Second-Generation Antipsychotics Cause a Rapid Switch to Fat Oxidation That Is Required for Survival in C57BL/6J Mice

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Some second-generation antipsychotics (SGAs) increase insulin resistance and fat oxidation, but counterintuitively they do not activate lipolysis. This seems unsustainable for meeting energy demands. Here, we measured dose-dependent effects of SGAs on rates of oxygen consumption (VO₂), respiratory exchange ratio (RER), and physical activity in C57BL/6J mice. The role of H₁-histamine receptors and consequences of blocking fat oxidation were also examined. Olanzapine, risperidone, and clozapine (2.5–10 mg/kg) elicited rapid drops in dark-cycle RER (~0.7) within minutes, whereas aripiprazole exerted only modest changes. Higher doses of olanzapine decreased VO₂, and this was associated with accumulation of glucose in plasma. Clozapine and risperidone also lowered VO₂, in contrast to aripiprazole, whereas all decreased physical activity. Astemizole and terfenadine had no significant effects on RER, VO₂, or physical activity. The VO₂ and RER effects appear independent of sedation/physical activity or H₁-receptors. CPT-1 inhibitors can enhance muscle glucose utilization and prevent fat oxidation. However, after etomoxir (2 × 30 mg/kg), a low dose of olanzapine that did not significantly affect VO₂ by itself caused precipitous drops in VO₂ and body temperature, leading to death within hours or a moribund state requiring euthanasia. One 30 mg/kg dose of either etomoxir or 2-tetradecylglycidate followed by olanzapine, risperidone, or clozapine, but not aripiprazole, dramatically lowered VO₂ and body temperature. Thus, mice treated with some SGAs shift their fuel utilization to mostly fat but are unable to switch back to glucose or meet their energy demands when either higher doses are used or when fat oxidation is blocked.

Key words: respiratory exchange ratio/fat oxidation/glucose/body temperature/risperidone/olanzapine/clozapine/histamine/oxygen consumption

Introduction

Second-generation antipsychotics (SGAs) including olanzapine, clozapine, and risperidone are commonly associated with metabolic side effects including insulin resistance, glucose intolerance, overeating, increased adiposity, metabolic syndrome, and diabetes. These side effects reduce quality of life and increase the risk of cardiovascular disease and mortality. Additionally, the diagnoses for which SGAs are prescribed along with their overall utilization in adults and children have increased dramatically, thus increasing the pool at risk. It is currently unclear whether children, a population in which diabetes appears to be more difficult to treat, are appropriately screened for these side effects. Another issue is that the weight gain and metabolic side effects contribute to reduced compliance, increasing the risk of psychosis and the potential for reduced efficacy of subsequent SGA treatment. Although some SGAs, like aripiprazole, have a lower incidence of adverse effects, they may not possess the same clinical efficacy as olanzapine, clozapine, and risperidone. So it is important to understand the mechanism of these effects in order to develop approaches or targets to reverse these trends.

Surprisingly, some of the glycemic effects of olanzapine and other SGAs manifest quickly. For example, elevated plasma glucose, glucose intolerance, or lower glucose infusion rates during a euglycemic clamp have been observed acutely in preclinical and clinical studies within minutes or days. Another metabolic disturbance, decreased circulating free fatty acids (FFA), has also been described after both acute (1 h to 3 days) and chronic (2–3 weeks) treatment. The converse effects of SGAs on glycemia and FFA are of interest for several reasons. First, these effects appear to precede significant weight gain. Second, the pattern of these changes among the SGAs tested so far is...
similar to the diabetes and weight-gain side-effect profile of these drugs. For example, FFA lowering was found after risperidone and olanzapine treatment but not after aripiprazole or haloperidol treatment in humans.10,12,17

In diabetes and other insulin-resistant states, peripheral tissues increasingly utilize fat rather than glucose as a fuel, leading to the accumulation of glucose in plasma. Similarly, in olanzapine-treated, ad libitum-fed rodents, a dramatic increase in FFA metabolic rate is observed (~2-fold), coinciding with a rapid fall in the dark-cycle respiratory exchange ratio (RER) to ~0.7, as well as glucose accumulation in plasma.13 RER measures provide a non-invasive assay for the rapid and robust shift in fuel utilization from glucose to fat observed after SGA treatment. It is unclear why RER lowering has not been reported in humans treated with SGAs; however in humans, calorimetry is usually performed in the fasted state, a time when RER is already lowered. Thus, in diabetes and insulin-resistant states and after acute SGA treatment, glucose accumulates in the plasma and, at least in animals, there is increased lipid oxidation. However, there are notable differences between these 2 situations. In insulin resistance and diabetes, FFA are mobilized due to increased adipose tissue lipolysis.19 FFA mobilization in this case is important to sustain metabolic rate and meet whole-body energy demands. In contrast, olanzapine does not increase glycerol rates of appearance in humans10 despite causing glucose intolerance within days.12 Furthermore, in both acute and chronic preclinical studies, basal and isoproterenol-stimulated lipolyses were actually impaired in vivo by olanzapine.13 From an energy-balance standpoint, increasing stimulated lipolyses were actually impaired in vivo by olanzapine, tetradecylglycidic acid (TDGA; CAS # 68170-97-8), were generous gifts of Neuland Laboratories Ltds and Janssen Research & Development, LLC, respectively. Clozapine (Alexis Biochemicals), risperidone (Amneal Pharmaceuticals), aripiprazole (Bristol-Myers Squibb), the noncompetitive CPT-1 inhibitor etoxomir (Sigma-Aldrich), and terfenadine and astemizole (Torcis Bioscience) were purchased from the indicated suppliers.

