Metabolic Dysregulation With Atypical Antipsychotics Occurs in the Absence of Underlying Disease
A Placebo-Controlled Study of Olanzapine and Risperidone in Dogs

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Atypical antipsychotics have been linked to weight gain, hyperglycemia, and diabetes. We examined the effects of atypical antipsychotics olanzapine (OLZ) and risperidone (RIS) versus placebo on adiposity, insulin sensitivity ($S_I$), and pancreatic $eta$-cell compensation. Dogs were fed ad libitum and given OLZ (15 mg/day; $n = 10$), RIS (5 mg/day; $n = 10$), or gelatin capsules ($n = 6$) for 4–6 weeks. OLZ resulted in substantial increases in adiposity: increased total body fat ($+91 \pm 20\%$; $P = 0.000001$) reflecting marked increases in subcutaneous ($+106 \pm 24\%$; $P = 0.0001$) and visceral ($+84 \pm 22\%$; $P = 0.000001$) adipose stores. Changes in adiposity with RIS were not different from that observed in the placebo group ($P > 0.33$). Only OLZ resulted in marked hepatic insulin resistance (hepatic $S_I$ [pre- versus postdrug]: $6.05 \pm 0.98$ vs. $1.53 \pm 0.93$ dl $\cdot$ min$^{-1}$ $\cdot$ kg$^{-1}$/[U$\cdot$ml], respectively; $P = 0.009$). $eta$-Cell sensitivity failed to upregulate during OLZ (pre-drug: $1.24 \pm 0.15$, post-drug: $1.07 \pm 0.25$ U $\cdot$ ml$^{-1}$/[mg$\cdot$dl]; $P = 0.6$). OLZ-induced $eta$-cell dysfunction was further demonstrated when $eta$-cell compensation was compared with a group of animals with adiposity and insulin resistance induced by moderate fat feeding alone ($+8\%$ of calories from fat; $n = 6$). These results may explain the diabetogenic effects of atypical antipsychotics and suggest that $eta$-cell compensation is under neural control. Diabetes 54: 862–871, 2005

The introduction of atypical antipsychotics in psychopharmacology represented a major advance in the treatment of schizophrenia, providing an effective therapy for both positive and negative symptoms of psychosis while minimizing the extrapyramidal effects characteristic of earlier therapeutic options. Indeed, these medications are widely prescribed (~3% of the U.S. population) for treatment of schizophrenia, as well as bipolar disorder, depression, and dementia.

In the face of their widespread use, concern has arisen regarding treatment-associated weight gain and apparent increased diabetes risk (1–3). It is unclear whether weight gain is a direct effect of the drug or secondary to behavioral changes, such as increased sedation associated with pharmacotherapy. But regardless of its causality, obesity is a significant public health concern and is a well-documented risk factor for type 2 diabetes as well as other chronic diseases, such as cancer and atherosclerosis (4–6). Nonetheless, diabetes risk with antipsychotic use has also been reported in the absence of significant weight gain (7–9).

Evidence linking atypical antipsychotics to metabolic dysregulation is largely based on case reports and retrospective analyses, which note a disproportionately greater number of patients developing fasting hyperglycemia and new-onset diabetes or exhibiting exacerbation of preexisting diabetes soon after the initiation of atypical antipsychotic treatment (10–15). Henderson et al. (14) reported that >30% of schizophrenic patients receiving clozapine developed diabetes within a 5-year follow-up, and those with preexisting diabetes required increased insulin dosing. Federal Drug Administration reports (10), based in part on the Medwatch Surveillance Program, provide further evidence of the excessive occurrence of new-onset diabetes and exacerbation of preexisting disease with clozapine compared with disease incidence in untreated individuals. More recently, olanzapine (OLZ) and risperidone (RIS), which collectively account for >80% of all drugs prescribed of their class of atypical antipsychotics, have also been associated with metabolic abnormalities, though OLZ is generally linked to greater relative risk for diabetes (11,16) and more marked obesity (3,11,17) compared with RIS (12,13,18).

