Routine exercise ameliorates the metabolic side-effects of treatment with the atypical antipsychotic drug olanzapine in rats

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Abstract

Second generation antipsychotic (SGA) drugs are effective treatments for psychosis. Common side-effects of SGAs include metabolic dysregulation and risk of cardiometabolic disorders. Metabolic side-effects, including glucose intolerance, can be accurately modelled in rodents. The benefits of interventions used for treating metabolic side-effects of SGAs are mostly unknown. In a 9 wk longitudinal study, female rats were given daily olanzapine (10 mg/kg s.c.) or vehicle. Animals were either sedentary or allowed 1 or 3 h daily access to a running wheel, with total wheel revolutions electronically quantified to reflect exercise intensity. Glucose tolerance tests were performed once weekly to measure glycemic control. Drug levels were measured at week 4. At week 9, abdominal fat and skeletal muscle levels of Glucose Transporter 4 (GLUT4) were measured. Exercise intensity progressively increased over time in all groups given access to running wheels; however, rats treated with olanzapine consistently exercised less than those given the vehicle. Olanzapine caused acute and persistent glucose intolerance throughout the study, which was markedly, though incompletely, ameliorated by exercise. Exercise did not affect glycemic regulation in vehicle-treated rats. Levels of GLUT4 in skeletal muscle were higher in both groups of exercising than in sedentary rats, and GLUT4 values were negatively correlated with glucose intolerance. Routine exercise reduced olanzapine-induced glucose intolerance and increased skeletal muscle levels of GLUT 4, the insulin-responsive transporter that mediates glucose uptake into cells. The current animal model is suitable for evaluating physiological pathways involved with glucose intolerance.

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Introduction

Antipsychotic drugs represent the principal pharmacological treatment for psychotic disorders. There has been a widespread recent increase in the use of antipsychotic drugs for additional psychiatric indications (McKean and Monasterio, 2012; Procyshyn et al., 2010), with 1 yr prevalence of drug use now exceeding 3.5% of the population in some countries (Chien et al., 2008). The newer, second generation antipsychotic drugs (SGAs) are associated with fewer of the neurological side-effects observed with the first generation drugs, but concerns have been raised about their metabolic side-effects. Numerous clinical studies have shown that SGAs cause weight gain, hyperlipidemia and insulin resistance, resulting in high rates of prediabetes (Reynolds et al., 2007) and cardiovascular disorders (Leung et al., 2012). For example, one recent study of adult psychiatric inpatients observed that nearly 50% of patients treated with antipsychotics met criteria for prediabetes or Type 2 diabetes mellitus (DM) (Manu et al., 2012). Importantly, the key symptoms of glucose intolerance and insulin resistance may be caused by SGAs independently of any weight gain. Studies have noted many new-onset cases of diabetes in the absence of major weight-gain in psychiatric
patients (Newcomer, 2005), while acute treatment with SGAs in non-psychiatric subjects causes rapid-onset glucose intolerance (Albaugh et al., 2011; Sacher et al., 2008; Vidarsdottir et al., 2010). These metabolic side-effects not only increase morbidity and mortality in patients treated with SGAs, but also contribute to lower rates of medication adherence (Rosenheck et al., 2009).

Options to control metabolic side-effects in patients who are treated with SGAs include the use of anti-diabetic medications. The most efficacious of these for opposing weight gain is metformin, although results have proven to be inconsistent (Baptista et al., 2008), while both sibutramine and topiramate can facilitate weight loss (Das et al., 2012; Fidorowicz et al., 2012). Metformin and rosiglitazone can also reduce glucose intolerance and insulin resistance in patients treated with SGAs (Baptista et al., 2009; Ehret et al., 2010). Nevertheless, both weight gain and glucose intolerance are only partially reversed by anti-diabetic drugs, and many of these medications carry additional health risks (Loke et al., 2011). Management of metabolic dysregulation, therefore, typically includes lifestyle modification. In particular, it is well established that interventions which include routine aerobic exercise-training can enhance insulin sensitivity in both healthy and insulin-resistant subjects (van Dijk et al., 2012). In non-psychiatric diabetic patients, exercise has been shown to exert its beneficial effects on hyperglycemia through multiple mechanisms, with one of the most consistent findings being an increase in levels of the glucose transporter 4 (GLUT4) in skeletal muscle (Hussey et al., 2012; O’Gorman et al., 2006). Exercise has been reported to benefit patients who experience SGA-induced weight gain (Park et al., 2011). However, the physiological pathways of the beneficial effects of exercise in patients with prediabetes or Type 2 DM whose etiology is based on treatment with SGAs remain almost entirely unknown.

