiglyl-carnitine from Ile, and methylbutyryl-carnitine from Ile). Consistent with the decrease in Tyr, there was a trend for a decrease in the carnitine species arising from glutaryl CoA (C5-DC).

Table 3 shows that olanzapine also elicited a 43%–78% rise in the muscle concentrations of long-chain species that most likely arise from 3-hydroxylation of palmitoleic, palmitic, oleic, and linoleic CoA esters during fatty acid metabolism. Consistently, there was also a trend (P = .055) for a peak that probably represents 3-hydroxytetradecenoyl carnitine (C14-OH). Other acyl-carnitine species that can also accumulate in chronic diabetes and “mitochondrial overload” were not impacted by acute treatment (eg, unmodified long-chain and medium-chain fatty acid carnitines and hydroxylated medium-chain acyl-carnitines, table 3). Figure 4 shows a graphical representation of the above findings with olanzapine (a color version is found in the Supplementary data as figure S4).
Effects of Other Atypical Antipsychotics in C57bl/6 Mice

To determine the broader relevance of the RER lowering in rat, the effects of olanzapine and other antipsychotics on RER were examined in another rodent model, C57BL6 mice. Mice were acclimated to the metabolic cages for 36 h or more and then during the dark cycle, when their RER was appropriately elevated in response to food deprivation from sedation/food restriction (figure 5A). As in rats, olanzapine rapidly lowered the dark cycle RER, declining to 0.7 or below within 30 min (figure 5A). To clarify that this effect was not secondary to sedation (animals being too sedated to eat), we examined the effect of vehicle and food restriction (figure 5A). As in rats, olanzapine was orally administered. As in rats, olanzapine administration can be observed in a rodent model. This study clearly demonstrates that the unexpected FFA lowering by olanzapine is also observed after acute administration. As confirmed here, the chronic effects of atypical antipsychotics on insulin sensitivity in rats can also be observed rapidly, supporting the conclusion that insulin resistance arises acutely in this model and is not simply secondary to changes in body composition or weight gain observed with chronic treatment.10,31,40 The same could be said for the lipid effects described in this report, which further suggests a new link between lipid and glucose metabolism in the development of the metabolic side effects of atypical antipsychotics.

Discussion

This study clearly demonstrates that the unexpected FFA lowering effect observed in humans after chronic olanzapine administration can be observed in a rodent model. We show that the FFA lowering by olanzapine is also observed after acute administration. As confirmed here, the chronic effects of atypical antipsychotics on insulin sensitivity in rats can also be observed rapidly, supporting the conclusion that insulin resistance arises acutely in this model and is not simply secondary to changes in body composition or weight gain observed with chronic treatment.10,31,40 The same could be said for the lipid effects described in this report, which further suggests a new link between lipid and glucose metabolism in the development of the metabolic side effects of atypical antipsychotics.

FFA lowering involved both impaired in vivo lipolysis and accelerated fat oxidation. The impairment of in vivo lipolysis is suggested from the reductions in plasma glycerol. Impaired lipolysis could contribute to the increased adiposity after chronic administration that others and we have observed after chronic administration that others and we observe.