It is indeed challenging to study the actions of antipsy-
chotic agents. Most prospective studies (19–21) have been performed in subjects with psychiatric disease. However, the use of such patients for prospective studies introduces confounding factors, such as comedication, comorbidity, and poorly defined effects of underlying disease per se. With respect to carbohydrate metabolism, disease per se can result in obesity and the associated risk for diabetes and other ailments (22–24). One possible approach to minimize such confounds is to study the effects of the drugs in healthy subjects (25). However, since there are well-known potential risks associated with administration of psychiatric drugs to normal subjects (26), it is often not possible to administer therapeutic drugs for extended periods of time.

In view of the overwhelming evidence that these agents may increase the risk for diabetes, it is incumbent to study those of the agents in an appropriate animal model. Also, because of the reports of diabetic ketoacidosis (10–12), which most often results from severe insulinopenia, it is important to include studies of pancreatic β-cell functionality. For these reasons, the present placebo-controlled study was performed in normal dogs to directly test whether chronic treatment with atypical antipsychotics results in obesity and glucose dysregulation.

The present study represents the first prospective analysis of the metabolic effects of two widely prescribed atypical antipsychotics in the absence of underlying disease and demonstrates clear differential effects of these two agents on body composition and carbohydrate metabolism. Most notably, we provide the first clear demonstration of a negative effect of an atypical antipsychotic agent on β-cell function.

RESEARCH DESIGN AND METHODS

Animal model: metabolic effects of antipsychotics. Procedures were performed on 26 male mongrel dogs (28.6 ± 0.6 kg). Dogs were individually housed in the University of Southern California Vivarium under controlled environmental conditions. At least 1 week before testing, dogs were surgically outfitted with chronic catheters in the jugular vein (advanced into the right atrium) for sampling of mixed central venous blood and a femoral vein for infusions. All procedures were approved by the University of Southern California Institutional Animal Care and Use Committee.

Diet and food intake. At least 1 week before surgery, dogs were established on a strict feeding regimen to permit assessment of daily food intake throughout the study to determine its contribution to any observed changes in body weight. During baseline and treatment periods, all dogs were fed ad libitum a diet of one can of Hill’s Prescription Diet (10% carbohydrate, 9% protein, and 8% fat) and a known quantity of dry chow (37% carbohydrate, 26% protein, and 15% fat) once per day. Quantity of uneaten food was removed, weighed, and recorded daily each morning before giving the next day’s food allotment. Any dog consuming its daily meal on 2 consecutive days was provided with an increased amount of dry chow for all subsequent days, although this rarely occurred since dogs were given dry chow in excess of typical daily intake to enable virtually ad libitum feeding. Food intake was presented as total calories consumed per week.

Study design. The study was divided into three phases: 1) baseline testing (pre-drug), 2) drug treatment period, and 3) post-drug period. Before study entry, dogs were randomly assigned to one of three treatment arms: OLOZ, RIS, or placebo. Predrug testing was performed in all dogs to quantify insulin sensitivity (S), glucagon sensitivity, and pancreatic β-cell function (see below for details). The order of experiments was randomized and performed in each animal over a 10-day period, with ≥2 days between experiments. After pre-drug testing, dogs were placed on the drug (or placebo) regimen for 4–6 weeks, after which all procedures performed during pre-drug phase were repeated (post-drug). Dogs of each drug were treated in a stepwise fashion by isocaloric moderate fat feeding. Dogs (n = 6; 27.5 ± 1.5 kg) were fed a weight-maintaining diet (3,885 kcal/day; 37.9% carbohydrate, 26.3% protein, and 35.8% fat) for 2 weeks before and during baseline testing. After baseline testing, dogs were placed on an isocaloric diet (i.e., total calories similar to baseline) with moderate supplementation of dietary fat (5 ± g/kg prediet body weight/day) to maintain body weight, which consisted of 3,945 kcal/day (32.9% carbohydrate, 22.9% protein, and 44.1% fat) for a total increase of ~8% daily calories from fat. This diet was maintained for 6 weeks and continued during postdosing. This diet was previously shown to cause a moderate increase in visceral and subcutaneous adiposity despite no increase in calories (30).

were administered orally once per day, 6–8 h after daily presentation of food (~2:00 P.M.). Doses were based on those used in typical treatment of patients, as well as on reported dopamine D2 receptor binding in the caudate nucleus (27). The OLOZ dose was chosen as the midpoint between zero and the dose associated with moderate toxicity (28). The RIS dose was subsequently chosen to parallel the approximate 3:1 (OLZ-to-RIS) ratio used in clinical trials with this drug. Doses of each drug were increased stepwise every day by the target dose by the end of the treatment period. The target dose for OLZ was 15 mg/kg/day and the target dose for RIS was 5 mg/day. All dogs (including placebo-treated animals) were video-taped for ~2–5 min before and 1–3 h and 24 h after first dosing at initial and target doses to facilitate monitoring of behavioral changes and possible movement disorders.