Fortunately, the metabolic side-effects of SGAs have been accurately modelled in preclinical rodent paradigms (Boyda et al., 2010a). Antipsychotics with greater metabolic liability in humans exert potent metabolic dysregulation in the animal paradigms (Boyda et al., 2010b, 2013b; Chintoh et al., 2009; Davey et al., 2012; Ferno et al., 2009; Houseknecht et al., 2007; Jassim et al., 2011; Skrede et al., 2012; Smith et al., 2008; Weston-Green et al., 2011), and these models are, therefore, useful in helping to understand the biological basis of metabolic side-effects. We and others have recently shown that specific classes of anti-diabetic drugs can partially reverse glucose intolerance in SGA-treated rats (Adeneye et al., 2011; Boyda et al., 2012b), similar to effects observed in humans. To our knowledge, there has never been a preclinical study of the effects of exercise on metabolic dysregulation caused by a SGA.

The purpose of the present study was, therefore, to determine whether chronic aerobic exercise could ameliorate the effects of a SGA on glucose intolerance. We and other research groups have shown previously that the drug olanzapine, which is one of the most commonly prescribed SGAs, causes robust and reliable glucose intolerance and insulin resistance which has been reported in both female and male rats (Baptista et al., 2002; Boyda et al., 2010a; Weston-Green et al., 2010). We therefore examined the effects of chronic aerobic exercise, using activity wheels, on metabolic dysregulation caused by daily treatment with olanzapine for 9 wk. Glucose sensitivity was assessed weekly using the glucose tolerance test. At the end of the study, changes in abdominal fat and levels of GLUT4 in skeletal muscle were also measured.

Method

Experimental animals

Adult female Sprague-Dawley rats (250–275 g; Charles-River, Canada) were pair-housed under ambient temperature (22±1 °C) with 12 h light-dark cycle (lights on 07:00 hours). Standard rat chow and water were available ad libitum, except prior to glucose tests. Animals were treated in accord with the ’Principles of laboratory animal care’ (NIH publication no. 85-23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm) and UBC’s Animal Care Committee.

Drug treatment

The dose of olanzapine (10 mg/kg) (TRC Inc, Canada) was based on our prior studies (Boyda et al., 2010b, 2012a, b, 2013a). Vehicle formulation was prepared daily (50% polyethylene-glycol 400, 40% distilled water and 10% ethanol, pH 7.6). Rat weights were recorded daily. Chow consumption was recorded weekly and measured on a cage-by-cage basis (n=2 per cage).

Weekly intraperitoneal glucose tolerance test (IGTT)

Prior to treatment, rats were subjected to a baseline intraperitoneal glucose tolerance test (IGTT). Fasted animals (16±2 h) were wrapped in a towel to minimize stress, and saphenous venous blood was procured to
measure baseline glucose levels at $t=0$ min and then at $t =15, 45, 75$ and $105$ min after an intraperitoneal (i.p.) injection of glucose ($1 \text{ g/kg.mL}^{-1}$) using a handheld glucometer (One Touch Ultra). This baseline was used to rank-order animals for subsequent randomization to treatment groups.

In the main experiment, on day 1 of week 1, fasted animals received a baseline glucose measurement, followed by i.p injection of either olanzapine or vehicle ($n=30$ per group). Sixty minutes after olanzapine or vehicle, rats were subjected to the IGTT, with glucose levels measured every 15 min for 120 min. Additionally, saphenous blood draws (200 $\mu l$) were obtained for plasma measurement of olanzapine levels ($t=60, 75, 120$ min) on Week 4; blood samples were centrifuged (10 000 r/min, 10 min, 4 °C) and stored at $-80^\circ$C. On day 1 of each subsequent week, the IGTT was repeated identically following daily exercise (see below). A separate group of rats ($n=6$) was treated with $10 \text{ mg/kg olanzapine and blood procured at 1, 4, 8 and 24 h}$ after treatment to determine pharmacokinetic levels.

**Exercise regimen and chronic drug treatment (see Fig. 1 for sequence of events)**

On day 2 of week 1, animals were randomized into six treatment groups ($n=8–10$ per group): olanzapine and no exercise (sedentary), olanzapine and 1 h exercise, olanzapine and 3 h exercise, vehicle and no exercise, vehicle and 1 h exercise, or vehicle and 3 h exercise. Rats assigned to sedentary conditions remained within their home cage, while exercise animals were placed in individual activity wheels (Med-Associates, USA). Running wheel activity (total wheel revolutions) was recorded via an external electronic LCD counter. After exercise, all groups rested for 30 min, followed by administration of either olanzapine or vehicle. This procedure was repeated daily every Mon–Fri, followed by a 2-d ‘wash-out’ period over the weekend to allow olanzapine levels to clear completely, for 9 consecutive weeks. As above, an IGTT was performed every Monday morning.

