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Olanzapine promotes fat accumulation in male rats by decreasing physical activity, repartitioning energy and increasing adipose tissue lipogenesis while impairing lipolysis

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Abstract

Olanzapine and other atypical antipsychotics cause metabolic side effects leading to obesity and diabetes; while these continue to be an important public health concern, their underlying mechanisms remain elusive. Therefore, an animal model of these side effects was developed in male Sprague-Dawley rats. Chronic administration of olanzapine elevated fasting glucose, impaired glucose and insulin tolerance, increased fat mass but, in contrast to female rats, did not increase body weight or food intake. Acute studies were conducted to delineate the mechanisms responsible for these effects. Olanzapine markedly decreased physical activity without a compensatory decline in food intake. It also acutely elevated fasting glucose, and worsened oral glucose and insulin tolerance, suggesting these effects are adiposity independent. Hyperinsulinemic-euglycemic clamp studies measuring ¹⁴C-2-deoxyglucose (¹⁴C-DOG) uptake revealed tissue-specific insulin resistance. Insulin sensitivity was impaired in skeletal muscle, but either unchanged or increased in adipose tissue depots. Consistent with the olanzapine-induced hyperglycemia there was a tendency for increased ¹⁴C-DOG uptake into fat depots of fed rats and, surprisingly, free fatty acid (FFA) uptake into fat depots was elevated approximately 2-fold. The increased glucose and FFA uptake into adipose tissue was coupled with increased adipose tissue lipogenesis. Finally, olanzapine lowered fasting plasma FFA and whereas it had no effect on isoproterenol-stimulated rises in plasma glucose, it blunted isoproterenol-stimulated *in vivo* lipolysis in fed rats. Collectively, these results suggest olanzapine exerts several metabolic effects that together favor increased accumulation of fuel into adipose tissue, thereby increasing adiposity.

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Keywords

atypical antipsychotics; insulin resistance; nutrient partitioning; hyperinsulinemic-euglycemic clamp; free fatty acid uptake; lipogenesis

Introduction

Atypical antipsychotic drugs are a mainstay of psychiatric pharmacotherapy and are used to treat a variety of psychiatric illnesses, most notably schizophrenia. These second generation drugs lack the extrapyramidal and other debilitating movement side effects that complicated treatment with first generation compounds. The popularity of the atypical antipsychotics, especially olanzapine, led to the observation that these drugs have distinct metabolic side effects that were not detected during preclinical testing; including, insulin resistance, diabetes, and obesity (1-6). The mechanisms underlying these metabolic side effects in humans are not known. However, case reports and other observational studies have suggested that effects of atypical antipsychotics on glucose homeostasis and insulin sensitivity precede weight gain in humans (7-9). Regardless, clinically significant weight gain is observed in almost one-third of patients treated with olanzapine, predisposing these patients to the metabolic syndrome and additional comorbid conditions (Reviewed in 6, 10). Drug therapy is typically continued in patients that are resistant to the weight-gain promoting effects of antipsychotics, though the weight gain-independent effects of these drugs are less well understood in humans. Indeed, several studies have suggested that atypical antipsychotics may perturb lipid as well as glucose metabolism (e.g. 11, 12, 13). Olanzapine, one of the most popular antipsychotics, confers the greatest potential for weight gain and is the focus of our current effort.

Numerous animal studies have examined the potential metabolic side effects of olanzapine, though many questions remain unanswered. A common finding is that, unlike the clinical situation, olanzapine-associated weight gain in rats is sex-dependent with female, but not male rats, displaying a robust increase in body weight gain (14-18). Body weight gain in female rats is strongly associated with hyperphagia, while effects of olanzapine in male rats appear to be limited to increased meal size without altering 24h food intake (19-22). However, recent reports (23, 24) have demonstrated that olanzapine does indeed have metabolic effects in male rats. When treated chronically (>3 weeks), olanzapine increased adiposity without altering food intake or body weight gain, similar to chronic (6 week) administration in dogs (25). The mechanism of the sexual dimorphism in rats and the increased adiposity in male rats and dogs is currently unknown, although these weight-gain independent effects in animals suggest that weight-gain resistant patients continued on olanzapine therapy may also be at increased metabolic risk.

In the following report, we confirm that chronic olanzapine administration leads to increased deposition of fat (adiposity) without a change in total body weight in male rats and demonstrate that these increases in fat mass can be detected as early as the seventh treatment day. After acute olanzapine treatment animals eat more food for their amount of physical activity, which declines. Flux of glucose and fatty acid fuels toward adipose tissue is

increased, which is associated with increased oxygen consumption that appears to be secondary to increased lipogenesis. The increased fuel uptake and storage is also associated with impaired lipolysis. Thus, olanzapine appears to increase adiposity by repartitioning excess nutrients toward adipose tissue and, by impairing mobilization of that stored energy, effectively traps it there. While many patients experience weight gain in response to olanzapine, our findings suggest a mechanism whereby olanzapine may silently increase metabolic risk by increasing adiposity without affecting body weight per se.

Materials and Methods

Animals

The animal facilities and protocol were reviewed and approved by the Institutional Animal Care and Use Committee of the Penn State Hershey College of Medicine. Male, Sprague-Dawley rats (~200-225 g) were purchased from Charles River Laboratories (Cambridge, MA) and maintained on a 12:12-h light/dark cycle with food (Harlan-Teklad Rodent Chow, no. 2018; Madison, WI) and water provided *ad libitum*. Animals were singly housed during chronic experiments with experimental groups matched for body weight. In acute studies, experimental groups were matched for body weight and housed in groups of five. Prior to the beginning of individual experiments, animals were routinely acclimated (> 1 week) to laboratory conditions by daily gavage and handling to reduce potential stress effects during experiments.

Drug Preparation/Administration

Olanzapine was a gift from Neuland Laboratories Ltd (Hyderabad, India) and Dr. Reddy's Laboratories Ltd (Hyderabad, India). Olanzapine was administered either by oral gavage (acute studies) or self-administered in cookie dough (chronic studies), similar to previous studies (14). For gavage, olanzapine solution was prepared by dissolving pure compound in 0.1 N HCl in distilled water, adjusting to pH 5.5 with 0.01 N NaOH, and adding distilled water to reach the desired concentration. Vehicle was similarly pH-adjusted, distilled water. For cookie dough self-administration, separate batches of dough were freshly prepared from a pre-prepared dry mix every few days as needed and refrigerated at 4°C.

Chronic Drug Studies

Animals were exposed chronically to olanzapine by self-administration of drug-containing cookie dough. Animals were provided 20 g dough/kg body weight in 1-ounce American portion-control cups (wax paper) from Hoffmaster (Oshkosh, WI; stock no. 60-100). Cookie dough was administered daily between 1600 and 1800h. At the time of drug administration, body weight and 24-hour food intake were measured. For chronic treatment, the dose of olanzapine was 4 mg/kg/day during days zero through six. On the seventh treatment day, the dose was increased to 8 mg/kg/day. The dose was again increased on day 14 to 12 mg/kg/day through the end of the study. This dosing scheme was adapted from studies in female rats, in which this ramped-dosing prolonged drug-induced increases in body weight gain (14).

Acute Drug Studies

Rats were randomly allocated to experimental groups. For acute oral glucose tolerance tests and insulin tolerance tests, dosing was identical to our previous studies in female rats (14). Briefly, one-third of the daily dose was administered on the first day between 0800 and 0900h, while the remaining two-thirds dose was administered before the dark cycle between 1600 and 1700h (total daily dose = 4 or 10 mg/kg). Food and water were provided *ad libitum*. The following morning, one-third of the daily dose was administered between 0700 and 0800h. At this point, animals were food-restricted while water remained available *ad libitum*. Four hours later the remaining two-thirds dose was administered (total dose = 4 or 10 mg/kg). One hour after the fourth and final drug or vehicle gavage, the glucose or insulin tolerance tests were performed.

In the calorimetry time course studies olanzapine (10 mg/kg) was provided at the time indicated in the figure in *ad libitum* fed animals, as indicated. For all other acute experiments, endpoints were measured after two doses of vehicle or drug (olanzapine = 4 or 10 mg/kg). The first dose of olanzapine or vehicle was administered before the start of the dark cycle at approximately 1800h. Food and water were provided *ad libitum* or animals were food-deprived. The following morning a second dose of vehicle or olanzapine was administered typically between 0700 and 0800h. The final drug or vehicle gavage was always given two hours prior to the beginning of any experiment or blood/tissue sampling.