Drug treatment period. During the time interval between pre- and post-dosing testing, body weight was measured and fasting blood samples drawn twice weekly for blood chemistry and hormones. Blood glucose levels were monitored daily. Blood samples were collected percutaneously from a cephalic vein, centrifuged, and plasma analyzed for glucose and lactate with an automated analyzer (YSI Model 230, Yellow Springs, Yellow Springs, OH). Remaining plasma was stored at −80°C for subsequent assay of insulin, free fatty acids (FFAs), glycerol, and triglycerides. Given reports of elevated hepatic enzymes with antipsychotic treatment (29), fasting blood samples were collected during pre- and post-dosing periods for determination of alanine aminotransferase and aspartate aminotransferase.

Experimental procedures. During pre-drug and post-drug periods, all animals underwent three procedures performed in random order: 1) abdominal magnetic resonance imaging (MRI) to assess treatment-induced changes in adiposity, 2) euglycemic hyperinsulinemic clamp (EGIC) to quantify hepatic and peripheral insulin sensitivity, and 3) stepwise β-cell stimulation test (STEP test) to assess pancreatic β-cell insulin response. All procedures were performed in overnight-fasted animals, and during all experiments (except MRIs), dogs were fully conscious, rested comfortably in a Pavlov sling, and were given free access to water.

Abdominal MRI. After an overnight fast, dogs were preanesthetized and sedated. Thirty 1-cm axial abdominal images were obtained using a General Electric 1.5 Tesla Horizon magnet. Images were analyzed by a laboratory technician who was blinded to treatment group and phase of study (basal, posttreatment). Analysis was performed using ScionImage software, which quantified fat tissue (pixel value: 0–120) and other tissues (pixel value: 121–256) in each slice. Total trunk fat and tissue were estimated as the integrated fat or tissue across all 30 slices. Visceral fat was defined as fat within the peritoneal cavity at ≤5 images from the slice at the level where the left renal artery branches from the abdominal aorta (i.e., from 11-cm abdominal range). All adiposity data are expressed in units of cm3 normalized to the volume of nonfat tissue.

Analysis of the clamp, one intracatheter was inserted percutaneously in the saphenous vein for infusion of glucose. At t = −180 min, a primed infusion of high-performance liquid chromatography-purified 3-H-glucose (tracer; 25 μCi + 0.25 μCi/min) was initiated via indwelling femoral ven catheter. At basal blood sampling, cyclical somatostatin (1 μg·min−1·kg−1) was infused intravenously to suppress endogenous insulin secretion. Following a 180-min period, glucose was replaced by exogenous infusion (0.15 mU·min−1·kg−1·kg−1). Hyperinsulinemia was induced by systemic insulin (regular purified pork; Lilly) infusion at 1.0 mU·min−1·kg−1 from 0 to 180 min. Euglycemia was maintained by a variable rate of 50% dextrose infusion spiked with 3-H-glucose (specific activity: 2.2 μCi/g) to avoid large fluctuations in plasma specific activity. Blood samples were drawn every 10 min from t = 0 to 60 min, every 15 min from t = 75 to 150 min, and every 10 min from 160 to 180 min. Plasma was stored for subsequent assay of insulin, tracer, FFAs, glycerol, and triglycerides.

STEP test. After basal blood sampling, glucose was clamped at three sequential glycemic levels by exogenous glucose infusion. Glucose was targeted to concentrations of 100 mg/dl (t = 0–50 min), 150 mg/dl (t = 60–149 min), and 200 mg/dl (t = 150–240 min).