Materials and Methods

Materials

Olanzapine and the noncompetitive CPT-1 inhibitor, tetracydeglycidic acid (TDGA; CAS # 68170-97-8), were generous gifts of Neuland Laboratories Ltds and Janssen Research & Development, LLC, respectively. Clozapine (Alexis Biochemicals), risperidone (Amneal Pharmaceuticals), aripiprazole (Bristol-Myers Squibb), the noncompetitive CPT-1 inhibitor etoxomir (Sigma-Aldrich), and terfenadine and astemizole (Torcis Bioscience) were purchased from the indicated suppliers.

Animals

All procedures were conducted after review and approval by the Penn State Hershey Institutional Animal Care and Use Committee (IACUC). The Animal Resource Program is operated by the Department of Comparative Medicine and is accredited by AAALAC International. All animal living conditions are consistent with standards laid forth in the Guide for the Care and Use of Laboratory Animals, 8th edition, published by the National Research Council.

Male C57BL/6J mice were from The Jackson Laboratory or from our colony back-crossed to mice from this supplier about every third generation. All of the mice tested had been in our facility for at least 3 weeks. The average body weight was 31 ± 0.3 g, and the ages ranged from 5 to 6 months. Mice were maintained on a 12:12 light–dark cycle (lights on at 0700h) with ambient room temperature at 20.6 ± 1°C. Food (Harlan-Teklad Rodent Chow, no. 2018; Harlan-Teklad) and water were provided ad libitum unless otherwise indicated. Food intake before the acute experiments described was 4.8 ± 0.1 g.

Body composition was measured in conscious animals using a Bruker LF90 proton-NMR Minispec (Bruker Optics). The mice had the following pretreatment body composition (mean ± SE): lean body mass, 71.4 ± 0.7 %; fat mass, 10.7 ± 0.6 %; and body fluid, 8.0 ± 0.1 %, and there was no significant effect of the acute treatments on body composition. Shapiro–Wilk test was used to test the hypothesis that these mice might display a lack of normality with regard to body composition. However, the results of that testing indicated that the body composition follows a normal distribution (lean mass:  P = .16; Fat mass:  P = .17; Fluid mass:  P = .55). Mice were routinely allocated to groups to minimize pretreatment differences in body weight.

Drug Preparation

All drugs were administered to mice via oral gavage that had been previously exposed to vehicle gavage treatments.
for at least 3 days. Olanzapine was prepared using 0.1N HCl adjusted to pH 5 using NaOH. Other SGAs and etomoxir were suspended in 2% polyethylene glycol and 98% carboxymethylcellulose as previously described.11,13 TDGA was prepared using 90 mg/ml of fatty acid–free bovine serum albumin in 0.9% NaCl.

SGAs were given at clinically relevant doses that induce varying levels of glycemia in rodents.16,24 Furthermore, the doses of SGAs chosen conform to recent criteria using dopamine D2 receptor occupancy to simulate human drug dosing.24–26 The dose of the noncompetitive CPT-1 inhibitors was based on previous literature and consultation with the supplier of TDGA.27,28 Where indicated, 1 or 2 doses of etomoxir or TDGA (both 30 mg/kg) were given 3 and 15 h prior to the SGA to induce presumptive full or partial blockade of CPT-1.25 Two doses are thought to provide a more complete inhibition of CPT-1. The doses of H1-antagonists in the current experiment were chosen based on their effective behavioral response in mice exposed to allergens.29

**VO₂ and Locomotor Activity**

Male C57BL/6J mice were acclimated to single cage housing for a week or more and then were accustomed to receiving daily gavages of 0.2 ml water for 4 to 7 days. They were then acclimated to Columbus Instruments (CI) metabolic chambers fitted with an air circulation fan for a minimum of 24 h and up to 96 h before studies. The CI system used does not have automated food intake, water intake, and spillage detection. The water bottles used were the same as in the single housing, and the food was provided on the plastic decking (~3 pellets per day) so that it was easy for the animals to find and was changed daily. We have compared this CI system to another that does have automated food intake detection. In our experience, the required acclimation time for mice, evaluated by measuring body weight, is less when the food is provided on the plastic decking compared with the automated system that we have access to. In our nonautomated CI system, the mice being acclimated to oral gavage have an elevated VO₂ that subsides over 6 h or so, thus, justifying the 24-h acclimation.

Researchers did not perform studies on the mice if they had lost weight by the dosing time, if 2100 h target RER values were not above 0.8, or if body weight–corrected VO₂ values were above 3500. In these cases, the animals were allowed to acclimate further until these objective endpoints were observed.

Vehicle-treated mice were tested concurrently with drug-treated mice for all replicates. VO₂, RER, and locomotor activity were measured as previously described.13 Briefly, O₂ and CO₂ concentrations were measured from sealed chambers to calculate O₂ consumption (VO₂) and CO₂ expiration (VCO₂) (Oxymax; Columbus Instruments). Each chamber was measured for 1 min at 15-min intervals. Flow rate was set to 0.6 l.p.m. RER was calculated by dividing VCO₂/VO₂. Locomotor activity was measured simultaneously using infrared technology (OPT-M3; Columbus Instruments). The number of light-beam breaks were counted along the x-axis and z-axis and averaged over 3 or 6 h of treatment.