Locomotor Activity and Energy Expenditure

Locomotor activity was measured using infrared technology (OPT-M3, Columbus Instruments). The counts of three-dimensional beam breaking (X total, X ambulatory or 'y', and Z) were measured for 24h. Energy expenditure was measured concurrently using indirect calorimetry (Oxymax, Columbus Instruments). Constant airflow (~8 ml/g/min) was drawn through the chamber and monitored by a mass-sensitive flow meter. O₂ and CO₂ concentrations were measured at the inlet and outlet of sealed chambers to calculate O₂ consumption and CO₂ expiration. Each chamber was measured for 1 minute at 15-minute intervals. Animals were acclimated to the specialized calorimetry cages and had *ad libitum* access to food and water throughout the 24h period.

Body Composition Analysis

Longitudinal changes in body composition were tracked non-invasively in conscious animals using a ¹H-NMR analyzer (Bruker LF90 proton-NMR Minispec; Bruker Optics; The Woodlands, TX) for rapid determination of total body fluid, lean and adipose tissue masses and then returned to their respective cages. Initially, groups were matched for total body adiposity and lean body mass (week 0) and then subsequent measurements were made weekly on days 7, 14, 21, 28 and 35.

Glucose and Insulin Tolerance Tests

Oral glucose tolerance tests (OGTT) or insulin tolerance tests (ITT) were performed after either chronic or acute drug treatment. Chronically exposed rats were food restricted for 14h, while acutely exposed animals were food restricted for approximately 5h prior to the OGTT

or ITT. Acutely exposed animals received drug according to the acute dosing regimen (see above) while chronically exposed animals received half the daily dose of drug (6 mg/kg) one hour prior to the start of the OGTT or ITT. During the OGTT, basal blood samples and glucose measurements were obtained and then glucose was given via oral gavage (Acute OGTT: 2.5 g glucose/kg; Chronic OGTT: 1.5 g glucose/kg); more glucose was used in acute studies since, at the time of the OGTT, animals were ~1 month younger than at the time of the OGTT following chronic treatment. Blood samples were collected via a tail snip at 30-min intervals for 120 minutes. Samples were then centrifuged at $1,800\times g$ for 10 min at 4°C ; plasma was separated and frozen at -80°C for further analysis. During the ITT, after basal blood samples were collected animals received an intraperitoneal (i.p.) injection of insulin (0.75 U/kg, Humulin-R, Lilly; Indianapolis, IN) and blood glucose was measured every 30 minutes for two hours.

Hyperinsulinemic-Euglycemic Clamp Experiments

Clamp experiments were performed in chronically catheterized animals. Prior to catheter implantation rats were housed in groups, but singly housed following surgery. Animals were anesthetized using volatile isoflurane (4% induction, 1.5% maintenance). Using aseptic surgical technique, a single arterial polyethylene catheter (PE-50) for blood sampling was implanted in the left carotid artery such that the tip lay in the aortic arch. A venous catheter (PE-50) with a silicon tip (silicon tubing; ID: 0.51 mm, OD: 0.94 mm; Braintree Scientific) was inserted in the right jugular vein for infusion of non-radioactive glucose and insulin. A second 'hybrid' venous catheter (PE-50/PE-10) was inserted for uninterrupted infusion of radiolabeled glucose (3- ^3H -glucose). At the conclusion of the operation, catheters were filled with a heparinized saline solution (200 U/ml) to prevent lumen thrombus formation and sealed with metal plugs. During postoperative recovery, animals were given subcutaneous, 37°C saline and intramuscular ceftriaxone (100 mg/kg). At least 4 days recovery was allowed before conducting clamp experiments. At this time body weight was greater than or within 5% of the pre-operative value. Animals not meeting this criterion were not used in clamp experiments.

All hyperinsulinemic-euglycemic clamp experiments were performed at the same time in the morning on conscious, unrestrained animals after 14h of food restriction to ensure animals were in the post-absorptive state. On the evening prior to clamp experiments, animals received either vehicle or olanzapine (10 mg/kg) via oral gavage between 1700 and 1800h and then food-restricted. The following morning animals received another gavage of vehicle or olanzapine (10 mg/kg) and then placed in small, dark cages for the clamp experiment. During the basal period ($t = -120$ to 0 min), saline was infused through the venous catheter and a tracer amount of tritiated glucose (3- ^3H -glucose; Perkin-Elmer, Waltham, MA) was infused as a primed-continuous infusion (10 μCi bolus, 0.2 $\mu\text{Ci}/\text{min}$) through the hybrid venous catheter for measurement of basal hepatic glucose output. At time zero ($t = 0$), a primed-continuous insulin infusion (75 mU/kg bolus, 1 mU/kg/min, 0.3% BSA) was started and glucose (20% dextrose) was co-infused to maintain euglycemia (~ 100 mg/dl). Blood glucose concentration was monitored every 10-15 minutes. Additionally, the rate of tritiated glucose infusion was increased to 0.4 $\mu\text{Ci}/\text{min}$ to maintain a constant specific activity for the measurement of insulin-stimulated glucose kinetics. Blood samples (< 250 μl) were

collected at -20, 0, 60, 120, 160 and 180 minutes for measurement of insulin and specific activity of plasma glucose.

Tissue Glucose Uptake

A tracer amount of 1-[¹⁴C]-2-deoxyglucose (8 μCi; MP Biochemicals, Irvine, CA) was given as an intravenous bolus during the steady-state phase (t = 140 min) of the hyperinsulinemic-euglycemic clamp for the determination of the *in vivo* rate of glucose uptake, or tracer was alternatively given as a bolus in 14h food-restricted or *ad libitum* fed animals. Serial blood samples were collected (t = 142, 145, 150, 160, 170, 180) to determine the area under the curve (AUC) of plasma 2-deoxyglucose activity during the final 40 minutes of the experiment, or similar *in vivo* labeling periods in food-restricted or fed animals. At the end of the clamp (t = 180 min), animals were euthanized and tissues quickly excised and immediately freeze-clamped in liquid nitrogen. Frozen tissues were powdered and then homogenized in ice-cold, 0.5 N perchloric acid (0.4 ml/100 mg tissue). Homogenized samples were centrifuged at 3,000 × g for 15 minutes. The supernatant was collected and neutralized with an equal molar amount of potassium hydroxide and then assayed for total ¹⁴C radioactivity using a dual-channel liquid scintillation counter (Beckman Coulter; Fullerton, CA). The ¹⁴C radioactivity in these samples represents the total counts from both the 2-deoxyglucose and the phosphorylated 2-deoxyglucose present in the tissue sample. An aliquot of the neutralized extract was subjected to Somogyi extraction, which removes the non-phosphorylated 2-deoxyglucose and then counted. Thus, total counts of phosphorylated 2-deoxyglucose were calculated as the total counts minus the counts remaining after Somogyi extraction.

Adipose Tissue Fatty Acid Uptake

Free fatty acid (FFA) uptake by adipose tissue depots was measured using the non-metabolizable fatty acid analog, radioisotopic beta-methyl-iodo-phenyl-pentadecanoic acid ([¹²⁵I]-BMIPP), as previously described and validated (26). At approximately 0700h, a tracer amount of [¹²⁵I]-BMIPP (10 μCi) was given as an intravenous bolus (t = 0 min). Serial blood samples (< 250 μl) were collected (t = 2, 5, 10, 20, 30, 40) to determine the AUC for plasma [¹²⁵I]-BMIPP during the 40 minute *in vivo* labeling period. After final blood samples were collected (t = 40 minutes), animals were euthanized and adipose depots, i.e. epididymal, pararenal and subcutaneous (over the abdomen) depots, were excised and freeze-clamped with aluminum tongs cooled in liquid nitrogen. Total [¹²⁵I]-BMIPP activity was measured in plasma (25 μl) and whole-tissue (~100 mg) for calculation of FFA metabolic rate. Tissue and plasma ¹²⁵I radioactivity was counted using a gamma counter (Beckman Coulter; Fullerton, CA).