Fat fed dogs: control for drug-induced adiposity. To determine the extent to which drug-associated changes in β-cell function were due to increased adiposity per se, we examined a separate group of dogs with obesity induced by isocaloric moderate fat feeding. Dogs (n = 6; 27.5 ± 1.5 kg) were fed a weight-maintaining diet (3,885 kcal/day; 37.9% carbohydrate, 26.3% protein, and 35.8% fat) for 2 weeks before and during baseline testing. After baseline testing, dogs were placed on an isocaloric diet (i.e., total calories similar to baseline) with moderate supplementation of dietary fat (5 ± g/kg prediet body weight/day) to maintain body weight, which consisted of 3,945 kcal/day (32.9% carbohydrate, 22.9% protein, and 44.1% fat) for a total increase of ~8% daily calories from fat. This diet was maintained for 6 weeks and continued during postdosing. This diet was previously shown to cause a moderate increase in visceral and subcutaneous adiposity despite no increase in calories (30).
TABLE 1
Effect of drug (or placebo) treatment on fasting plasma values

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>Insulin (μU/ml)</th>
<th>FFAs (mmol/l)</th>
<th>Glycerol (mmol/l)</th>
<th>Triglycerides (mmol/l)</th>
<th>Lactate (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predrug</td>
<td>98 ± 1</td>
<td>13.7 ± 2.3</td>
<td>0.62 ± 0.07</td>
<td>0.291 ± 0.025</td>
<td>8.69 ± 0.55</td>
</tr>
<tr>
<td>Postdrug*</td>
<td>96 ± 1†</td>
<td>18.2 ± 3.0†</td>
<td>0.79 ± 0.07</td>
<td>0.277 ± 0.027</td>
<td>7.37 ± 0.41</td>
</tr>
<tr>
<td>Predrug</td>
<td>98 ± 1</td>
<td>10.3 ± 1</td>
<td>0.73 ± 0.07</td>
<td>0.351 ± 0.026</td>
<td>7.76 ± 1.04</td>
</tr>
<tr>
<td>Postdrug†</td>
<td>97 ± 1</td>
<td>13.0 ± 1.1†</td>
<td>0.58 ± 0.05</td>
<td>0.297 ± 0.023§</td>
<td>8.43 ± 0.49</td>
</tr>
<tr>
<td>Predrug</td>
<td>92 ± 2‡</td>
<td>10.5 ± 1.6</td>
<td>0.54 ± 0.07</td>
<td>0.366 ± 0.029</td>
<td>8.13 ± 0.39</td>
</tr>
<tr>
<td>Postdrug‡</td>
<td>91 ± 2</td>
<td>13.0 ± 1.7†</td>
<td>0.45 ± 0.08</td>
<td>0.332 ± 0.020</td>
<td>7.68 ± 0.70</td>
</tr>
</tbody>
</table>

Data are means ± SE. *Values reported are mean of week 6 of drug treatment period; †P < 0.05 vs. pre-drug; ‡P = 0.015 vs. OLZ and RIS (by ANOVA); §P = 0.011 vs. pre-drug.

Data from diet group represents a subset of results currently under review (31). Before and after fat feeding, each dog underwent procedures described above (MRI, EGC, and STEP tests), performed in a manner except for insulin infusion rate during clamps (1.5 mU·min⁻¹·kg⁻¹). Blood sample collection and tracer analysis were collected in lithium- and heparin-coated tubes to which EDTA was added. Samples were measured for FFAs, glycerol, and triglycerides were collected in tubes containing paraoxan (to suppress lipoprotein lipase) and EDTA. All samples were centrifuged immediately and plasma stored at −80°C for subsequent assays. Plasma glucose was assayed online in duplicate by the glucose oxidase technique on an automated analyzer, with intra-assay coefficient of variation (CV) 1%, respectively. Plasma FFAs were measured using kits that utilize a procedure (GPO-Trinder; Sigma, St. Louis, MO). Measurement of [3-3H]-glucose was performed after deproteination of samples as previously described (32).

Data analysis and calculations. After data smoothing (33), rates of glucose disappearance (Ra) and hepatic glucose output (HGO) during EGC were calculated with Steele's equations, as modified for use with labeled glucose infusion (34). Measures of insulin sensitivity from clamps were calculated as previously described (35). Whole-body insulin sensitivity (SIclamp combined actions of insulin to stimulate glucose uptake and suppress endogenous glucose production) was defined as $SI_{clamp} = \frac{\Delta GINF}{\Delta GINS \times GLU_w}$, where $\Delta GINF$ is the increase in glucose infusion rate and plasma insulin measured during exogenous insulin infusion (i.e., steady state minus basal period) and $GLU_w$ is the observed change in endogenous glucose production. Peripheral insulin sensitivity ($SI_{p\ clamp}$) was defined as $\frac{\Delta R_G}{\Delta GINS \times GLU_w}$, where $\Delta R_G$ is the change in glucose uptake ($R_G$) from basal to steady state. Hepatic insulin sensitivity was calculated as the absolute value of $\frac{\Delta GINF}{\Delta GINS \times GLU_w}$, where $\Delta HGO$ is the observed change in endogenous glucose production or HGO (basal minus steady state).