**Determination of plasma olanzapine concentration**

Standard solutions of olanzapine (200–40000 ng/ml) were prepared by dilution of a stock 1 mg/ml solution with 0.1 $\mu$M orthophosphoric acid (OPA) to generate a standard curve. The internal standard (IS) solution consisted of 2000 ng/ml clozapine in OPA. A modified version of the single-step liquid–liquid acid solution back-extraction with wash technique was used (Zhang et al., 2007). Samples were prepared with 2.5 $\mu$l of 2000 ng/ml IS, 50 $\mu$l ultrapure water and 5 $\mu$l of 0.5 M dibasic sodium phosphate added to 47.5 $\mu$l plasma. 800 $\mu$l of 70:30 (v/v) diisopropyl ether:pentane was added followed by vortex mixing, centrifugation and transfer of organic layer to 100 $\mu$l OPA. Samples were centrifuged and the organic layer discarded. Diisopropyl ether:pentane (40 $\mu$l of 70:30 v/v) was added, followed by mixing, centrifugation, and removal of the residual organic layer. The remaining acidic aqueous layer was injected into the high performance liquid chromatography (HPLC) system.

**HPLC-UVD**

Olanzapine levels were analysed by HPLC coupled to ultraviolet detection (UVD). A Shimadzu series HPLC system, including a SPD-20A UV/Vis detector, separated analytes on a C18-EPS column (Grace, USA). Mobile phase (Sigma Aldrich, USA) contained 75% ultrapure water, 25% acetonitrile, 0.05 M potassium dihydrogenphosphate, and 0.2% triethylamine, pH-adjusted to 3.4. A 20 $\mu$l injection was loaded at 1.0 ml/min. The UV-detector program was a 0–10 min sequence at 255 nm and 10–20 min sequence at 245 nm for acquisition of olanzapine and IS, respectively.

**Tissue removal**

Animals were euthanized 24 h after the final IGTT via sodium pentobarbital overdose. Kidneys, adrenal glands, whole heart and intra-abdominal fat (perirenal, retroperitoneal, inguinal) was dissected out and weighed. Gastrocnemius muscle was extracted and stored at $-80^\circ$C for analysis.

**Skeletal muscle tissue preparation and Western blot analysis**

Frozen gastrocnemius muscle was homogenized in ice-cold lysis buffer (50 mM Tris-HCl at pH6.8, 1 mM EDTA, 0.2% sodium dodecyl sulfate, and 1 $\mu$g/ml protease inhibitor cocktail (Sigma-Aldrich, USA)). Homogenates were centrifuged (3000 r/min, 20 min, 4 °C) and total protein concentration determined by DC Protein Assay (Bio-Rad Laboratories, USA). Triplicate samples of 20 $\mu$g of protein were separated by 10%-SDS-polyacrylamide gel-electrophoresis and transferred to activated polyvinylidene difluoride membranes. Equal amounts of lysates from 3T3-L1 cells expressing GLUT4 (sc-2243, Santa Cruz Biotechnology, USA) served as positive controls. Membranes were blocked in 5% milk in Tris-buffered saline containing 1% Tween-20 for 1 h at room temperature, followed by overnight incubation at 4°C.
with anti-GLUT4 mouse monoclonal antibody (ab65267, Abcam, USA) diluted 1:1000. Blots were washed and incubated with peroxidase-conjugated goat anti-mouse IgG secondary antibody (1:5000 dilution; Jackson Laboratories, USA). Protein bands were viewed by chemiluminescence (PerkinElmer, USA) and captured using a LAS-3000 imager (Fujifilm Medical Systems, USA). Band intensities were quantified by densitometric analysis.

**Statistical analysis**

Data were analysed by ANOVA or t-test with significance set at \( p<0.05 \). Tukey’s post-hoc tests were used for follow-up analysis of significant main effects, using SPSS software v.18 (Chicago, USA). Correlations were conducted using Pearson’s Correlation.