Table 3. Effect of Acute Olanzapine on Acyl-Carnitines in Fed Rat Gastrocnemiusa

<table>
<thead>
<tr>
<th>Acyl-Carnitine</th>
<th>Control</th>
<th>Olanzapine</th>
<th>P Value</th>
<th>Acyl-Carnitine</th>
<th>Control</th>
<th>Olanzapine</th>
<th>P Value</th>
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<tr>
<td>C2</td>
<td>1238 ± 92</td>
<td>1150 ± 82</td>
<td>.02</td>
<td>C12-OH/C10-DC</td>
<td>431 ± 69</td>
<td>570 ± 89</td>
<td>.0005</td>
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<tr>
<td>C3</td>
<td>14.8 ± 1.08</td>
<td>18.9 ± 0.92</td>
<td>.02</td>
<td>C14:2</td>
<td>313 ± 58</td>
<td>315 ± 43</td>
<td>.005</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C14:1</td>
<td>541 ± 135</td>
<td>530 ± 94</td>
<td>.03</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C14</td>
<td>1447 ± 262</td>
<td>1188 ± 200</td>
<td>.02</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>C14:1-OH/C12:1-DC</td>
<td>971 ± 146</td>
<td>1186 ± 170</td>
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<td>C14-OH/C12-DC</td>
<td>911 ± 142</td>
<td>1269 ± 160</td>
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<td>C16:2</td>
<td>457 ± 74</td>
<td>427 ± 50</td>
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<td>C16:1</td>
<td>1230 ± 164</td>
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<td>C16</td>
<td>3941 ± 454</td>
<td>3706 ± 366</td>
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<td>C16:1-OH/C14:1-DC</td>
<td>1779 ± 252</td>
<td>2596 ± 336</td>
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<td>C16-OH/C14-DC</td>
<td>1849 ± 192</td>
<td>2650 ± 327</td>
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<td>C18:2</td>
<td>3708 ± 404</td>
<td>4196 ± 409</td>
<td>.02</td>
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<td>C18:1</td>
<td>4765 ± 761</td>
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<td>2067 ± 183</td>
<td>2041 ± 105</td>
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<td>C18:2-OH</td>
<td>2531 ± 273</td>
<td>4517 ± 620</td>
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<td>5642 ± 581</td>
<td>8632 ± 845</td>
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<td>C18-OH/C16-DC</td>
<td>652 ± 74</td>
<td>795 ± 82</td>
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<td>C20:4</td>
<td>858 ± 113</td>
<td>973 ± 92</td>
<td>.02</td>
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<td></td>
<td>C20</td>
<td>100 ± 14</td>
<td>115 ± 15</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C20:1-OH/C18:1-DC</td>
<td>96 ± 14</td>
<td>87 ± 17</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>C20-OH/C18-DC</td>
<td>110 ± 12</td>
<td>117 ± 13</td>
<td>.02</td>
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<td></td>
<td>C22</td>
<td>46 ± 8.1</td>
<td>60 ± 6.2</td>
<td>.02</td>
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</tbody>
</table>

Note: All data represent the mean ± SE. P values < .1 are shown (n = 10/group).

Metabolic Side Effects of Atypical Antipsychotics
have observed. However, in our in vivo studies, we noted that olanzapine had a disproportionate effect on FFA and glycerol in different experiments and different cohorts of animals, suggesting an additional component. Our data further demonstrate that olanzapine also acutely lowers FFA by dramatically increasing whole-body lipid disposal and FFA uptake into most peripheral tissues, except heart. This effect was robust and affected sufficient numbers of peripheral tissues as needed to cause rapid reductions in the whole-body RER of fed rats, improved lipid tolerance, and accumulation of carnitine derivatives of β-oxidation intermediates associated with elevated fat metabolism in diabetes. Consistent with these findings, Ferno et al reported that clozapine caused hepatosteatosis and activated hepatic lipid metabolism genes usually associated with activation of the fatty acid responsive transcription factor, peroxisome proliferator-activated receptor-α.

Healthy humans and animals fed a low fat diet can readily shift between different metabolic states, eg, fed and fasted states, associated with the change in fuel usage by peripheral tissues. The ability to easily switch between these has been termed “metabolic flexibility” and is readily observable by changes in the RER during fasting and with refeeding in animals and humans. Olanzapine acutely blunted this metabolic flexibility in rats, as it and other side effect prone atypical antipsychotics did in mice. Olanzapine acutely supplanted the appearance of these states with a hybrid state that has elements of each. For example, olanzapine impaired lipolysis and elevated insulin and glucose as might be expected under prandial conditions. However, the major fuel being used by peripheral tissues appears to be lipid as expected during food-deprived conditions.

**Olanzapine-Induced FFA Lowering—Good or Bad?**

Conventional wisdom would suggest increasing fat oxidation should be beneficial for health. For example, increasing fat oxidation was associated with improved insulin sensitivity in mice overexpressing muscle CPT1. However, this is an area of current controversy. For example, whereas increased lipid oxidation capacity was noted after high fat feeding and diet-induced obesity in mice, this increase did not decrease fat accumulation in muscle that may activate signaling pathways promoting insulin resistance. Other more recent studies have also

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**Fig. 4.** Schematic Representation of Findings. Olanzapine lowered fasting plasma glycerol implicating impaired lipolysis; however, FFA was depressed lower. FFA lowering appears secondary not only to impaired lipolysis but also increased uptake FFA by most peripheral tissues and elevated lipid oxidation. The key regulator of fat oxidation was decreased along with muscle TCA cycle intermediates, and anapleurotic metabolites contributing to the first strip of the TCA cycle. Measured metabolites are indicated by black text. A color version of this figure is in the Supplementary data as figure S4.