Pancreatic β-cell sensitivity to glucose was calculated from STEP tests as the slope of the line relating plasma insulin to glucose concentrations (β-cell slope), using the mean of the final 20–30 min from each glycemic period of each variable (i.e., 40–60, 120–150, and 210–240 min). Incremental area under the curve was calculated by the trapezoidal rule. It is known that there is a stereotypical hyperbolic relationship between insulin secretion and insulin sensitivity, such that any reduction in sensitivity (insulin resistance) is normally compensated for by an increase in β-cell responsiveness (36,37). Therefore, to assess the ability of the β-cells to compensate for adiposity-driven insulin resistance, we also normalized β-cell response to insulin sensitivity (36), as reductions in insulin sensitivity would be expected to prompt upregulation of β-cell function. Pancreatic β-cell function is expressed as the disposition index (DI) (36,37) calculated as $SI_{clamp} \times \beta$-cell slope.

All statistics (t tests and ANOVA, with Tukey’s post hoc analysis when overall significance was detected) were performed using MINITAB statistical software (version 13.32; Minitab, State College, PA). Data are reported as means ± SE. Statistical significance was set at P ≤ 0.05.

RESULTS
Basal values. The pre-drug characteristics of each treatment group are shown in Table 1. Fasting glucose was similar between OLZ and RIS (98 ± 1 mg/dl for both) but was slightly lower in the placebo group (92 ± 2 mg/dl; P = 0.015 by ANOVA). In the pre-drug state, there were no differences in fasting insulin, FFAs, glycerol, triglycerides, or lactate among groups (P > 0.14). In response to drug (or placebo) treatment, fasting hyperglycemia did not develop in any dog. Rather, glucose was marginally lower after OLZ (P = 0.05) but clearly remained in the physiologic range within which counterregulatory responses are absent. In contrast, insulin was elevated from pre-drug in all groups (P = 0.033, P = 0.01, and P = 0.02 for OLZ, RIS, and placebo, respectively). FFAs, glycerol, and lactate concentrations were not appreciably changed in any treatment group, although there was a trend toward reduced FFAs after treatment with RIS (P = 0.078). Triglycerides were unchanged by OLZ (P = 0.44) but significantly reduced by RIS (P = 0.011). Except for insulin, fasting plasma values were not changed significantly by placebo treatment.

Body weight and food intake. There were modest

![FIG. 1. Time course of body weight in dogs treated with OLZ (A), RIS (B), or placebo (C). Week 0 represents the mean of 2 weeks before drug (or placebo) administration and is shown as dashed line. Statistics denote comparison to respective pre-drug values. *P < 0.05; †P < 0.01; ‡P < 0.001.](image_url)
changes in body weight in all groups (Fig. 1). OLZ-treated dogs exhibited an initial decline in weight of 0.7 ± 0.2 kg (−2.5 ± 0.6% pre-drug weight) within 1 week of drug treatment (P = 0.001; Fig. 1A), which occurred despite increased food intake during that time (P = 0.015; Fig. 2A). Weight normalized by week 2 and steadily increased to maximum weight gain of +1.7 ± 0.4 kg (+5.9 ± 1.2% of pre-drug weight) at week 6 (P = 0.001 vs. pre-drug). Body weight changes during RIS appeared similar in pattern to OLZ though smaller in magnitude (Fig. 1B). Following transitory weight loss after 1 week of RIS treatment (0.7 ± 0.2 kg, −2.4 ± 0.8% pre-drug weight; P = 0.016), weight returned to basal levels within 2 weeks and exhibited a marginal nonsignificant upward trend to maximum recorded weight by week 6 (P = 0.09 vs. pre-drug). Three dogs exhibited net weight loss after RIS (week 6 versus pre-drug). Placebo-treated dogs exhibited an upward trend in body weight; by week 6, weight gain in placebo-treated dogs was +1.5 ± 0.3 kg, or +4.8 ± 1.0% of pre-drug weight (P = 0.006).