There is current controversy about how to normalize VO₂ or energy expenditure data when groups of animals with different body compositions are being compared.30,31 Such data are usually collected over time and then averaged for some time period (eg, 24 h, dark cycle or light cycle, energy expenditure or VO₂). However, these approaches that employ ANCOVA do not seem to be appropriate for displaying and comparing repeated measures over time in the same animals or comparisons to vehicle-treated mice with the same body composition, genotype, and diet. Mice in this study were all lean with a narrow range of body weight and composition. In pilot studies, we found that body composition did not change over the time course we were studying in animals treated with olanzapine, data not shown. Even et al.31 addressed a second situation applicable to our study where adult, lean animals of the same body composition were being compared. They indicate that when lean rodents are being studied, a normalization procedure is needed even if the animals are all of the same genotype, and they showed that body weight is a better normalize compared with an exponent of body weight or fat-free mass for this purpose. This was validated in their study by plotting the body weight (x) against body weight–corrected energy expenditure (y). Although Even et al.31 indicated that using body weight for a denominator was valid for lean adult rats and mice, only rat data was shown. To help validate body weight normalization of our VO₂ data, we used their approach by plotting the body weight of vehicle-treated mice against body weight–corrected dark-cycle VO₂ values. We included mice at the lighter and heavier end of the spectrum. The data were fitted to a linear equation F(X) = MX + B and analyzed to determine the correlation coefficient using GraphPad Prism 6.0 software. The results of this analysis are described and support the use of body weight for our comparisons.

**Blood Glucose and Body Temperature**

Glucose was measured from tail blood in duplicate using an Ascencia Contour blood glucose meter (Bayer Health Care LCC). Rectal body temperature was measured using a NIST-certified electronic thermometer (Fluke Corporation) with a mouse thermocouple probe from Harvard Apparatus.

**Statistical Analysis**

Data are expressed as the mean ± SEM. Multiple comparisons were performed using one-way ANOVA.
with significance at $P < .05$ using GraphPad Prism computer software (GraphPad Software). To determine when $VO_2$ and RER were changing after SGA treatment, data were analyzed using repeated measures ANOVA (GraphPad Prism) with time and treatment as main effects. Experimental treatment values were compared with that of controls using a Dunnett’s post test and the vehicle group as the indicated control. Because in many instances drug treatments decreased both $VO_2$ and activity, an ANCOVA was performed using XLSTAT software (Addinsoft). We assessed the relationship between body weight–corrected $VO_2$ and activity using the total number of beam breaks along the $x$-axis (XTOT) summed over 3 h, and we compared this to the 3-h average of $VO_2$ during this same time. We chose to use data from 3 h after treatment because this is when repeated measures ANOVA detected differences in $VO_2$ between most doses of SGAs and vehicle. We also compared 3-h XTOT with the 3-h average of $VO_2$ after treatment subtracted from the 3-h average of $VO_2$ before treatment ($VO_2$ difference) to account for potential differences in $VO_2$ prior to treatment. Differences among groups were analyzed using Bonferroni’s multiple comparison post test where appropriate.

Results

Olanzapine Has Converse, Dose-Dependent Effects on Plasma Glucose and RER

Recent studies from our group and others have shown that olanzapine acutely raises plasma glucose in Sprague-Dawley rats and FVB/N mice within 1–2 h. An acute glycemic effect of olanzapine is also observed in euglycemic clamp studies performed on rats. In this study, in male C57BL/6J mice, olanzapine dose dependently increased plasma glucose 1 h after oral administration (Figure 1). Glucose was elevated 43% at the 5 mg/kg dose and 61% at 10 mg/kg. We did not evaluate higher doses; however, in acute rat studies by Assie et al., the acute glycemic effects had not been saturated by 40 mg/kg.

Olanzapine lowers plasma FFA, increases FFA metabolic rate, and dramatically lowers dark-cycle RER. These findings support the idea that olanzapine increases fat oxidation. Here, we also monitored changes in dark-cycle RER as a noninvasive approach to monitor changes in major fuel selection. At 5 mg/kg, olanzapine caused a sustained lowering of RER to ~0.7, which began to recover around 8 h later. A dose of 2.5 mg/kg was somewhat less efficacious, lowering RER early on and exhibiting a faster recovery (Figure 2A). Repeated measures ANOVA indicated that compared with vehicle treatment, 2.5 mg/kg olanzapine caused a significant decrease in RER between 2.25 and 8.5 h compared with vehicle ($P < .0001$ to $P < .001$) with effects lasting longer at the higher dose of at 5 mg/kg (2.25–13 h; $P < .0001$). Thus, hyperglycemia after olanzapine (Figure 1) is associated with a dose-dependent transition to fat oxidation as measured by this drop in the RER (figure 2A).