**Results**

**Weekly IGTT**

Groups did not differ on the initial baseline IGTT values. Analysis of the weekly IGTT values throughout Weeks 1–9 by repeated-measures ANOVA indicated highly significant main effects of time (\( F(8, 400)=3.48, p=0.001 \)), olanzapine treatment (\( F(1, 50)=275.78, p<0.001 \)) and exercise status (sedentary, 1 or 3 h) (\( F(2, 50)=10.02, p<0.001 \)). Post-hoc analysis indicated that IGTT values remained stable in vehicle-treated rats, and did not change over time or differ between exercise treatments. Olanzapine treatment caused pronounced, immediate glucose intolerance, evident as a large increase in the ‘area-under-the-curve’ of glucose levels in the IGTT (see Fig. 2). Glucose levels in olanzapine-treated rats did not differ between groups based on exercise treatment during the first three weeks, but after the fourth weekly IGTT, both the 1 and 3 h exercise groups displayed significantly lower IGTT values than the sedentary olanzapine-treated rats, and these group differences remained lower until the end of the study (see Fig. 3). IGTT values in the two exercise groups always remained significantly higher than vehicle-treated rats, indicating only a partial amelioration of olanzapine’s effects on glucose tolerance. IGTT values showed slightly less improvement in the 3 h than the 1 h group, but this effect was not significant (\( p=0.56 \)).

**Weight gain and food consumption**

Repeated-measures ANOVA indicated a significant main effect of time on body weights (\( F(8, 400)=216.81, p<0.001 \)), as all groups showed weight gain over time (Fig. 4a). However, there was no effect of olanzapine treatment (\( F(1, 50)=0.89, \) NS) or exercise status (\( F(1, 50)=0.03, \) NS), indicating no systematic effect of either of these factors on weight gain.

By contrast, there was a significant main effect of time (\( F(8, 400)=234.37, p<0.001 \)), olanzapine treatment (\( F(1, 50)=5.89, p<0.05 \)) and exercise (\( F(1, 50)=4.07, p<0.05 \)).
Effects of exercise on olanzapine-treated rats

Fig. 2. The effects of exercise on olanzapine-induced glucose intolerance for 9 consecutive weeks. Adult female rats \((n=8–10\text{ per group})\) were exposed to 3 h exercise (3 h), 1 h exercise (1 h) or sedentary conditions (S), followed by chronic administration of either olanzapine (10 mg/kg, s.c.) or vehicle for 5 consecutive days. This was followed by a 48 h ‘wash-out’ period. Subsequently, fasted animals then received a challenge intraperitoneal injection of either olanzapine (10 mg/kg) or vehicle. Glucose levels were recorded prior to treatment (Time 0) and then at 60 min. All rats were subjected to a glucose tolerance test (1 g/\text{ml.kg}^{-1} \text{ of glucose, i.p.}), and blood glucose levels were measured every 15 min for a 2 h duration. This procedure was repeated for an additional 9 wk. Total cumulative glucose levels for each treatment group are summed as ‘area under the curve’ by graph insets (top right of each graph). Representative data shown for weeks 2, 4, 6 and 8 (see Fig. 3 for all week values). Values represent group means±S.E.M. \(* p<0.05\) vs. both exercise groups, \(* * p<0.001\) vs. both exercise groups, \(* * * p<0.0001\).

Effects of exercise on olanzapine-treated rats

\(p<0.05\) on weekly food consumption (Fig. 4b). When collapsed across weeks, sedentary vehicle-treated rats ate the least, vehicle-treated exercising and olanzapine-treated sedentary rats ate more, and olanzapine-treated exercising rats ate the most. This was not due to differences in body weight, as an ANCOVA using midpoint bodyweights as covariate indicated all main effects remained significant.

Activity (wheel revolutions)

There were significant main effects of time \((F(8, 264)=22.34, p<0.001)\), exercise \((F(1, 33)=9.48, p<0.005)\) and olanzapine treatment \((F(1, 33)=10.02, p<0.005)\) on activity levels. As expected, rats in the 3 h groups ran more than the 1 h groups, and all groups showed increased activity over time (Table 1).
Interestingly, olanzapine-treated rats were less active than vehicle-treated animals throughout the study. This effect was not affected by the 2 d break over the weekend (i.e. levels of activity on the Monday did not differ from the rest of the week). Post-hoc tests indicated that olanzapine treated rats did not differ significantly from vehicle controls at any time point in the 1 h exercise groups. However, locomotor activity was significantly lower in olanzapine treated animals at weeks 1, 2, 5–7 and 9 in the 3 h exercise group.

Olanzapine levels

Plasma olanzapine levels were measured on week 4, at 60, 75 and 120 min after drug treatment (Table 2). There was a main effect of time on olanzapine levels ($F(2, 50)=12.55, p<0.001$) as drug levels decreased from 60 to 120 min after injection. There was no main effect of exercise on plasma olanzapine levels ($F(2, 25)=0.65$, NS).