**Fig. 5.** Effect of Antipsychotic Drugs on Respiratory Exchange Ratio (RER) in ad libitum Fed Mice during the Dark Cycle. Male mice were given a single dose of antipsychotic drug or vehicle via oral gavage (indicated by arrows) during the dark cycle following adequate food intake and normal rise in RER. Mice retained ad libitum access to food and water, except for a separate time-matched control group that was food restricted following vehicle gavage (A–D). The effects of olanzapine (OLZ), sulpiride (SUL), risperidone (RIS), clozapine (CLO), ziprasidone (ZIP), aripiprazole (ARI), and haloperidol (HAL) in ad libitum fed mice were examined and compared with vehicle-treated mice that were either fed ad libitum or food restricted. Data represent the mean ± SE (n = 5–6). Some of the same data are shown in different panels for easier comparison of time courses.
noted that obesity and diabetes are marked by indices of elevated fat metabolism already, and these are associated with increased reactive oxygen species and mitochondrial overload leading to incomplete fat oxidation.\textsuperscript{47–49} Indeed, in vitro and mouse studies have shown that decreasing fat oxidation improves muscle glucose uptake.\textsuperscript{50,51} Therefore, the utility of further increases in lipid oxidation in obesity has begun to be questioned. Notably, efforts that were underway in industry that focused on developing therapeutics to increase fat oxidation (eg, mixed ACC inhibitors to lower malonyl-CoA) appear to be shifting to blockers of fat oxidation (eg, CPT1 inhibitors). Because atypical antipsychotics cause severe metabolic side effects leading to obesity and diabetes, our findings add support to the idea that increased fat oxidation may contribute to metabolic dysfunction and may be therefore detrimental for obesity.

The increased FFA oxidation observed after acute olanzapine treatment was associated with some but not all indices of mitochondrial overload that have been observed in obesity and diabetes. For example, we did not observe increased accumulation of long-chain and medium-chain acyl-carnitines in muscle. Further studies are needed to determine whether these worsen with longer treatments. On the other hand, we did observe rises in muscle long-chained 3-OH-acyl-carnitines that become elevated in diabetes. Their presence has been taken as evidence that when fatty acid metabolism is highly active, 3-ketoacyl-CoA thiolase (3-KAT) may become limiting.\textsuperscript{43} Other signature metabolites associated with mitochondrial overload were also observed. There was, for example, evidence of backed-up metabolism of glucose (elevated plasma glucose and insulin, lower GIR, and worsened insulin tolerance) and BCAAs (elevated muscle Val and accumulation of carnitine esters of BCAA intermediates in the muscle).

Based on these findings, it is tempting to speculate that fat metabolism blockers (eg, CPT1 inhibitors) or supplementation of anapleurotic amino acids might be useful to ameliorate some metabolic side effects of atypical antipsychotics.

**Implications for Insulin Resistance and Cardiac Side Effects**

Chronic treatment with side effect prone atypical antipsychotics can result in obesity, and obesity is typically associated with insulin resistance. However, as mentioned earlier, in high-fidelity animal models, rapid, weight gain independent effects on insulin resistance have been observed.\textsuperscript{10–12} According to the Randle theory, major substrates compete for oxidation in the mitochondria.\textsuperscript{52,53} Based on our findings reported here, it is tempting to speculate that substrate competition from elevated fat metabolism may underlie the acute hyperglycemia that others and we have observed. Consistent with a substrate competition mechanism, we did not observe any acute effects of olanzapine on AKT phosphorylation at a site that correlates with elevated activity. We are not aware of any in vitro studies using relevant therapeutic concentrations of olanzapine (eg, 70–350 nM), where insulin resistance has been attributed to known mechanisms such as AKT inhibition or Ser phosphorylation of IRS-1. However, we recognize that the mechanism of the insulin resistance that occurs with these drugs acutely may shift to other mechanisms as lipid metabolites accumulate in muscle and as obesity-associated inflammation develops.

Two observations argue against the converse possibility that olanzapine causes acute insulin resistance that in turn accelerates FFA oxidation. First, olanzapine also increased FFA uptake into tissues that are not dependent on insulin for glucose uptake—namely kidney, liver, gut, and skin. A second caveat involves timing of the olanzapine effect. A 50% reduction in GIR following an i.v. bolus of olanzapine (or clozapine) occurred after \( \sim 90 \) min, with maximal effects appearing after 2 h\textsuperscript{10}; another study showed similar results.\textsuperscript{32} Here, the time to half-maximal effect on RER was more rapid, \( \sim 15–30 \) min following the same dose of olanzapine with maximal effects (RER 0.7) after \( \sim 1 \) h. Notably, we provided olanzapine orally, not intravenously, which should delay peripheral bioavailability compared with an i.v. bolus. These findings suggest that the switch to lipid oxidation is not secondary to an acute effect of olanzapine on glucose disposal.