Effects of Risperidone, Clozapine, and Aripiprazole on RER

In their study, Assie et al. found that clozapine and risperidone also efficaciously raised glucose, whereas comparatively, aripiprazole had only marginal affects that were not dose related. Here, we examined the dose-dependent effects of risperidone, clozapine, and aripiprazole on RER (figures 2B–D). At 2.5 and 5 mg/kg, risperidone had RER lowering effects (figure 2B), decreasing the dark-cycle RER to ~0.7 within 1 h after gavage (Repeated measures ANOVA: vehicle and 2.5 mg/kg risperidone were significantly different between 2.5 and 13 h [$P < .0001$]; vehicle and 5 mg/kg RIS were different between 2.75 and 13 h [$P < .0001$]). Comparable to the lower dose of olanzapine (figure 2A), 5 and 10 mg/kg clozapine had a similar RER nadir (~0.7) and similar rates of recovery (figure 2C; Repeated measures ANOVA: vehicle and 5 mg/kg clozapine were different between 2 and 8 h [$P < .0001$ to $P < .05$] and 10 mg/kg clozapine was different from vehicle treatment between 2 and 8.25 h [$P < .0001$ to $P < .05$]). Compared with these other SGAs, aripiprazole caused smaller effects on RER as we previously reported. The RER after a 1 mg/kg aripiprazole dose was not significantly different from vehicle at any time point ($P > .05$). However, at 5 and 10 mg/kg, aripiprazole lowered RER, albeit with less efficacy compared with the other SGAs (figure 2D; Repeated measures ANOVA: vehicle and 5 mg/kg aripiprazole were different between...
2.25 and 10.5 h \( P < .0001 \) to \( P < .01 \), whereas 10 mg/kg aripiprazole was different from vehicle between 2.5 and 13 h \( P < .0001 \) to \( P < .01 \)).

Some SGAs Lower Whole-Body Respiration at Higher Doses

Next, we examined the time-course effects of different doses of SGAs on VO\(_2\). As described in the methods, we used an approach from Even et al.\(^{31}\) to validate the use of body weight as a normalizer for the VO\(_2\) values. Using the approach from their article, we plotted the average dark-cycle body weight–corrected VO\(_2\) of vehicle-treated mice against their body weight (figure 3). We included mice at the lighter and heavier end of the spectrum such as those used in this study. We found that the relationship between the body weight and the per weight VO\(_2\) for our mice appears similar across the range of weights we used (the fitted line had a very low \( r^2 \) and has a very slight negative slope with a ratio of M to B less than 0.001), which is consistent with the rat data obtained by Even et al.\(^{31}\)

Notably, the average body weight of all of the animals used was \( \sim 31 \) g. For these reasons, we decided to use body weight as a normalizer for our VO\(_2\) measurements and analyzed the time courses for differences using ANOVA repeated measures as with the RER data.

The dose-dependent effects of olanzapine on body weight–corrected VO\(_2\) is shown in figure 4A. The lower dose of olanzapine (2.5 mg/kg) that dramatically lowered RER had no significant effect on VO\(_2\) (compare figure 2A with figure 4A). In contrast, at 5 mg/kg, olanzapine, which also lowered RER, caused a roughly 50% decrease in the VO\(_2\) (figure 4A; repeated measures ANOVA indicated treatment differences from vehicle after 5 mg/kg olanzapine between 2.25 and 6.25 h \( P < .0001 \) to \( P < .05 \); it should be noted that at the higher dose, the ratio of VCO\(_2\)/VO\(_2\) (RER) is based on a much lower VCO\(_2\) and VO\(_2\).)

The dose-dependent actions of risperidone, clozapine, and aripiprazole on VO\(_2\) were also examined (figures 4B–D). Although 2.5 and 5 mg/kg doses of risperidone had similar efficacy of lowering RER (figure 2B), they exhibited dose-dependent effects on VO\(_2\) (figure 4B),...
decreasing the VO$_2$ by ~33% and 52%, respectively, within 1 h after gavage. Repeated measures ANOVA for 2.5 mg/kg risperidone was significant compared with vehicle-treated mice between 1.75 and 2 h ($P < .0001$ to $P < .05$), whereas 5 mg/kg risperidone was different from vehicle between 1.75 and 3.5 h ($P < .0001$ to $P < .05$). Although 5 and 10 mg/kg clozapine had a similar RER nadir, the higher dose led to a ~64% transient reduction in the VO$_2$ compared with a ~40% reduction at the lower dose (figure 4C; repeated measures ANOVA: 5 mg/kg clozapine compared with vehicle was significantly lower between 2 and 4.5 h [$P < .001$ to $P < .05$], and for 10 mg/kg clozapine, a difference was observed between 1.75 and 4.5 h [$P < .0001$ to $P < .05$]). None of the aripiprazole doses affected VO$_2$ at any time point (figure 4D).

H1-Antihistamines Do Not Affect RER, VO$_2$, or Physical Activity

Blocking of H1-histamine receptors has been implicated in some metabolic side effects of SGAs.$^{34-37}$ Therefore, we examined the effect of 2 H1-blocking exemplars, astemizole (Hismanal) and terfenadine (Seldane), using effective H1-blocking doses from previous literature. Neither

**Fig. 3.** Comparison of body weight to body weight–corrected dark-cycle rates of oxygen consumption in lean vehicle-treated C57BL/6J mice used in our study. Average dark-cycle body weight–corrected rates of oxygen consumption was calculated for vehicle-treated mice and plotted against their different body weights as described in the Materials and Methods. The data were fitted to a line to calculate the correlation coefficient, slope, and intercept.

**Fig. 4.** Effects of second-generation antipsychotics on rates of oxygen consumption. Rates of oxygen consumption shown for the same mice from figure 2 before and after vehicle (☐) or indicated doses (closed symbols) of (A) olanzapine (OLZ), (B) risperidone (RIS), (C) clozapine (CLZ), or (D) aripiprazole (ARI) at $t = 2100$ h (indicated by the arrow). Gray background indicates dark cycle; white background indicates light cycle. Mice had access to food and water ad libitum before and after gavage. Mean and SE are shown ($n = 5–11$/grp).
astemizole (3 mg/kg) nor terfenadine (10 mg/kg) had any significant effect on RER or VO₂ (figure 5). While there was a tendency for reduced physical activity after terfenadine administration, neither terfenadine nor astemizole elicited a statistically significant decrease in physical activity (table 1).