Tissue weights

There was no effect of exercise or olanzapine treatment on kidney, adrenal or heart weights (Table 3). However, there was a significant main effect of olanzapine treatment on the amount of perirenal ($F(1, 50)=23.63, p<0.001$), retroperitoneal ($F(1, 50)=11.01, p<0.005$) and inguinal ($F(1, 50)=5.06, p<0.05$) fat. Post-hoc analysis indicated that the olanzapine/sedentary and olanzapine/3 h exercise groups had significantly more perirenal fat than all of the vehicle-treated groups, while retroperitoneal fat was greater in both the olanzapine/sedentary and olanzapine/1 h exercise groups than all vehicle-treated animals. Inguinal fat was greater in the olanzapine/3 h group compared to all vehicle-treated groups.

GLUT4 levels

A discrete band with an apparent molecular weight of ~50 kDa was immunodetected in the Western
Rats were exposed to either sedentary, 1 or 3 h of daily exercise, followed by a single daily injection of olanzapine or vehicle. Activity counts were recorded after each daily exercise, followed by a single daily injection of olanzapine or vehicle. Values represented as means±S.E.M. at t=1h or 3h. The ANOVA indicated a main effect of exercise (F(2, 50)=25.00, p<0.001) on GLUT4 levels in the gastrocnemius muscle, as well as an exercise×olanzapine-treatment interaction (F(2, 50)=4.51, p<0.05). Post-hoc analysis indicated that in vehicle-treated rats, levels of GLUT4 were non-significantly (p=0.09) increased after 1 h of exercise, and elevated further after 3 h of exercise (p<0.001), compared to GLUT4 levels in sedentary rats. In comparison, both the 1 h and 3 h exercise groups who received olanzapine showed higher GLUT4 levels than olanzapine-treated sedentary rats (p<0.001) (Fig. 5b). In olanzapine-treated rats, there was a strong negative correlation (r=−0.57, p<0.005) between GLUT4 levels and glucose levels on the final IGTT, indicating that higher GLUT4 levels were associated with decreased glucose intolerance (Fig. 6).

Table 1. Mean activity levels for olanzapine-treated rats exposed to 1 h or 3 h of daily exercise

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Treatment</th>
<th>Activity counts (1 h)</th>
<th>Activity counts (3 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEH</td>
<td>OLA</td>
<td>VEH</td>
</tr>
<tr>
<td>1</td>
<td>1458±283</td>
<td>1333±212</td>
<td>2789±342</td>
</tr>
<tr>
<td>2</td>
<td>1967±290</td>
<td>1315±206</td>
<td>3606±632</td>
</tr>
<tr>
<td>3</td>
<td>2593±389</td>
<td>1466±284</td>
<td>5241±1448</td>
</tr>
<tr>
<td>4</td>
<td>2974±491</td>
<td>1423±345</td>
<td>4937±1209</td>
</tr>
<tr>
<td>5</td>
<td>2546±175</td>
<td>1830±344</td>
<td>6177±917</td>
</tr>
<tr>
<td>6</td>
<td>2362±216</td>
<td>1454±272</td>
<td>5931±1240</td>
</tr>
<tr>
<td>7</td>
<td>3029±476</td>
<td>1864±290</td>
<td>7622±1366</td>
</tr>
<tr>
<td>8</td>
<td>2745±532</td>
<td>1693±294</td>
<td>6028±1117</td>
</tr>
<tr>
<td>9</td>
<td>2615±543</td>
<td>1759±295</td>
<td>6249±895</td>
</tr>
</tbody>
</table>

VEH=Vehicle; OLA=Olanzapine (10 mg/kg).
Rats were exposed to either sedentary, 1 or 3 h of daily exercise, followed by a single daily injection of olanzapine or vehicle. Activity counts were recorded after each daily session, five times per week for nine consecutive weeks. Values represented as weekly means±S.E.M. at t=1 h or 3 h.

Table 2. Mean concentration of plasma olanzapine after 4 weeks of daily exercise in female rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Treatment</th>
<th>Plasma [OLA] ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>OLA – S</td>
<td>2220±295</td>
</tr>
<tr>
<td>75</td>
<td>OLA – S</td>
<td>2086±269</td>
</tr>
<tr>
<td>120</td>
<td>OLA – S</td>
<td>1884±235</td>
</tr>
<tr>
<td></td>
<td>OLA – 1 h</td>
<td>1918±300</td>
</tr>
<tr>
<td></td>
<td>OLA – 3 h</td>
<td>1934±316</td>
</tr>
</tbody>
</table>

[ ]=concentration; OLA=Olanzapine 10 mg/kg; S=Sedentary.
Rats were exposed to either sedentary conditions, 1 h or 3 h of daily exercise, followed by a single daily injection of olanzapine or vehicle. Values represented as means±S.E.M. at t=60, 75 and 120 min during the IGTT on Week 4. No significant differences between groups.