Tissue Lipogenesis

Measurement of *in vivo* lipogenesis using ³H₂O was measured and calculated as previously described (27), with the exception that animals were not anesthetized during the isotope-labeling period. Male Sprague Dawley rats were housed two per cage and randomized to control or olanzapine groups with *ad libitum* access to water and chow. The mean body weights between the two groups did not significantly differ (Con: 340 ± 3 g vs. Olz: 340 ± 6

g). Olanzapine (10 mg/kg) or vehicle was administered by oral gavage at 0800 and 2000h on the first day of treatment. On the morning of the second day animals received a third dose of drug or vehicle and then received 4.5 mCi of $^3\text{H}_2\text{O}$ dissolved in saline 15 min following oral gavage. Two hours following the radioisotope injection, tissues samples were quickly excised under isoflurane anesthesia and immediately freeze-clamped in liquid nitrogen and stored at -84°C .

Tissues were subsequently powdered and 150-300 mg was transferred to liquid nitrogen cooled screw cap glass centrifuge tubes. Tubes were then warmed to ice temperatures and total lipid was extracted using a two-step Bligh and Dyer extraction using the yield increasing modification: mixture of 1M saline and 0.2 M phosphoric acid instead of water (28, 29). Results are reported as mol ^3H incorporated into total lipid per hour based on individual plasma specific activities corrected for estimated plasma water (92%).

Isoproterenol Challenge Test

To gauge adipose tissue lipolytic capacity an isoproterenol challenge test was performed. Isoproterenol (Isuprel[®]) was purchased from Abbott Laboratories (Abbott Park, Illinois) and diluted in sterile normal saline immediately before use. Animals were given *ad libitum* access to food and water and received the final vehicle or olanzapine gavage 2h prior to the start of the challenge test. After collection of basal blood samples, an i.p. injection (2 ml/kg) of isoproterenol (0.05 mg/kg) was given to vehicle and olanzapine-treated animals. Serial blood samples were collected at 30-minute intervals for two hours to measure blood glucose and to prepare plasma by centrifugation for FFA and glycerol analysis.

Analytical Procedures and Metabolite Measurements

Ascensia[®] Contour[®] blood glucometers were chosen to measure whole blood glucose (Bayer Healthcare LLC; Mishawaka, IN) because of the small sample size required (~ 0.6 μl) and correction for differences in hematocrit. Plasma insulin concentrations were determined by ELISA kit for rat insulin (Merckodia; Uppsala, Sweden). Glucose specific activity was determined on plasma deproteinized with equal molar volumes of barium hydroxide and zinc sulfate. Samples were incubated at 4°C overnight and then dried to remove $^3\text{H}_2\text{O}$. Counts remaining represent the radioactivity from plasma 3- ^3H -glucose. Colorimetric assays were used to measure FFAs (Wako Diagnostics, Richmond, VA) and glycerol (Cayman Chemical, Ann Arbor, MI).

Calculations

In chronic feeding studies 'Food-Conversion Efficiency' was calculated as the 'change in body weight' divided by the 'cumulative food intake' (30). During euglycemic clamp studies the glucose rates of appearance (R_a) and disappearance (R_d) were calculated using the isotope dilution method (31). Briefly, glucose turnover rate was calculated as the ratio of the rate of ^3H -glucose infusion (dpm/min) to the glucose specific activity (dpm/mg). Hepatic glucose output (HGO) in the basal state was defined as the tracer determined rate of glucose turnover. During the steady-state portion of the clamp experiment ($t = 120$ to 180 min) the rates of glucose appearance and disappearance are equal and thus the residual hepatic glucose output during the clamp equals the total glucose turnover minus the

exogenous glucose infusion rate (GIR). The glucose metabolic rate, R_g , (nmol / g tissue / min) in each tissue was determined by taking the total counts of phosphorylated 2-deoxyglucose and dividing by the AUC of plasma ^{14}C -2-deoxyglucose during the 40 minute *in vivo* labeling period and multiplying by the plasma glucose concentration (32, 33). Under non-euglycemic clamp conditions, tissue metabolic clearance rate for glucose was calculated by dividing the calculated R_g by the plasma glucose concentration. The adipose tissue FFA metabolic rate, R_f , was calculated by taking the total tissue counts of [^{125}I]-BMIPP and dividing by the AUC of [^{125}I]-BMIPP during the 40 minute *in vivo* labeling period and multiplying by the mean plasma FFA concentration (26). For insulin tolerance tests, the inverse area-under-the-curve (ITT-AUC) was calculated by integrating the decline in the plasma glucose relative to time 0, as blood glucose normally decreases following insulin injection.

Statistical Analyses

For all results, data are expressed as the mean \pm standard error. To calculate statistical significance ($P < 0.05$), Student's t-test or one-way analysis of variation (ANOVA) with Bonferroni's multiple comparison post-test was used when appropriate. A Welch correction was applied if the variances between groups were significantly different. Asterisks indicate a particular level of significance ($***P < 0.001$, $**P < 0.01$, $*P < 0.05$). Sample sizes for each experiment are included in the respective figure legends. All statistical analyses and data manipulations were made using GraphPad Prism or InStat computer software (GraphPad Software, San Diego, CA).

Results

Effects of Chronic Olanzapine on Male Rats

Previous work has demonstrated that olanzapine increases adiposity over an extended time course (~ 3 weeks), though the mechanism of this effect is not clear (23, 24). Similar to previous reports, neither body weight gain (Fig 1A) nor 24h food-intake (Fig 1B) differed between vehicle- and olanzapine-treated rats throughout the study. Moreover, food-conversion efficiency was not different during the first week of treatment [23.1 ± 1.4 vs. 21.8 ± 1.8 %, for control and olanzapine treated groups, respectively] or after three weeks of treatment [17.0 ± 0.8 vs. 15.1 ± 1.4 %]. In contrast to weight gain and food intake, olanzapine increased percent of total body fat by the seventh day of treatment (Fig 1C). The increase in total body fat relative to control animals was maintained for the duration of the study but reached a plateau at three weeks. Percentages of adipose, lean and fluid masses over the course of the study are shown in Table 1. Accordingly, as olanzapine did not change body weight gain during chronic administration, the observed increase in fat mass was balanced by a decrease in total body lean mass (Table 1).

Physical activity was measured during the third week of olanzapine or vehicle treatment, as decreased activity has been implicated in olanzapine-induced obesity in female rats (15, 34). However, locomotor activity did not differ between experimental groups during the third treatment week [$\text{'X dimension' } 25,820 \pm 1,121$ vs. $22,445 \pm 1,616$; $\text{'Y dimension' } 14,589 \pm$

820 vs. 12, 201 \pm 1,095; 'Z dimension' 2,431 \pm 285 vs. 1,899 \pm 278 (n = 10), all values 'Control vs. Olanzapine', respectively].

Increased adiposity is strongly associated with insulin resistance, and thus we challenged animals with an oral glucose tolerance test after 4 weeks of olanzapine treatment. Consistent with their increased adiposity, the olanzapine-treated animals displayed hyperglycemia (Fig 1D) and hyperinsulinemia (Fig 1E) after 14h of food-restriction. Glucose and insulin concentrations remained significantly elevated compared to vehicle-treated controls throughout the OGTT. Moreover, the AUC_{Glucose} was significantly elevated by olanzapine [194 \pm 78 vs. 401 \pm 134 (mM·min), P<0.01], suggesting decreased whole-body insulin sensitivity. To confirm such changes, an insulin tolerance test was performed on day 42 of drug treatment. Once again, drug-treated animals showed elevated plasma glucose following 14h food-restriction [5.0 \pm 0.2 vs. 6.1 \pm 0.2 mM, P<0.001]. Olanzapine blunted the fall in blood glucose following insulin injection (Fig 1F), with glucose concentrations remaining elevated compared to control levels at 60, 90 and 120 minutes post-injection. The inverse area under the ITT curve (ITT-AUC) was lower in the olanzapine group compared to control animals [6,226 \pm 564 vs. 3,797 \pm 569 (min·% Baseline), P<0.01], which further suggests decreased insulin sensitivity.