After clozapine and olanzapine gavage in mice, RER dropped as low as 0.63 in the mouse chambers. If RER is a true reflection of the combined respiratory quotient of all the tissues, it would not be expected to drop below 0.7. We paid special attention to our gas standardization so that is not likely to be an explanation for this problem. Notably with food deprivation RER did not drop below the expected 0.7. It is unclear therefore if the particularly low RER we observed with olanzapine and clozapine in mice represents an effect of these drugs on things that would affect \( O_2 \) and \( CO_2 \) delivery to the sensor, eg, blood pH, plasma potassium or bicarbonate concentrations, or inaccuracy of the instrument design for the mouse calorimeter.

FFA uptake was not increased in the heart, despite increased cardiac work, posited to be an anticholinergic side effect. Heart and skeletal muscles are most severely affected by insulin resistance after acute olanzapine.\textsuperscript{31} Here, we also observed that FFA mobilization associated with food deprivation was impaired by olanzapine. With most of the peripheral tissues switching to use of the heart’s preferred fuel and loss of the ability to mobilize that fuel in times of stress or food deprivation, olanzapine may put the heart at greater risk for arrhythmias associated with impaired energy production, such as torsades.\textsuperscript{17} Interestingly, the specific tachycardia elicited by olanzapine, torsades, is also observed in severe diarrhea, chronic
alcoholism and starvation, other situations of metabolic stress, and decreased RER. Thus, the possibility that the increased lipid metabolism by noncardiac tissues we observed may facilitate sudden cardiac death should be considered.

Malonyl-CoA Reduction

The increased FFA oxidation after olanzapine may be secondary to decreasing the major negative regulator of CPT1, malonyl-CoA, as opposed to direct inhibitory effects on ACC1/2 or indirect effects via AMPK activation. We cannot exclude other possibilities, however, as side effect prone atypical antipsychotics have a number of known targets and presumably unknown targets as well. For example, most contain piperidine or piperazine pharmacophores upon which many pharmaceuticals are built including ligands of enzymes involved in lipid metabolism (eg, ranolazine, trimetazidine, antrafenine) and cell signaling (eg, sildenafil, vardenafil, imatinib).

Further studies are needed to determine the mechanism by which malonyl-CoA is reduced in muscle. Its precursor, citrate was also decreased, as were anapleurotic intermediates and related amino acids required for malonyl-CoA production in the first span of the TCA cycle. Flux studies will be needed to understand whether these anapleurotic metabolites are responsible for the lower TCA cycle intermediates or are a consequence of lower TCA intermediate concentrations.

Additionally, further studies are needed to demonstrate potential malonyl-CoA lowering in the other affected tissues. While FFA uptake was not observed in brain as expected, we cannot rule out that this might occur in select brain regions where endothelium is more fenestrated (eg, arcuate nucleus and area postrema). Within some hypothalamic neurons, alterations in malonyl-CoA may be a hunger signal and thereby involved in the orexigenic effects of olanzapine.

Summary

Collectively, the present study presents novel data related to the mechanism of a metabolic side effect of olanzapine observed in humans, i.e. FFA lowering. We have also shown that acute olanzapine induces metabolic inflexibility by causing a rapid shift in the major fuel being oxidized within peripheral tissues from mostly carbohydrate to mostly fat while insidiously preventing the mobilization of that fuel. After food deprivation, and perhaps by extension between meals, these actions of olanzapine more rapidly deplete lipid fuel than would otherwise occur. The shift in fuel utilization appears to precede the development of insulin resistance and, therefore, has the potential to explain its development and conceivably may also be involved in some of the other known side effects of these drugs.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org

Funding

V.L.A. is the recipient of a Seed Grant from the American Medical Association, Foundation, National Institutes of Health (DK062880 to C.J.L.; DK072909 to C.H.L.; AA12814, GM39277 to T.C.V.); Pennsylvania Department of Health using Tobacco Settlement Funds.

Acknowledgments

We especially thank Drs Christopher B. Newgard and Dr James R. Bain at the Sarah W. Stedman Nutrition and Metabolism Center at Duke University Medical Center for their organizing metabolomic analysis of organic acids and acyl-carnitine profiles in muscle tissues. We are also grateful for the expert technical assistance of Beth Halle Gern, Stephanie Goshorn, Jamie G. Spencer, Anne Pruznak, and Rachel Lantry. The Department of Health specifically disclaims responsibility for any analyses, interpretations, or conclusions. The authors declare no conflicts of interest.

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