Changes in food intake suggest possible mechanisms of weight gain observed in this study (Fig. 2). OLZ caused increased food intake; dogs consumed a significant excess of calories during treatment (16,024 ± 6,240 kcal over baseline; P = 0.031). However, such changes were similar to that induced by placebo, as evidenced by the tendency for increased caloric intake in control dogs (19,355 ± 12,885 kcal over baseline; P = 0.82 vs. OLZ). Food intake was not appreciably altered by RIS (−9,599 ± 6,111 kcal over baseline; P = 0.17), suggesting that even modest weight changes with RIS treatment could not be accounted for by increased consumption of calories. Food intake during RIS was significantly lower than that observed during OLZ treatment (P = 0.007).

**Adiposity**. Unremarkable changes in body weight did not reflect substantial increases in adiposity with drug treatment (Fig. 3). Fat deposition was most dramatic in OLZ-treated dogs, where total trunk fat increased in all dogs, from 24.9 ± 3.2 to 43.4 ± 3.9 cm³ (P < 0.000001). Both fat depots enlarged: the visceral adipose compartment expanded by 66% (pre-drug: 13.1 ± 1.3, post-drug: 21.8 ± 1.1 cm³; P < 0.000001), while subcutaneous fat increased 83% (pre-drug: 11.8 ± 2.0, post-drug: 21.6 ± 3.1 cm³; P = 0.0001).

Adiposity changes with RIS were less than with OLZ and more variable (Fig. 3). RIS increased total fat by 45%, from 21.9 ± 3.0 to 31.8 ± 2.8 cm³ (P = 0.005), with two dogs exhibiting reduced fat volume. Visceral fat was increased in most dogs (group increase = +52%; pre-drug: 11.4 ± 0.8, post-drug: 17.3 ± 1.2 cm³, P = 0.001), while the subcutaneous adipose depot was only marginally enlarged 38% (pre-drug: 10.5 ± 2.4, post-drug: 14.4 ± 1.8 cm³; P = 0.053). Dogs given placebo exhibited increases of 27–30% in total (P = 0.042), visceral (P = 0.046), and subcutaneous (P = 0.044) fat depots.

Compared with placebo, changes in adiposity with OLZ were markedly greater in total (P = 0.0088), visceral (P = 0.025), and subcutaneous (P = 0.0078) compartments. Changes with RIS were not different from placebo (P > 0.33). Compared with RIS, OLZ resulted in a 2-to-2.5-fold greater accumulation of total (OLZ: 18.5 ± 1.8, RIS: 9.9 ± 2.7 cm³; P = 0.018) and subcutaneous fat (OLZ: 9.8 ± 1.5, RIS: 4.0 ± 1.8 cm³; P = 0.024) and a tendency toward a greater increase in visceral fat (OLZ: 8.7 ± 0.9, RIS: 5.9 ± 1.3 cm³; P = 0.091). For a given observed change in body weight, OLZ-treated dogs exhibited greater total fat deposition than dogs treated with either RIS or placebo (not shown).