Effect of Acute Olanzapine on Locomotor Activity and Energy Expenditure

Because primary disturbances in energy expenditure might increase adiposity and weight gain over a similar time period, we examined potential drug effects on these parameters. Locomotor activity and energy expenditure were measured following acute oral olanzapine administration (Fig 2B-C, indicated by arrows) at the beginning of the dark cycle (shaded area) and then again at the beginning of the light cycle on the following morning. As seen in Figure 2A, olanzapine significantly decreased locomotor activity in all dimensions (x, y, z) during the dark and light cycles, but had no effect on 24h food intake (data not shown); thus relative food intake was more than expected for the amount of physical activity. Leptin, an adipocyte-derived hormone and known satiety factor, has been posited as contributing to the hyperphagia observed in female rats following olanzapine treatment (14). Consistent with those observations, plasma leptin concentration was also decreased following acute olanzapine-treated male rats [5h Fast: Control = 5.29 \pm 0.55 (n = 9), Olz = 3.16 \pm 0.44 (n = 10), P<0.01]. Thus, decreases in plasma leptin may also contribute to the relative increase in food intake.

O₂ consumption (Fig 2B) and CO₂ production (Fig 2C) were measured throughout a complete dark and light cycle. Unexpectedly, the olanzapine-treated group had a similar VO₂ (Fig 2D) and VCO₂ (Fig 2E) relative to vehicle-treated controls during the dark cycle, while these parameters were significantly elevated during the light cycle. Rectal temperature was also measured in separate groups of animals before and 2h after olanzapine gavage, though no change from baseline body temperature was detectable (data not shown). Thus, energy expenditure is not decreased following olanzapine administration, even though drug-treated animals have less locomotor activity. These results demonstrate that olanzapine-treated rats use some of the excess caloric intake to support a process other than locomotor activity.

Effects of Acute Olanzapine on Glucose Tolerance and Insulin Action

The worsening glucose and insulin tolerance observed after chronic treatment with olanzapine might be secondary to increased adiposity. To test this hypothesis, OGTTs and ITTs were conducted in separate cohorts of rats on the second treatment day before significant accumulation of adipose tissue. As seen in Figure 3, acute olanzapine-treated rats displayed mild hyperglycemia (Fig 3A) and hyperinsulinemia (Fig 3B) after 5h of food-restriction following a low dose of olanzapine (4 mg/kg/day), suggesting insulin resistance. During the OGTT, glucose concentration tended to be higher relative to control values, with baseline and 120 minute blood glucose measurements being significantly elevated in the olanzapine group. Plasma insulin was similar between experimental groups throughout the OGTT, even though increased circulating insulin was expected given the mild elevations in blood glucose. To better assess insulin sensitivity, an insulin tolerance test (Fig 3C) was conducted in vehicle-treated rats or in rats injected with one of two doses of olanzapine (4 or 10 mg/kg/day). Olanzapine administration led to a dose-dependent decrease in inverse ITT-AUC (Fig 3C). The 4 mg/kg/day dose decreased the inverse ITT-AUC relative to the control group [$6,054 \pm 344$ vs. $4,436 \pm 415$ (min · % Change), $P < 0.01$], while the 10 mg/kg/day dose further decreased the inverse ITT-AUC [$4,436 \pm 415$ vs. $1,514 \pm 296$ (min · % Change), $P < 0.001$]. Together these data demonstrate that insulin resistance following olanzapine administration precedes and is therefore independent of the change in body composition observed in the chronically treated animals.

Since drug-induced insulin resistance precedes the changes in body composition, it is tempting to speculate that it might play a role in the development of the olanzapine-induced adiposity. For example, Caro *et al* (35) proposed a mechanism of obesity wherein tissue-specific insulin resistance, in which muscle but not fat was affected, would lead to shunting of substrates away from muscle and towards fat. To test this hypothesis, hyperinsulinemic-euglycemic clamp studies using tracer methodology were conducted on the second treatment day. Plasma insulin concentration was maintained at a mild, hyperinsulinemic level to examine drug effects on peripheral tissues as well as hepatic insulin sensitivity [Plasma insulin: 200 ± 28 vs. 205 ± 25 pM in control vs. olanzapine treated animals, respectively]. During the basal state (14h food-restricted), the tracer-determined rate of glucose turnover, which is equivalent to hepatic glucose output (Fig 3D), did not differ between experimental groups. Moreover, insulin-mediated suppression of hepatic glucose output, a measure of hepatic insulin sensitivity, was similar in the control and olanzapine groups. Consistent with two previous studies (36, 37), the tracer-calculated rate of whole-body glucose disposal during the clamp was significantly decreased in the olanzapine group (Fig 3E). Insulin-mediated glucose disposal was increased by ~85% in control animals, while olanzapine-treated animals showed no increase in whole-body glucose disposal, confirming the insulin resistance suggested by the glucose and insulin tolerance tests.

To determine the tissue(s) responsible for the insulin resistance, tissue glucose metabolic rates (R_g) were calculated for muscle, heart, liver and adipose tissues during the steady-state portion of the euglycemic clamp (Table 2). Fed insulin concentrations were not significantly different (data not shown) and the plasma insulin was clamped to approximate the insulin in the fed state. Consistent with the decrease in insulin-stimulated whole-body glucose

disposal, R_g was decreased by 39 % in the heart as well as by 31% in gastrocnemius and 40% in soleus, which represent both fast- and slow-twitch skeletal muscle fibers, respectively. Unlike skeletal muscle R_g , a 53% increase in subcutaneous adipose tissue glucose uptake was observed relative to control animals, while epididymal and pararenal adipose tissue R_g remained unchanged. Thus, olanzapine acutely perturbs insulin sensitivity in a tissue-specific manner, decreasing insulin sensitivity in muscle tissue while either increasing or not affecting sensitivity in adipose depots.

Tissue-specific glucose uptake was also measured in 14h food-restricted animals, under similar conditions as the basal period of the clamp studies. Tissue metabolic clearance rate instead of tissue R_g is shown in Table 2, as olanzapine elevated glucose and insulin concentrations under these conditions. Despite the insulin resistance, tissue clearance of glucose by skeletal muscle and two adipose depots was not affected, presumably because the plasma glucose and insulin were elevated. However, clearance by subcutaneous adipose tissue was increased. Basal uptake was also increased in the heart.

Effects of Olanzapine on Adipose Tissue FFA Flux

In 14h food-restricted animals, acute olanzapine treatment decreased plasma FFA (Fig 4A), as well as glycerol [17.5 ± 1.3 vs. 9.6 ± 1.1 , $P < 0.001$]. This was surprising as insulin resistance is normally associated with elevated FFA. To examine the mechanism underlying this effect and to further elucidate the mechanisms of elevated adiposity development in this model, we measured the uptake of FFA into adipose tissue, rates of *in vivo* lipogenesis, and FFA mobilization following a mild-dose isoproterenol challenge under *ad libitum* fed conditions in which FFA levels were not different (data not shown).

FFA metabolic rates were measured using the non-metabolizable FFA analog ^{125}I -BMIPP in *ad libitum* fed animals (Fig 4B). Olanzapine increased FFA metabolic rate 2-fold in epididymal, pararenal, and subcutaneous adipose depots.

Given that adipose tissue uptake of FFA and/or glucose tended to be increased by olanzapine, we hypothesized that lipogenesis might be also elevated, which could potentially explain the elevated oxygen consumption and chronic increases in fat mass in drug-treated animals. To test this hypothesis we measured *in vivo* lipolysis in fed rats by measuring adipose tissue incorporation of $^3\text{H}_2\text{O}$ into triglyceride. As seen in Figure 4C, a trend for increased epididymal adipose tissue lipogenesis was detectable ($P=0.1$), though significant increases in lipogenesis ($P < 0.0001$) were detectable in both the retroperitoneal and subcutaneous adipose tissue depots (75% and 266% higher, respectively).