SGAs Including Aripiprazole Acutely Decrease Physical Activity

The calorimeter is fitted with 2 light sensor arrays to measure horizontal (X) and vertical (Z) light-beam breaks that were monitored in the above experiments. All of the SGAs tested decreased X movement 50% or more during the dark cycle compared with corresponding vehicle controls, with some also affecting rearing behavior (Z), which was more variable between the controls (table 1). Activity levels neither increased nor plummeted immediately following vehicle gavage, reflecting our acclimation procedure. Thus, any change in activity likely represents a drug treatment effect. Presumably, this decrease in physical activity represents sedation by the drugs. Aripiprazole, which did not affect VO₂ (figure 3D), is included in the drugs that caused this decrease in physical activity (table 1). This observation would seem to disassociate the sedative/physical activity and VO₂ effects of the SGAs, an interaction that is presented later in the analysis.

This apparent lack of association between physical activity and VO₂ was also found in another analysis using ANCOVA. In many instances, SGA treatment decreased VO₂ and physical activity. ANOVA repeated measures analyses suggested that some SGA doses had consistent effects on body weight–corrected VO₂ at least over 3 h after gavage. Therefore, we used ANCOVA analysis to examine the potential relationship between vehicle and drug effects on physical activity and VO₂ using a 3-h average of the physical activity and VO₂ after gavage (data not shown). We found no interaction between XTOT and VO₂ for olanzapine (P = .49), risperidone (P = .45), clozapine (P = .60), aripiprazole (P = .86), terfenadine (P = .70), or astemizole (P = .66). However, consistent with repeated measures ANOVA, there was a treatment effect for olanzapine (P = .001), risperidone (P = .03), and clozapine (P = .008). Furthermore, the coefficient of determination (R²) for each regression line was less than 0.1 for most of the VO₂ and XTOT interactions tested including the vehicles, indicating that VO₂ is not a good predictor of total activity and vice versa. We extended the

Fig. 5. Lack of effects of H1-antihistamines on respiratory exchange ratio or rates of oxygen consumption. Using the same protocol from figures 2 and 3, the effects of indicated single doses of either (A and B) astemizole (ASTEM) or (C and D) terfenadine (TERF) on (A, C) respiratory exchange ratio and (B, D) rates of oxygen consumption were measured. Repeated measures ANOVA did not detect any differences between vehicle (VEH) and antihistamines on respiratory exchange ratio or rates of oxygen consumption at any time point (P > .05). Oral gavages were performed at t = 2100 h, indicated by an arrow. Gray background indicates dark cycle; white background indicates light cycle. Mice had access to food and water ad libitum before and after gavage. Mean and SE are shown (n = 7–10/grp).
CPT-1 inhibitors tend to enhance glucose metabolism.\textsuperscript{17,21–23,43} We questioned whether CPT-1 blockade could prevent the SGA-induced increase in fat metabolism, which might in turn convert the animals back to using more glucose as a fuel. To test this, we used 2 CPT-1 noncompetitive antagonists, etomoxir and TDGA. A previous study showed that two 30 mg/kg doses of a CPT-1 inhibitor within 24 h produces an effective noncompetitive CPT-1 blockade.\textsuperscript{28}

In pilot studies, CPT-1 blockade did not increase the dark-cycle RER significantly after treatment with either etomoxir (figure 6) or TDGA (data not shown). In one previous study, 2 doses of etomoxir (30 mg/kg) raised light-cycle RER measurements from 0.77 to 0.89 in fasted male C57BL/6J mice tested in the light cycle,\textsuperscript{29} conditions that are ideal to observe an increase in the RER. Such food-deprived conditions are typical for calorimetry in humans as well. However, our studies were performed on ad libitum-fed mice during the dark cycle in order to raise the RER close to 0.89 or above in order to provide ideal conditions for observing RER lowering. Under these conditions, not observing a further rise in RER may represent a ceiling affect.

In the first experiment, we provided 2 doses of etomoxir (both 30 mg/kg) 15 and 3 h before dark-cycle administration of 2.5 mg/kg olanzapine or vehicle (ETX-ETX-OLZ; figure 6). The ETX-ETX-OLZ treatment was compared to a vehicle treatment in which animals received vehicle instead of etomoxir but still received 2.5 mg/kg of olanzapine (VEH-VEH-OLZ; figure 6). Notably, the lower 2.5 mg/kg dose of olanzapine did not affect VO$_2$ in earlier studies (figure 4A) but did lower RER (figure 2A).

Unexpectedly, on the morning following this treatment regimen, some of the animals were found to have died during the night. Those living were in a moribund state with body temperatures approaching room temperature (figure 6C). Analysis of the calorimetry data (table 2 and figure 6) showed that shortly after olanzapine treatment, VO$_2$ in the surviving ETX-ETX-OLZ–treated mice had dropped by ~90% within hours. The RER (figure 6A) also decreased but was for a much smaller VO$_2$ (figure 6B) and VCO$_2$ (not shown). This lowering of the VO$_2$ with the RER of ~0.7 implies that the animals could not switch to an alternate fuel after ETX-ETX-OLZ treatment and were mostly metabolizing fat using the residual CPT-1 activity that the etomoxir treatment had failed to block.