Discussion
In the present study, we assessed the effects of routine exercise on the metabolic side-effects of chronic treatment with the SGA drug olanzapine. Rats that were treated daily with 10 mg/kg of olanzapine (Mon–Fri) exhibited pronounced glucose intolerance in the IGTT, which remained stable in magnitude when tested weekly over a 9 wk period. Animals treated with olanzapine that were able to exercise daily for either 1 h or 3 h from Mon–Fri displayed a significant reduction in glucose intolerance by the fourth week of exercise, and this effect lasted until the end of the study. There was no effect of exercise on glucose tolerance in vehicle-treated rats. There was no effect of olanzapine treatment on weight gain, despite greater food consumption in olanzapine-treated animals, although animals treated with the SGA exhibited significantly greater amounts of abdominal fat at the end of the study. Activity levels were lower in olanzapine-treated rats, but still remained high in absolute terms. At the conclusion of the study, animals that had been allowed to exercise had significantly higher levels of GLUT4 in skeletal muscle, and higher levels of this protein in olanzapine-treated rats were strongly associated with decreased glucose intolerance.

The findings with the IGTT reinforce the powerful effects of the SGA olanzapine in animal models of glucose dysregulation (Albaugh et al., 2006; Boyda et al., 2010b; Chintoh et al., 2008b; Cooper et al., 2005; Houseknecht et al., 2007; Martins et al., 2011; Patil et al., 2006; Smith et al., 2011; Victoriano et al., 2009), which parallel the effects seen in humans.
The glucose tolerance test evaluates glucose ‘intolerance’ by measuring the capacity of the fasted subject to return glucose levels to baseline after a glucose challenge; the procedure is commonly used in both clinical and preclinical studies of prediabetes and Type 2 DM (Monzillo and Hamdy, 2003). The glucose tolerance test is viewed positively for its physiological relevance and practicality in measuring loss of glycemic control, and for its accuracy in predicting how glucose will be regulated after a meal (Muniyappa et al., 2008). From our previous studies, we have established that the use of an intraperitoneal glucose load vs. oral or intravenous routes provides more consistent effects with less inter-individual variation, while minimizing distress to animals with chronic dosing. However, it should be noted that with this
technique, glucose is loaded directly into peripheral tissues and does not stimulate intestinal secretion of insulinotropic hormones. Importantly, the IGTT permitted us to conduct repeated, weekly measures of glycemic control, which other procedures, such as the hyperinsulinemic–euglycemic clamp (HIEC), would not have made possible over an extended period. We have shown previously in rats that there is a strong correlation between olanzapine-induced glucose intolerance measured with the IGTT and olanzapine-induced insulin resistance measured by the HIEC (Boyd et al., 2013a). The magnitude of glucose intolerance we observed is consistent with known effects in humans. For olanzapine, a majority of preclinical studies observe some degree of weight gain, but these are typically in shorter duration studies, during the first 2–3 wk of treatment (Albaugh et al., 2006; Pouzet et al., 2003; Skrede et al., 2012). Longer studies are less likely to report significant increases in weight (Chintoh et al., 2008a; Fell et al., 2008). A casual examination of the longitudinal pattern of body weights in our study (Fig. 4a) hints that olanzapine-treated rats exhibited greater body weight from weeks 2–5, but not thereafter. Nevertheless, we did observe significant increases in adiposity at the conclusion of the study in olanzapine-treated animals. A number of recent preclinical studies have reported increased adiposity in white adipose tissue (WAT) depots after repeated olanzapine treatment (Davey et al., 2012; Mann et al., 2012; Skrede et al., 2012; Weston-Green et al., 2011). In our study, we noted that olanzapine tended to increase visceral fat (perirenal and retroperitoneal) to a greater degree than subcutaneous (inguinal) fat. However, regional increases in WAT were dependent on exercise treatment in a complex manner that we cannot currently explain, and will require further study. It is possible that olanzapine treated rats did not display higher body weight, despite greater food consumption and

Fig. 6. Negative correlation between GLUT4 protein levels in rat gastrocnemius muscle and blood glucose AUC in IGTT. After 9 weeks of olanzapine treatment, gastrocnemius muscle samples (n = 27) from animals that received sedentary conditions (S), 1 h or 3 h exercise were assessed for total GLUT4 protein content. Pearson’s correlation analysis was conducted on data obtained from GLUT4 protein levels vs. blood glucose area under the curve (AUC). Values represent group means of the arbitrary units (AU) obtained from skeletal muscle immunoblots vs. individual blood glucose AUC values obtained from Week 9 of the intraperitoneal glucose tolerance test (IGTT).