Adipocytes and hepatocytes mobilize, respectively, stored fatty acids and glucose fuels in the post-absorptive state, during longer periods of food-deprivation, or in times of stress when catecholamines are elevated. Lipolytic impairment could serve as an additional pathway leading to the chronic changes in body composition by trapping stored fuels, and thus we tested lipolytic capacity *in vivo* with an isoproterenol challenge test in fed animals in which the FFA are not different. Following injection of a low dose of isoproterenol, rises of plasma glycerol (Fig 4D) and free fatty acids (Fig 4E) were blunted by olanzapine. Even though lipolysis was attenuated, peak isoproterenol-stimulated hepatic glucose output

remained intact (Fig 4F). Thus, the effects of olanzapine on lipolysis probably do not involve the early steps in the beta-adrenergic signaling pathway shared by the lipolytic and hepatic glucose output response to isoproterenol. Overall, through the above actions, olanzapine seems to concurrently promote energy repartitioning to and trapping within triglyceride stores of adipose tissue.

Discussion

In this paper we confirm that chronic olanzapine administration in male rats increases total body adiposity independent of a change in either body weight gain or food intake. The increased fat mass was detectable by ¹H-NMR within the first week of drug administration and is associated with impaired oral glucose tolerance and insulin resistance. The present study extends these original findings by demonstrating that olanzapine has at least four acute metabolic effects that may act in concert to favor adipose tissue deposition and development of insulin resistance. First, olanzapine decreases physical activity without a compensatory reduction in food intake, resulting in a relative over-nutrition in drug-treated animals. This was associated with a decline in the satiety hormone leptin. Second, olanzapine causes tissue-specific changes in insulin sensitivity that support a tendency for increased glucose uptake into adipose tissue. Third, FFA uptake into adipose tissue was also increased, along with drug-stimulated increases in adipose tissue lipogenesis in some depots. Lastly, olanzapine impaired *in vivo* lipolysis during an isoproterenol challenge, which suggests that adipose tissue is functionally trapping newly synthesized and stored metabolic fuels. These acute effects of olanzapine are present on the second treatment day and are associated with detectable increases in adipose tissue by the seventh treatment day. In this model, the accumulation of adipose tissue is self-limiting, reaching a plateau after approximately three weeks of dose ramping, but most likely further worsens the drug-induced insulin resistance and glucose intolerance observed before body composition is affected.

Decreased Physical Activity without a Compensatory Decrease in Food Intake

A prolonged decrease in physical activity without reduction of food intake favors the accumulation of adipose tissue by excess nutrient storage. In contrast to humans, olanzapine-induced weight gain and hyperphagia in rats is sexually dimorphic. Studies have shown that female rats, but not males, are susceptible to drug-induced weight gain (e.g. 14, 34), and our results are consistent with those findings (23, 24). However, while female rats show decreased activity and increased food intake (15, 34), male rats show decreased activity *without* changes in food intake. Decreased locomotor activity was not observed at three weeks, suggesting this effect lessens with time. Interestingly, percent body fat also reached plateau over a similar time course. Thus, in the short term, male rats have a relative over-nutrition for their given level of activity. It is tempting to speculate this mismatch is due to the drug-induced reduction in plasma leptin concentrations we observed here and previously in females. While obesity drives higher plasma leptin concentrations in humans it has been noted that plasma leptin concentrations tend to be lower in patients receiving olanzapine than expected based on BMI (38). The reason for lack of weight gain despite eating more food than expected for physical activity appears to be due to maintained energy expenditure. The energy-consuming process might represent activation of an adaptive

thermogenesis. However, that should decrease body weight, which is not consistent with deposition of adipose tissue and loss of lean mass that occurs with chronic olanzapine treatment. A more likely alternative is that energy expenditure is increased, at least in part, by male rats expending energy for de novo lipogenesis, which is supported by the observed increase in body fat and elevated lipogenesis found in several adipose tissue depots. In individuals consuming a mixed macronutrient diet (carbohydrates, fat, protein) de novo lipogenesis usually accounts for a small fraction of total energy expenditure (<5%), as most stored lipid is recycled from dietary sources (39). However, de novo lipogenesis from carbohydrate precursors can account for upwards of ~20-30% of energy expenditure (40, 41). The standard rat chow used in this study is ~6% crude fat and, therefore, increased adiposity would be thought to come mostly from carbohydrate precursors. It is noteworthy that adipose tissue did not exhibit the insulin resistance observed in skeletal muscle and therefore was under different conditions of either increased glucose uptake or a trend for increased glucose uptake. Moreover, the percent increase of lipogenesis observed in subcutaneous adipose tissue was much greater than that of epididymal or retroperitoneal fat, consistent with the apparent increase in insulin sensitivity observed in the subcutaneous depot as measured by the increased insulin-stimulated glucose uptake during a euglycemic clamp.

Increased Energy Uptake Into Fat

Tissue-specific insulin resistance has been posited to underlie the pathogenesis of obesity in humans and monogenetic obesities in rodents (35). We noted such tissue-specific insulin resistance after acute olanzapine. Under some conditions, a tendency or significant increase in glucose uptake into fat was observed. Additionally, there was a large increase in FFA uptake into adipose tissue in fed animals. In a recent study we showed that other peripheral tissues were similarly affected, and this may explain the sparing of glucose/hyperglycemia and apparent muscle insulin resistance after acute olanzapine (14). Increased glucose and FFA uptake appears to be an additional mechanism through which olanzapine contributes to increased adiposity during chronic treatment.

It should be emphasized that these effects are acute and therefore not secondary to increased adiposity, but may instead promote the accumulation of fat seen with chronic treatment. Previous studies have documented weight gain-independent insulin resistance during hyperinsulinemic-euglycemic clamp studies (36, 37). Consistent with those reports, we demonstrate a similar decrease in insulin-stimulated whole-body glucose disposal (Fig 3E). Furthermore, this decreased glucose disposal is due to skeletal muscle insulin resistance (Table 2), a conclusion suggested from previous studies but most likely undetected due to small sample size (37). In contrast, glucose uptake in epididymal and pararenal adipose tissue was unchanged, while subcutaneous adipose tissue showed increased glucose flux. The mechanism by which skeletal muscle becomes insulin resistant and not adipose tissue is currently unknown. Moreover, the differential effects on glucose flux in visceral and subcutaneous adipose depots is not known, but may be due to intrinsic differences between these distinct tissue subtypes (42-44). Because olanzapine elevated plasma glucose and insulin in the fed and food-restricted states, excess glucose should be repartitioned to adipose tissue with the prevailing differences in tissue insulin sensitivity.

Increased adipose tissue glucose uptake relative to skeletal muscle would be expected to increase adipocyte triglyceride storage, which is further potentiated by the nearly 2-fold increase in FFA flux present in visceral and subcutaneous fat depots. The increased FFA flux likely contributes to triglyceride storage by providing FFAs for esterification to phosphoglycerol moieties from glycolysis. These changes in glucose and FFA flux are consistent with increased adiposity and adipocyte hypertrophy previously reported (45). Because tissue FFA uptake is both passive and regulated by insulin-sensitive fatty acid transporters (46), the mechanism of the increased tissue FFA uptake may involve either increased fatty acid oxidation, stimulation of fatty acid transporters by a direct drug effect, or by drug-induced increases in plasma insulin affecting lipogenic pathways. Increased VO_2 during the light cycle supports the theory of increased lipogenesis.

Impaired Fuel Mobilization from Adipose Tissue

Another factor contributing to increased adiposity during olanzapine administration is impaired mobilization of stored fuels. Normally, stored adipose triglyceride is mobilized via lipolysis during the post-absorptive state to conserve glucose fuel for the brain. Alternatively, FFA are mobilized in times of stress by elevated plasma catecholamines. Olanzapine blunted increases in plasma FFA and glycerol in 14h food-restrict rats, suggesting lipolytic impairment that was confirmed by decreased *in vivo* lipolysis after injected isoproterenol in fed animals when FFA were not significantly different between the experimental groups. Consistent with these findings, decreased plasma FFA have also been reported in patients and healthy volunteers following acute (2-3 weeks) olanzapine treatment (12, 13). Moreover, catecholamine-stimulated lipolysis is attenuated in adipose tissue harvested from chronically treated rats (45), suggesting a primary defect in the adipocyte. The mechanism of the decreased lipolysis is currently unknown, though several hypotheses may be considered. Decreased sympathetic tone will reduce lipolysis (47), however, this is likely not responsible because the response to exogenous catecholamines remains intact. Previous radioligand binding studies have not detected binding of olanzapine to β_2 -adrenergic receptors (48) and hepatic glucose output, which is regulated by β_2 -adrenergic receptors in response to isoproterenol, was not affected. It is possible that olanzapine inhibits β_3 -adrenergic receptors that are found only on adipose tissue. As many of the early steps in β -adrenergic signaling are shared between β_2 - and β_3 -adrenergic receptors in fat and β_2 -adrenergic receptors in liver, it seems unlikely that these are the target of the impairment.