To further investigate the effects of CPT-1 blockade on SGA metabolic responses, we explored the effects of a presumptive partial CPT-1 blockade (ie, only a single dose of either etomoxir or TDGA 3 h before SGA or vehicle). Here again, lower doses of SGAs that had minimal effects on VO$_2$ by themselves were used (figure 7). Figures 7A and B show that treatment with a single dose of either etomoxir or TDGA followed by vehicle (ETX-VEH or TDGA-VEH) had no effect on VO$_2$ compared with 2 consecutive vehicle treatments (VEH-VEH). However, the combination of a single dose of either etomoxir or TDGA followed by 2.5 mg/kg olanzapine led to reduced VO$_2$ and body temperature (figures 7A–C). Thus, etomoxir and TDGA were equally effective at causing this adverse VO$_2$ lowering when combined with olanzapine. Although all of the mice treated with the lower cumulative dose CPT-1 inhibitor and SGA combination treatment survived through the next morning, their morbimund state and reduced body temperature (figure 7C) continued, requiring humane euthanasia the next day.

The activity data in tables 1 and 2 were acquired at different times, with a different cohort of mice, so we did not

**Table 1. Six-Hour Cumulative Physical Activity (Light Array Beam Breaks) After Treatment**

<table>
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<th>Drug</th>
<th>Dose (mg/kg)</th>
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<th>Activity on Z-axis</th>
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<td>812 ± 176</td>
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<td>821 ± 232**</td>
<td>225 ± 74*</td>
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<td>1938 ± 479**</td>
<td>324 ± 73**</td>
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<td>263 ± 51**</td>
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<tr>
<td>Aripiprazole</td>
<td>1</td>
<td>2280 ± 428*</td>
<td>626 ± 224</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2887 ± 444*</td>
<td>623 ± 131</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3647 ± 924*</td>
<td>867 ± 218</td>
<td>7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>5463 ± 1386</td>
<td>1556 ± 567</td>
<td>8</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>10</td>
<td>3428 ± 861</td>
<td>1021 ± 337</td>
<td>9</td>
</tr>
<tr>
<td>Astemizole</td>
<td>3</td>
<td>6109 ± 1258</td>
<td>1360 ± 405</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: Activity was measured using infrared beam breaks during the dark phase of the light/dark cycle. Data are mean ± SE for 6-h cumulative activity after oral gavage at 2100h. Asterisks indicate number of beam breaks different from vehicle (*$P < .05$, **$P < .01$, ***$P < .001$).

analysis to include ANCOVA testing for the difference in VO$_2$ before and after treatment, and again, we detected no interaction between VO$_2$ difference and XTOT activity for any of the SGAs or antihistamines ($P > .05$).

These findings suggest that a decrease in VO$_2$ does not appear to be related to any changes in physical activity as a measure of the level of sedation. This should not be surprising because the physical activity is only one contributor to daily caloric requirements.

**Effects of Noncompetitive Fat Oxidation Inhibition**

CPT1 is the rate controlling step in fat oxidation. It is negatively regulated by intracellular concentrations of malonyl CoA. In previous studies, we found that olanzapine increased the FFA metabolic rate and this was associated with a rapid decline in malonyl CoA.\textsuperscript{13} Recent studies have suggested that increased FFA oxidation in diabetes may be “too much of a good thing” and contributes to diabetes pathology.\textsuperscript{38–42} CPT-1 inhibitors tend to enhance glucose metabolism.\textsuperscript{17,21–23,43} We questioned whether CPT-1 blockade could prevent the SGA-induced increase in fat metabolism, which might in turn convert the animals back to using more glucose as a fuel. To test this, we used 2 CPT-1 noncompetitive antagonists, etomoxir and TDGA. A previous study showed that two 30 mg/kg doses of a CPT-1 inhibitor within 24 h produces an effective noncompetitive CPT-1 blockade.\textsuperscript{28}
Fig. 6. Effects of 2 doses of etomoxir (ETX) in combination with olanzapine (OLZ) on rates of oxygen consumption, respiratory exchange ratio, and body temperature. Male C57BL/6J mice acclimated to single housing, gavage treatment, and calorimetry cages as in figures 2 and 3. ETX (30 mg/kg) or vehicle (VEH) were given twice via oral gavage 15 h (not shown) and 3 h before a single dose of (A–C) 2.5 mg/kg OLZ or (C) VEH at 2100 h. Mean ± SE of the (A) respiratory exchange ratio and (B) rates of oxygen consumption are shown (n = 4 grp). Repeated measures ANOVA detected differences between VEH-VEH-OLZ and ETX-ETX-OLZ between 7.5 and 13 h (*P < .0001 to **P < .05) for rates of oxygen consumption. No differences were detected between VEH-VEH-OLZ and ETX-ETX-OLZ for respiratory exchange ratio (P > .05). Gray background indicates dark cycle and white background indicates light cycle times. Mice had access to food and water ad libitum before and after gavage. Rectal body temperature (C) was measured in remaining live mice the following light cycle (0900 h) when the mice treated with ETX and OLZ (ETX-ETX-OLZ, n = 3) were observed to be deceased or in a moribund state compared with OLZ- or VEH-treated mice. Asterisks indicate statistically different from vehicle at P < .0001.