with rodents. Weight gain and hyperlipidemia demonstrate the greatest inconsistency when compared to humans (Boyd et al., 2010a). Nevertheless, heterogeneity of metabolic side-effects has also been reported in clinical studies (Newcomer, 2005). For example, patients with schizophrenia who are antipsychotic drug naïve exhibit metabolic abnormalities, which may be influenced by smoking habits, poor diet and sedentary lifestyle (Coccurello and Moles, 2010). Additional factors include gender and age differences, antipsychotic polypharmacy and frequent switching between antipsychotic medications (Buckley and Correll, 2008; McIntyre and Jerrell, 2008). The use of animal models, though, has provided important insights into the underlying mechanisms involved, which may include central and peripheral receptor targets such as serotonergic 5-HT2C (satiety and food intake), histaminergic H1 (regulation of energy homeostasis) and adrenergic β2 vs. 3 (lipolysis) pathways (Coccurello and Moles, 2010).

In the current study, we did not observe a significant effect of olanzapine treatment on weight gain. Data regarding the effects of SGAs on weight gain in rodents have been much less consistent than in humans. We have previously reviewed in detail many of the studies that examined weight gain in rats after chronic SGA treatment (Boyd et al., 2010a). Some SGAs, such as clozapine, do not induce weight gain, despite causing substantial weight gain in humans. For olanzapine, a majority of preclinical studies observe some degree of weight gain, but these are typically in shorter duration studies, during the first 2–3 wk of treatment (Boyd et al., 2006; Pouzet et al., 2003; Skrede et al., 2012). Longer studies are less likely to report significant increases in weight (Chintoh et al., 2008a; Fell et al., 2008). A casual examination of the longitudinal pattern of body weights in our study (Fig. 4a) hints that olanzapine-treated rats exhibited greater body weight from weeks 2–5, but not thereafter. Nevertheless, we did observe significant increases in adiposity at the conclusion of the study in olanzapine-treated animals. A number of recent preclinical studies have reported increased adiposity in white adipose tissue (WAT) depots after repeated olanzapine treatment (Davey et al., 2012; Mann et al., 2012; Skrede et al., 2012; Weston-Green et al., 2011). In our study, we noted that olanzapine tended to increase visceral fat (perirenal and retroperitoneal) to a greater degree than subcutaneous (inguinal) fat. However, regional increases in WAT were dependent on exercise treatment in a complex manner that we cannot currently explain, and will require further study. It is possible that olanzapine treated rats did not display higher body weight, despite greater food consumption and
adiposity, because of overall lower activity levels, which could result in lower muscle mass which would offset increases in adiposity. This remains to be determined empirically.

An important novel finding is that routine exercise increased levels of GLUT4 in the gastrocnemius muscle of SGA-treated animals. This effect was significant for both exercise groups in olanzapine-treated rats, as levels of GLUT4 almost doubled compared to sedentary rats, which parallels the observation that both 1 h and 3 h of exercise were equally effective in mitigating glucose intolerance. There is a large body of evidence indicating that the beneficial effects of exercise on glucose intolerance and insulin resistance in patients with Type 2 DM may be mediated partly through the activity and increased expression of GLUT4 (Wang et al., 2009). The role of GLUT4 is manifold, and its beneficial effects on insulin resistance and hyperglycemia may involve its translocation to the cell membrane in addition to increased gene and protein expression in skeletal muscle and other tissues. Additional stimulants of GLUT4 expression may also play a direct role in enhanced glucose uptake and improved glucose tolerance. Mice treated with daily peripheral injections of brain-derived neurotrophic factor (BDNF) exhibited significantly enhanced levels of GLUT4 protein expression in gastrocnemius muscle with concurrent hypophagia (Suwa et al., 2010). We observed a strong and highly significant negative relationship between gastrocnemius GLUT4 levels and glucose intolerance in the IGTT, whereby higher levels of GLUT4 were associated with decreased glucose intolerance. As these findings are only correlational, the causal role of GLUT4 on olanzapine-induced glucose intolerance will require additional future study with techniques that directly modify expression of GLUT4, such as the muscle-specific GLUT4 knockout mouse (Fam et al., 2012). Unlike the extensive Type 2 DM literature, the role of skeletal GLUT4 expression in patients treated with SGAs who exhibit metabolic dysregulation has not been examined. Nevertheless, it is of interest that a recent genetic study observed an association between a polymorphism of the TBC1 domain family member 1 protein (a Rab-GTPase activating molecule that regulates GLUT4 trafficking) and antipsychotic-induced weight gain in patients treated with olanzapine or clozapine (Brandl et al., 2013). Furthermore, exercise therapy has been positively correlated with increased circulating BDNF levels in overweight and obese, non-diabetic patients with and without schizophrenia (Araya et al., 2013; Kuo et al., 2012). The present study therefore provides additional impetus to study the GLUT4 in this population, despite an etiology of metabolic dysregulation differing from Type 2 DM in non-SGA treated patients. It should be noted that rats treated with olanzapine exhibited lower levels of activity than controls, and this was significant in the 3 h exercise group at specific time points. This may reflect a sedating property of the olanzapine, acting either directly or through central changes. We believe that this better reflects the clinical picture, where many patients treated with antipsychotic drugs, including olanzapine, show sedation (Mitchell et al., 2006).