In summary, olanzapine increases the accumulation of fat mass in male rats independent of a change in body weight gain and food intake. Current findings demonstrate that olanzapine has at least five effects that collectively predispose male rats to increased adiposity, namely decreased physical activity, tissue-specific insulin resistance leading to repartitioning of glucose fuel, increased uptake of FFA into fat, increased adipose tissue lipogenesis and impaired lipolysis which traps excess fuel taken up as well as newly formed triglyceride. Further studies are needed to identify the molecular mechanisms leading to these early drug effects and determine their relevance to antipsychotic-induced weight gain and insulin resistance seen clinically.

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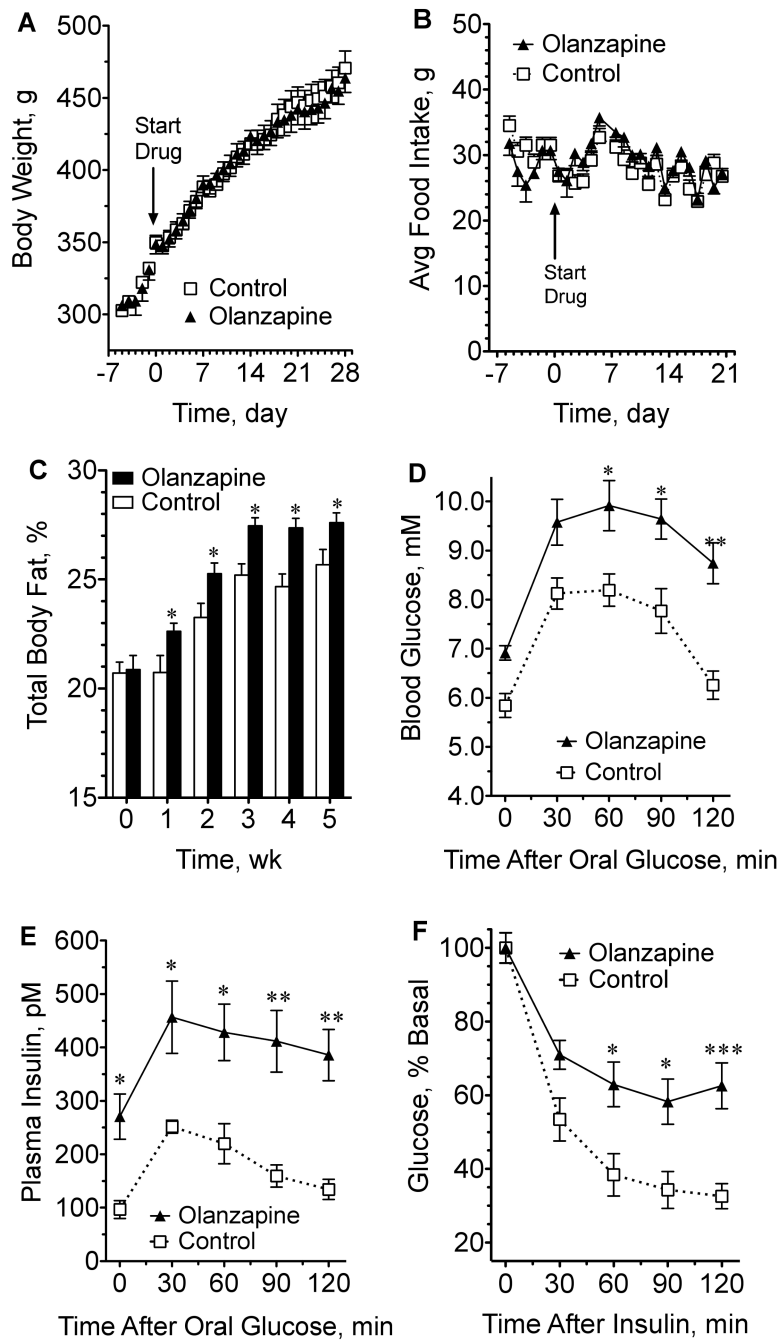


Figure 1.

Effects of chronic olanzapine on body weight, food intake, adiposity, oral glucose tolerance and insulin tolerance. Animals were trained to eat drug-free cookie dough and then allocated to experimental groups for chronic olanzapine or vehicle treatment as indicated (see ‘methods’ for detailed dosing protocol). Data represent the mean \pm S.E. ($n = 8-10$). Asterisks indicate a significant difference (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) in the olanzapine group compared to the control group for each measurement. (A-B) Body weight and 24h food intake were measured daily and data represent the mean \pm S.E. (C) Percent total body

adiposity was measured weekly by $^1\text{H-NMR}$ for 5 weeks. (D-E) On day 28 of olanzapine or vehicle treatment an oral glucose tolerance test was performed. Animals were food-restricted for 14h and administered a half-dose of olanzapine (6 mg/kg) or vehicle 1h prior to beginning the OGTT. Baseline blood samples were collected 1h after the gavage and then a glucose solution (1.5 g/kg) administered orally. Serial blood samples were collected at 30-min intervals for 2h following glucose gavage for measurement of (D) blood glucose and (E) plasma insulin. Data represent the mean \pm S.E. ($n = 5-10$). (F) On day 42 of olanzapine treatment, an insulin tolerance test was performed. Animals were food-restricted for 14h prior to the test and given a half-dose of olanzapine (6 mg/kg) or vehicle one hour prior to the challenge. The response to injected insulin was measured for 120 min as the change in baseline blood glucose.