Table 2. Six-Hour Cumulative Physical Activity (Light Array Beam Breaks) After First Treatment With Etomoxir or Vehicle Followed by Second Treatment With Second-Generation Antipsychotics or Vehicle as Indicated

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>Dose (mg/kg)</th>
<th>Treatment 2</th>
<th>Dose (mg/kg)</th>
<th>Activity on X-axis</th>
<th>Activity on Z-axis</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>Vehicle</td>
<td>0</td>
<td>7575 ± 1914</td>
<td>2076 ± 680</td>
<td>6</td>
</tr>
<tr>
<td>Etomoxir</td>
<td>30</td>
<td>Olanzapine</td>
<td>2.5</td>
<td>920 ± 180**</td>
<td>273 ± 54*</td>
<td>4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>Vehicle</td>
<td>0</td>
<td>7349 ± 2094</td>
<td>2009 ± 818</td>
<td>6</td>
</tr>
<tr>
<td>Etomoxir</td>
<td>30</td>
<td>Risperidone</td>
<td>2.5</td>
<td>1370 ± 594*</td>
<td>402 ± 118</td>
<td>6</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>Vehicle</td>
<td>0</td>
<td>7236 ± 1729</td>
<td>1204 ± 437</td>
<td>6</td>
</tr>
<tr>
<td>Etomoxir</td>
<td>30</td>
<td>Clozapine</td>
<td>3</td>
<td>1393 ± 266**</td>
<td>244 ± 51*</td>
<td>6</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>Vehicle</td>
<td>0</td>
<td>7813 ± 1753</td>
<td>1353 ± 469</td>
<td>6</td>
</tr>
<tr>
<td>Etomoxir</td>
<td>30</td>
<td>Aripiprazole</td>
<td>1</td>
<td>3207 ± 825*</td>
<td>503 ± 165</td>
<td>7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>Vehicle</td>
<td>0</td>
<td>7089 ± 1689</td>
<td>1305 ± 574</td>
<td>6</td>
</tr>
<tr>
<td>Etomoxir</td>
<td>30</td>
<td>Vehicle</td>
<td>0</td>
<td>4529 ± 809</td>
<td>1123 ± 296</td>
<td>10</td>
</tr>
</tbody>
</table>

Note: Activity was measured using infrared beam breaks during the dark cycle. Data are mean ± SE for 6-h cumulative activity after oral gavage at 2100h as in table 1. Etomoxir, TDGA, or vehicle was given at 1800h (Treatment 1), followed by second-generation antipsychotics or vehicle at 2100h (Treatment 2). Data are mean ± SE for 6-h cumulative activity after oral gavage at 2100h. Asterisks indicate number of beam breaks different from vehicle (*P < .05, **P < .01).
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Discussion

In this study, olanzapine acutely and dose dependently increased accumulation of glucose in the plasma of male...
C57BL/6J mice consistent with our previous results and those of Assie et al.\textsuperscript{16} in rats. This was associated with dose-dependent changes in fat oxidation as reflected in rapidly falling RER values, consistent with our previous studies showing that olanzapine acutely increases fat oxidation in most peripheral tissues of the rat.\textsuperscript{13} However, in our previous studies, we also showed that olanzapine impairs lipolysis and increases lipogenesis in adipose tissue. These factors appeared to underlie lower FFA concentrations. As mentioned, switching peripheral tissues to fat oxidation while impairing FFA mobilization would seem to be an unsustainable situation from a metabolic standpoint. Our current study also supports this idea. In the current experiments, olanzapine, risperidone, and clozapine rapidly and dose-dependently lowered RER values, consistent with increased fat oxidation. These compounds also affected whole-body cellular respiration as reflected in the VO\textsubscript{2}. However, this effect occurred at higher doses than those needed to observe effects on RER, and it was possible to identify doses that did not have a significant effect on VO\textsubscript{2} but lowered RER. These findings are consistent with the idea that switching fat metabolism without a supporting increase in FFA rate of appearance is not sustainable from an energy-balance perspective. Aripiprazole did not affect VO\textsubscript{2} and had smaller effects on RER. Overall, aripiprazole has greatly reduced incidence of metabolic side effects compared with olanzapine, risperidone, and clozapine; for review, see Ref. 44.

As mentioned already, the effects of olanzapine on RER and VO\textsubscript{2} occurred in conjunction with a dose-dependent accumulation of glucose in the plasma; dose-related increases in blood glucose have also been observed previously in rats acutely treated with olanzapine, risperidone, and clozapine, but not aripiprazole.\textsuperscript{16}

H1-histamine receptors have been linked to some of the metabolic side effects of atypical antipsychotics.\textsuperscript{5,34–36} However, 2 example antihistamines tested here, astemizole and terfenadine, did not cause any effects on RER or VO\textsubscript{2}. It seems unlikely, therefore, that histamine receptors are involved in these responses.