Alternative mechanisms may be considered with regards to the beneficial effects of exercise on olanzapine-induced glucose intolerance. It is unlikely that exercise increased the metabolism of olanzapine, such as through up-regulation of cytochrome P450 1A2 (Vistisen et al., 1991), thereby lowering plasma levels of the drug. We observed no significant difference in olanzapine levels between exercising and sedentary rats on Week 4, which is when exercise first demonstrated a significant improvement in glucose intolerance. Overall levels of visceral fat were lower in exercising rats, and this type of fat is much more strongly associated with metabolic dysregulation than subcutaneous fat (Hamdy et al., 2006). It is possible that the beneficial effects of exercise occurred partly because they decreased or prevented the deposition of visceral fat, and this in turn improved glucose intolerance. However, glucose intolerance was present after first exposure to olanzapine, and did not worsen over time. Thus, the relationship between visceral fat and glucose intolerance remains uncertain, given that increases in visceral fat would be expected to occur over a matter of weeks, thus representing a less likely substrate for exercise than the GLUT4.

To our knowledge, the present study is the first to demonstrate beneficial effects of exercise on glucose intolerance caused by treatment with an SGA in otherwise normal animals. The study is consistent with our previous finding that the olanzapine-induced glucose intolerance can be ameliorated, but not fully reversed, by treatment with the biguanide drug metformin and the thiazolidinedione drug rosiglitazone (Boyda et al., 2012b). One group previously demonstrated that treatment with a high dose of the original first generation antipsychotic (FGA) drug chlorpromazine increased hepatic-specific insulin resistance, and exercise partially recovered insulin sensitivity in male rats (Park et al., 2007). However, to induce this effect, all rats first had to be 90% ‘pancreatectomized’ to induce a severe state of chronic hyperglycemia and loss of

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glucose-induced insulin release. Following a delay of 5–6 d for surgical recovery in preparation for the HIEC, the final assessment of metabolic indices was conducted only at a single time point at the end of the experiment. While useful as a model of the effects of an antipsychotic drug in patients with diabetes, pancreatectomized animals may not represent the most valid animal paradigm to investigate the impact of exercise intervention on drug-induced side-effects in the vast majority of psychiatric patients, who do not have severe loss of pancreatic function prior to starting drug treatment. Furthermore, olanzapine remains one of the most commonly used antipsychotic drugs worldwide, but use of chlorpromazine remains relatively infrequent. It is uncertain whether the beneficial effects of exercise that we observed would also have been noted in male rats. SGA-induced insulin resistance has been observed in both sexes, although weight gain is noted more commonly in females (Boyda et al., 2010a; Weston-Green et al., 2010). A possible role for gender effects, therefore, should be the focus of future studies.

In summary, the present study demonstrates the utility of routine aerobic exercise for the glucose intolerance caused by the widely-used SGA drug olanzapine. The current model suggests a potential role for GLUT4 in these effects, which indicates an important lead for clinical studies. The longitudinal design of the paradigm will allow for future studies to measure biochemical changes over time both before and after the beneficial effects of exercise become evident, to characterize key pathways for future therapeutic development. There is increasing concern that in previously healthy individuals, SGAs can cause weight-independent and drug-specific effects on glucose intolerance and insulin resistance. The impact of these effects is widespread; for instance, a major recent clinical trial of antipsychotic drugs noted that in body mass index-matched patients treated with SGAs, men were 85% and women 137% more likely to have metabolic syndrome than the normal subjects not treated with antipsychotics (McEvoy et al., 2005). In light of our current preclinical findings, the beneficial effects of routine aerobic exercise on metabolic dysregulation may not fully translate to psychiatric patients, simply due to the rigorous nature of the exercise protocol (5 d per week/1 or 3 h per day). However, several lines of clinical evidence suggest that short bouts of intense exercise performed at least once a week (>10 min) in healthy, but sedentary participants decreases weight gain, improves skeletal muscle insulin sensitivity and glucose tolerance (Boutcher, 2011; Short et al., 2012), and may prove useful to reduce metabolic dysregulation in the current target population. Given, in absolute terms, the very large – and increasing – number of patients treated with SGAs, understanding better the interventions that can ameliorate the metabolic side-effects of these drugs should remain a priority.

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Statement of Interest

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