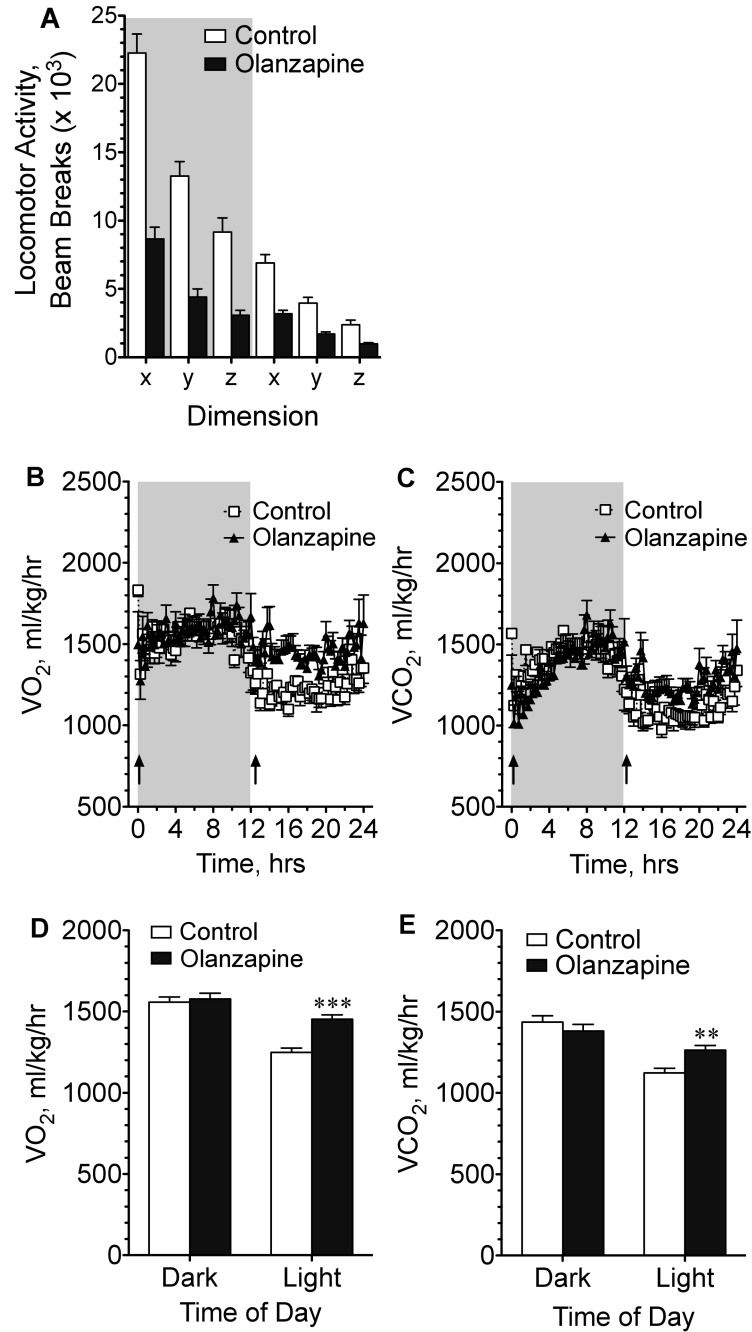


Figure 2. Energy expenditure and locomotor activity following acute olanzapine administration. Animals were placed in specialized chambers to measure locomotor activity and energy expenditure using indirect calorimetry. Following acclimation, olanzapine (10 mg/kg) or vehicle solution was administered by oral gavage (indicated by arrows). Animals retained *ad libitum* access to food and water for the duration of the experiment. (A) VO_2 and (B) VCO_2 were measured at 15-minute intervals for 24h. (C) 24h locomotor activity was measured as an indicator of physical activity. Background shading or lack thereof indicates the dark and

light cycles, respectively. Locomotor activity was significantly different from control in each dimension during the dark and light cycles ($P < 0.001$). (D) Average VO_2 and (E) Average VCO_2 for the dark and light cycles were calculated. All data represent the mean \pm S.E. ($n = 12$), asterisks indicate significant differences ($***P < 0.001$, $**P < 0.01$).

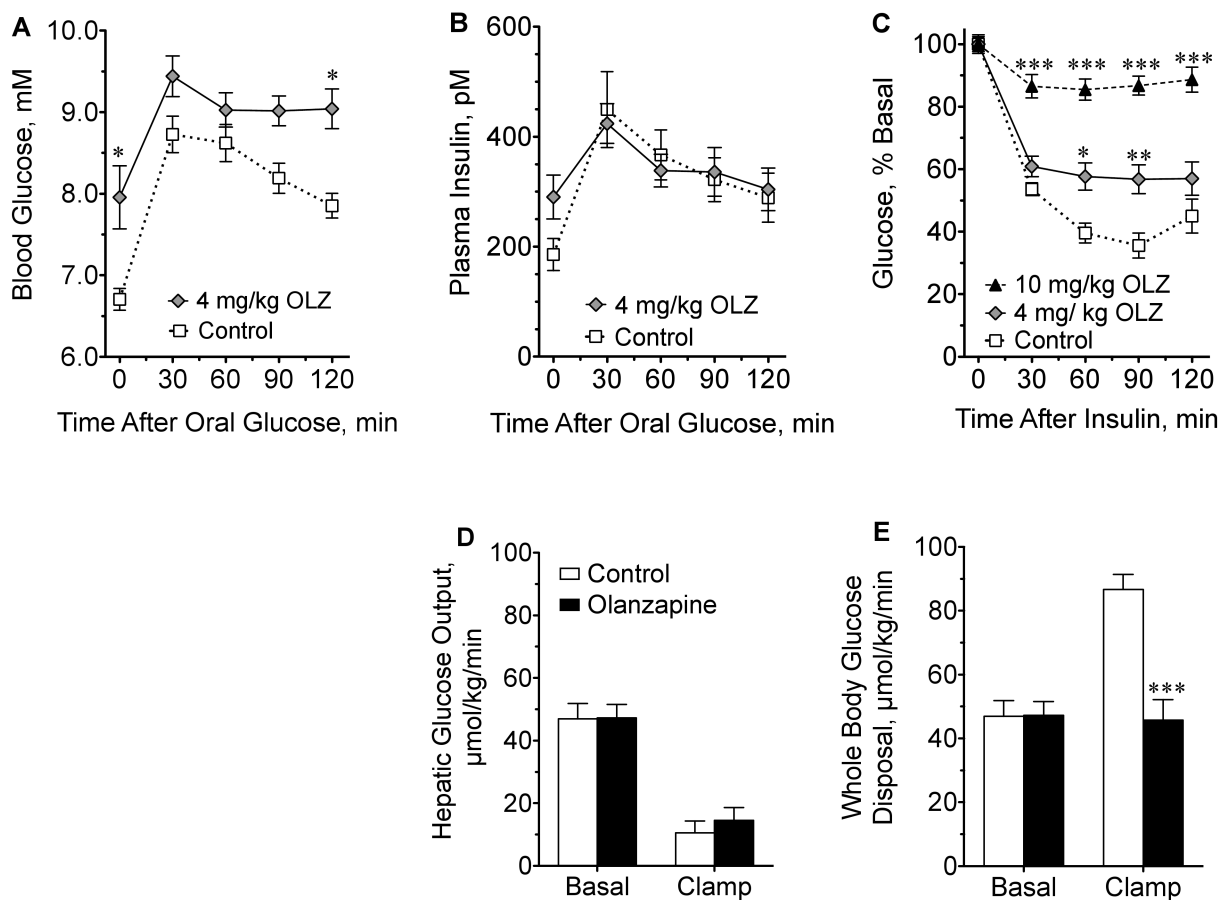
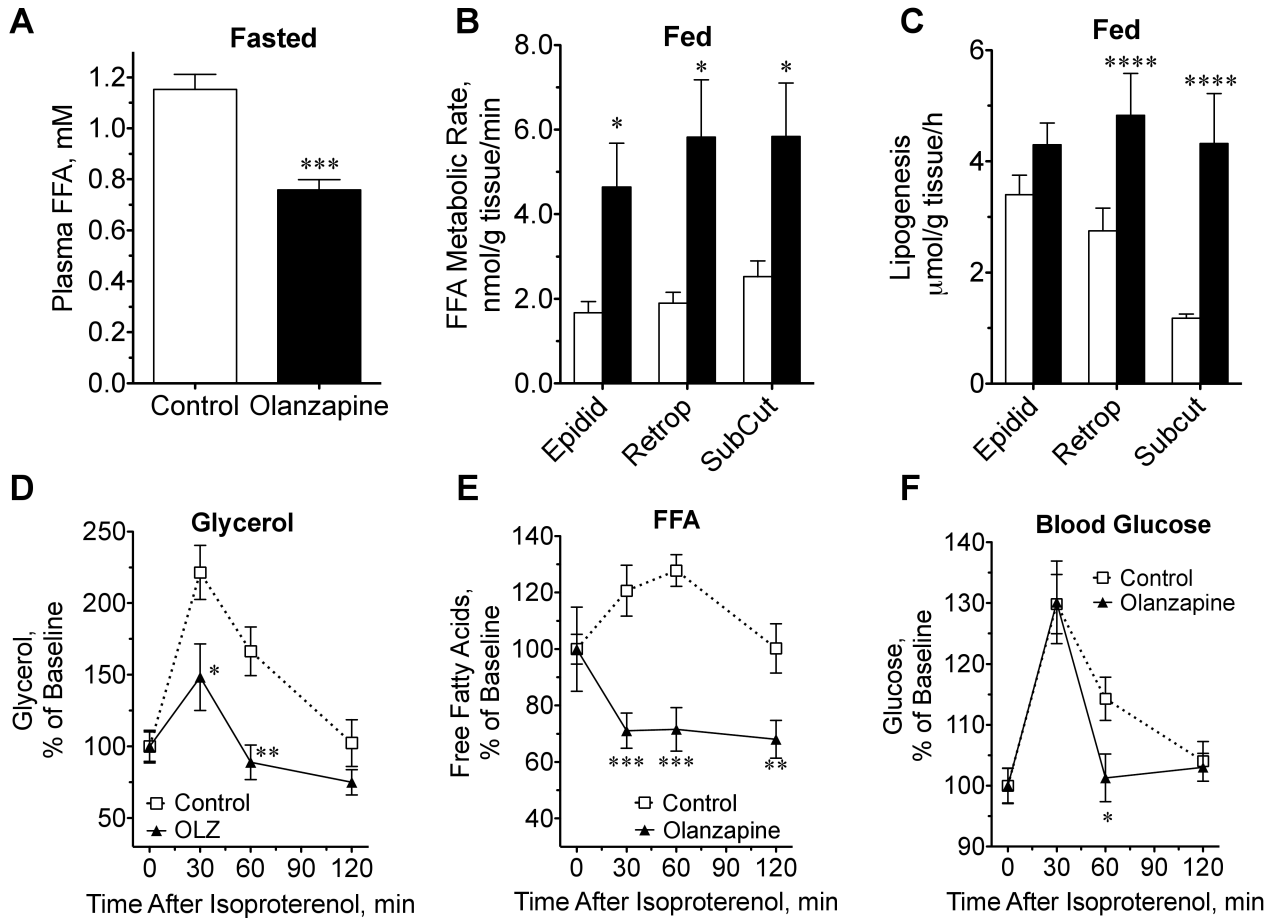


Figure 3.