As mentioned earlier, there are differences between the metabolic changes elicited by SGAs and metabolic syndrome. One difference concerns the glucose intolerance. In contrast to traditional glucose intolerance associated with insulin resistance, olanzapine does not appear to impair insulin-stimulated increases in skeletal muscle AKT activation during a euglycemic clamp.\textsuperscript{13}

Another difference is that although impaired lipolysis in the rodent model has been used to help explain SGA-induced adiposity,\textsuperscript{11} it represents a sharp difference from traditional type II diabetes where FFA utilization and mobilization rise in the early stages and later. After olanzapine treatment, glycerol rates of appearance are not affected in humans,\textsuperscript{17} and in rats, basal and isoproterenol-stimulated glycerol concentrations are lower, not higher,\textsuperscript{13} despite increased FFA oxidation. Given these differences, it may not be productive to interpret the acute metabolic effects of SGAs using the traditional framework of diabetes pathogeny. Indeed, the metabolic unsustainability of olanzapine increasing fat oxidation while impairing intracellular lipolysis is certainly disconcerting from a physiological or diabetes pathophysiological perspective. However, these findings could explain the decrease in VO\textsubscript{2} we observed with higher doses of SGAs. If some SGAs are switching the major fuel in mice to fat and preventing fat mobilization, that should lead to exhaustion of plasma FFA (FFA lowering). We observed this previously with olanzapine in rodents,\textsuperscript{11,13} and in humans FFA lowering is also observed.\textsuperscript{10,12,17} SGAs have a very short half-life in rodents, so it is tempting to speculate that with longer exposures facilitated by higher doses, plasma concentrations of FFA may reach a critical concentration that is insufficient to maintain energy requirements leading to the observed drops in the VO\textsubscript{2} at higher SGA doses. It does not seem likely that the VO\textsubscript{2} lowering is secondary to sedation because decreased physical activity was also observed in animals treated with aripiprazole, which did not affect VO\textsubscript{2}, and ANCOVA testing failed to reveal an interaction between these variables after any treatment. Furthermore, physical activity only constitutes approximately 15% to 30% of daily energy expenditure with resting metabolic rate comprising 60% to 75%.\textsuperscript{45} With such a small contribution to daily energy expenditure, it is not surprising that no interaction of physical activity with VO\textsubscript{2} was detected.

We initially viewed the converse effects of olanzapine on fat oxidation and glucose intolerance as a potentially reversible substrate competition due to an affect that primarily increased fat oxidation.\textsuperscript{13} Fatty acids and glucose are the major fuels for oxidative phosphorylation in skeletal muscle. Early studies in muscle demonstrated that provision of fatty acid as a fuel decreased glucose oxidation,\textsuperscript{46} and later, the ability of FFA to diminish glucose uptake and glycolysis was demonstrated.\textsuperscript{19} The converse relationship also exists; increasing glucose oxidation diminishes fat oxidation.\textsuperscript{47} This relationship between glucose and fat oxidation has been termed the Randle Cycle.\textsuperscript{19} Consistently, previous studies have shown that TDGA could increase glucose oxidation in vitro and enhance clearance or decrease production of glucose in vivo.\textsuperscript{17,21–23} However, when we tested the ability of CPT-1 inhibitors to prevent SGA-induced shifts in fat oxidation, the results were unexpected. CPT-1 blockade, instead, synergistically and dramatically lowered VO\textsubscript{2}, and body temperature and caused a moribund state or death when combined with olanzapine. Similar results occurred with risperidone and clozapine, but not aripiprazole. Thus, with higher doses of olanzapine, risperidone, or clozapine or with a combination of lower doses and even partial CPT-1 blockade, there does not seem to be sufficient oxidative capacity for the mice to meet their...
energy demands, accounting for the falling VO₂. Our findings imply that the SGAs are not simply making the conditions ideal to promote FFA oxidation. FFAs are apparently being increasingly oxidized because the SGAs are preventing glucose from being used as a fuel, and the degree of this effect increases with dose that is consistent with that of Assie et al.¹⁶

Thus, the usual substrate competition countermeasures that should occur to promote glucose oxidation when fat oxidation is blocked appear to be impaired after SGA treatment. Alternatively, SGAs may be impairing a step preceding the points of this counter-regulation in glucose metabolism. These usual counter-regulatory steps are thought to include pyruvate dehydrogenase and the regulation of phosphofructokinase by intracellular citrate concentrations (muscle citrate was lowered by SGA treatment in our previous study).¹³

Alternatively, SGAs could interfere with glucose transport. Consistently, risperidone and clozapine were reported to decrease glucose transport in “pruneural” PC12 and L6 muscle cells.⁴⁸–⁵⁰ Similarly, risperidone, clozapine, and olanzapine decrease glucose transport in differentiated 3T3L-1 adipocytes.⁵¹ A caveat is that subsequent studies questioned the clinical relevance of the SGA concentrations used in those in vitro experiments.⁵²

Furthermore, we have shown that multiple tissues with different glucose transporters have elevated FFA metabolic rates.¹³ This would mean that SGAs would have to affect multiple glucose transporters to bring about these effects. Although possible, it is unlikely, and that may raise a caution flag about this idea. An alternative could be that olanzapine, risperidone, and clozapine interfere with an early step in glycolysis to bring about these effects or work through a central mechanism that impacts peripheral glucose metabolism. These are testable hypotheses we are exploring.

In conclusion, our findings indicate that the mechanism underlying the effects of SGAs on RER and VO₂ are not likely to involve H1-histamine receptors. Our findings suggest potential mechanisms for how SGAs conversely affect plasma FFA and glucose. Further studies are needed to investigate the potential sites responsible for these effects including glucose transport and early steps in glycolysis. CPT-1 is currently considered a potential therapeutic target for diabetes drug development. This seems reasonable based on previous studies on etomoxir or TDGA.⁷⁻¹⁹⁻²¹ However, if ongoing efforts with CPT-1 inhibitors lead to new drugs in the future, our studies would seem to suggest that those taking SGAs should avoid them.

Acknowledgments
The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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