Effects of acute olanzapine on oral glucose tolerance, insulin tolerance and whole-body insulin sensitivity. Asterisks indicate significant differences compared to control (* $P < 0.05$, *** $P < 0.001$). (A, B) An oral glucose tolerance test was conducted on the second treatment day after acute olanzapine (4 mg/kg) gavage (see 'Methods' for dosing protocol). Animals were food-restricted for 5h prior to the OGTT and received a final olanzapine or vehicle gavage 1h prior to the start of the OGTT. Baseline blood samples were collected at 1h following gavage and then glucose solution (2.5 g/kg) administered orally. Serial blood samples were collected at 30-min intervals for 2h following glucose gavage for measurement of (A) blood glucose and (B) plasma insulin. Data represent the mean \pm S.E. ($n = 12$). (C) An insulin tolerance test was performed in another group of animals on the second treatment day. Animals received high dose olanzapine (10 mg/kg), low dose olanzapine (4 mg/kg) or vehicle solution by oral gavage 1h prior to beginning the tolerance test. Baseline blood samples were collected and then insulin (0.75 U/kg, i.p.) injected. Blood glucose was measured for 120 min as an indicator of insulin sensitivity. Data represent the mean \pm S.E. ($n = 18-20$). (D-E) Hyperinsulinemic-euglycemic clamp studies were conducted after acute olanzapine administration (10 mg/kg) (D) Hepatic glucose output was measured during basal (14h food-restricted) and clamp conditions. Basal hepatic glucose output (HGO) was equal to the total glucose turnover during the basal period, while the clamp was the residual HGO during the final 40 minutes of the clamp. (E) Whole-body glucose

disposal, a measure of whole-body insulin action, was measured during hyperinsulinemic-euglycemic clamp conditions and compared to basal glucose turnover. Data represent the mean \pm S.E. of glucose turnover during the last 40 minutes of the clamp experiment (n = 10-14). (F) Adipose tissue glucose uptake in *ad libitum* fed animals was measured after acute olanzapine administration (10 mg/kg). Data represent the mean \pm S.E. (n = 8).

**Figure 4.**

Acute effects of olanzapine on circulating and adipose tissue FFA uptake, lipogenesis, and mobilization of FFA and glucose in response to an isoproterenol challenge. (A) Plasma FFAs were measured in 14h food-restricted animals following olanzapine (10 mg/kg) gavage. (B) In a separate cohort, FFA uptake into adipose tissue was measured on the second day of olanzapine treatment using a non-metabolizable FFA analog (^{125}I -BMIPP) in animals that had *ad libitum* access to food and water. Two hours after the final olanzapine dose (10 mg/kg), an intravenous bolus of the FFA tracer was given and blood samples were collected during a 40-min *in vivo* labeling period. Epididymal, pararenal and subcutaneous fat pads were harvested and measured for tracer uptake, an index of adipocyte FFA uptake. (C) In another cohort of animals adipose tissue lipogenesis was measured in the well-fed state after acute olanzapine (10 mg/kg). Following final administration of olanzapine, $^3\text{H}_2\text{O}$ was administered via i.p. injection and tissue samples were collected after a 120-min *in vivo* labeling period. Epididymal, retroperitoneal and subcutaneous adipose tissues were excised, extracted for total lipid and then counted for ^3H content as an index of lipogenesis. (D-F) An isoproterenol challenge test was conducted to assess the effects of olanzapine (10 mg/kg) on isoproterenol-stimulated lipolysis and hepatic glucose output. Animals had *ad libitum* access to food and water. Baseline blood samples were collected and then isoproterenol (0.01 mg/kg) administered. Serial blood samples at 30-min intervals for 2h were collected to

measure the lipolytic response, as measured by the change in (C) FFA and (D) glycerol from isoproterenol-stimulated lipolysis, as well as the (E) hepatic glycolytic response. Data represent the mean \pm S.E. (n = 8-10). Asterisks indicate significant differences compared to time-matched control values (*P<0.05, ***P<0.001, ****P<0.0001).

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Table 1

Minispec Proton-NMR body composition data during chronic olanzapine treatment (4-12 mg/kg) in male rats.

Time	Weight Component	Control (%)	Olanzapine (%)
Week 0	Adipose	20.7 ± 0.5	20.9 ± 0.6
	Lean	65.1 ± 0.7	65.8 ± 0.5
	Fluid	6.3 ± 0.2	6.2 ± 0.2
Week 1	Adipose	20.7 ± 0.8	22.6 ± 0.4*
	Lean	59.2 ± 1.4	58.3 ± 1.3
	Fluid	5.3 ± 0.2	5.0 ± 0.2
Week 2	Adipose	23.3 ± 0.7	25.3 ± 0.5*
	Lean	63.5 ± 0.8	62.1 ± 0.6
	Fluid	5.7 ± 0.3	5.3 ± 0.2
Week 3	Adipose	25.2 ± 0.5	27.5 ± 0.4**
	Lean	63.4 ± 0.3	62.1 ± 0.4*
	Fluid	5.7 ± 0.1	5.6 ± 0.1
Week 4	Adipose	24.7 ± 0.6	27.4 ± 0.4**
	Lean	61.3 ± 0.9	60.5 ± 0.7
	Fluid	5.5 ± 0.3	5.2 ± 0.2
Week 5	Adipose	25.7 ± 0.7	27.6 ± 0.5*
	Lean	62.8 ± 0.6	61.3 ± 0.3*
	Fluid	6.2 ± 0.1	6.2 ± 0.1

Values are means ± SE; n = 10 rats per group. Data represent the average component mass in percent of total body weight. Asterisks indicate significantly different (*P<0.05, **P<0.01) compared to measurements from time-matched control animals.

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Table 2

Tissue metabolic clearance rates and insulin-stimulated glucose metabolic rates for individual tissues during either basal or euglycemic-hyperinsulinemic clamp conditions in male rats on the second day of acute olanzapine treatment (10 mg/kg).

Tissue	Basal Conditions Metabolic Clearance Rate (ml/g tissue/min)		% Change from Control	Clamp Conditions \bar{C} Glucose Metabolic Rate (mmol/g tissue/min)		% Change From Control
	Control	Olanzapine		Control	Olanzapine	
Gastrocnemius	1.28 ± 0.10	1.62 ± 0.24	ns	198 ± 20	136 ± 11	-31%*
Soleus	2.36 ± 0.28	3.20 ± 0.46	ns	381 ± 25	229 ± 36	-40%**
Heart	36.5 ± 5.6	124.9 ± 24.5	+242%***	375 ± 37.0	221 ± 26.7	-39%**
Epididymal Adipose	0.67 ± 0.09	0.59 ± 0.10	ns	51 ± 6	51 ± 8	ns
Pararenal Adipose	0.67 ± 0.12	0.74 ± 0.09	ns	65 ± 13	75 ± 8	ns
Subcutaneous Adipose	1.62 ± 0.25	2.68 ± 0.37	+65%*	89 ± 8	136 ± 16	+53%*
Liver	10.71 ± 1.46	11.04 ± 2.05	ns	195 ± 23	202 ± 16	ns

Values are means ± SE; n = 6-14 per tissue; asterisks indicate significant differences (*P<0.05, **P<0.01) compared to Control. ns = not significantly different from control