**Insulin sensitivity**. Before treatment, dogs exhibited a wide range of insulin sensitivity, consistent with the wide range of pretreatment adiposity (38). There were no differences among groups at basal (P > 0.8). Whole-body insulin sensitivity (SI_{clamp}) declined to zero after both OLZ and RIS (OLZ: −6.2 ± 3.6, RIS: −6.9 ± 5.8 dm³·min⁻¹·kg⁻¹/[µU/ml]; P > 0.1) but not for the placebo group (+3.3 ± 5.8 dm³·min⁻¹·kg⁻¹/[µU/ml]; pre-drug: 25.6 ± 5.2, post-drug: 28.9 ± 6.2 dm³·min⁻¹·kg⁻¹/[µU/ml]; P = 0.6). Peripheral sensitivity (SI_{p,clamp}; Fig. 4) was similar before treatment (OLZ: 24.3 ± 4.4, RIS: 24.7 ± 6.3, and placebo: 20.1 ± 4.2 dm³·min⁻¹·kg⁻¹/[µU/ml]; P > 0.84) and not significantly altered by treatment in any group (OLZ: 23.3 ± 6.4, RIS: 19.9 ± 1.9, and placebo: 25.3 ± 5.7 dm³·min⁻¹·kg⁻¹/[µU/ml]; P > 0.3 compared with respective pre-drug value). However, liver sensitivity to insulin was impaired by OLZ. Before OLZ, HGO was suppressed >90% during clamps (basal: 2.5 ± 0.2, steady state: 0.2 ± 0.5 mg·min⁻¹·kg⁻¹, P < 0.0001). Comparable hyperinsulinemia failed to significantly suppress HGO after OLZ treatment (basal: 2.3 ± 0.1, steady state: 1.8 ± 0.4 mg·min⁻¹·kg⁻¹, P = 0.12). Thus, hepatic insulin sensitivity was markedly diminished by OLZ, from 6.1 ± 1.0 to 1.5 ± 0.9 dl·min⁻¹·kg⁻¹/[µU/ml] (P = 0.009; Fig. 5).
Unlike OLZ, hepatic insulin sensitivity was not significantly affected by RIS (pre-drug: 4.3 ± 0.8, post-drug: 3.0 ± 0.9 dl·min⁻¹·kg⁻¹·[μU/ml]; P = 0.35). Likewise, there was no significant change in hepatic insulin sensitivity in placebo-treated dogs (pre-drug: 5.5 ± 1.3, post-drug: 3.3 ± 0.4 dl·min⁻¹·kg⁻¹·[μU/ml]; P = 0.12; Fig. 5), although a trend toward liver resistance was observed in the majority of placebo animals.

**Pancreatic β-cell function.** OLZ had little overall effect on insulin response to graded hyperglycemia (Fig. 6A), although post-drug insulin response was higher at first glycemic target (P = 0.005). RIS treatment resulted in greater circulating insulin during STEP tests, which was significantly higher than the pre-drug response at the first two glucose plateaus (P = 0.015 and P = 0.007, respectively). Insulin response was unaffected by placebo.

Changes in insulin sensitivity and adiposity should elicit a compensatory upregulation of β-cell sensitivity to glucose. However, despite SIClamp reduction, treatment with OLZ had no measurable effect on insulin response to graded hyperglycemia (pre-drug: 1.24 ± 0.15, post-drug: 1.07 ± 0.25 μU·ml⁻¹/[mg/dl]; P = 0.58; Fig. 6B), with 8 of
10 dogs actually demonstrating a paradoxical decline in insulin response after OLZ. DI tended to decline with OLZ (pre-drug: 35.7 ± 4.2 vs. 24.8 ± 6.6; P = 0.22). Conversely, RIS-treated animals, faced with similar insulin resistance, upregulated β-cell function by 50% (pre-drug: 0.64 ± 0.11, post-drug: 0.97 ± 0.10 μU·ml⁻¹/[mg/dl]; P = 0.038; Fig. 6B), and DI remained constant (pre-drug: 19.8 ± 5.0, post-drug: 21.8 ± 2.7, P = 0.74). Insulin response to hyperglycemia was unaffected by placebo, consistent with no change in SIclamp. Likewise, no appreciable change in DI was observed with placebo treatment (pre-drug: 33.3 ± 7.9, post-drug: 37.9 ± 16.7; P = 0.80; Fig. 6B).

Diet-induced insulin resistance. We compared changes in pancreatic function during OLZ with those observed in a separate group of dogs with increased adiposity and resistance induced by increased fat in the diet but no treatment with atypical antipsychotics. Baseline (pre-fat feeding) body weight (27.5 ± 1.5 kg) and adiposity (total: 21.0 ± 5.2 cm³, visceral: 13.5 ± 3.1 cm³, and subcutaneous: 7.6 ± 2.2 cm³) as well as fasting insulin (14 ± 3 μU/ml) were similar to pretreatment values of all other experimental groups (P > 0.1 by ANOVA), while fasting glucose was lower than OLZ and RIS (ANOVA with post hoc analysis; P = 0.001 for both comparisons) but not placebo (P = 0.4).

Increased dietary fat (~8% daily calories from fat [FAT]) induced a marked truncal adiposity pattern very similar to that observed with OLZ. Fat feeding almost doubled total adipose tissue volume (21.0 ± 5.2 vs. 35.8 ± 7.3 cm³; P = 0.60 vs. changes after OLZ) and enlarged visceral (OLZ: +8.7 ± 0.9 cm³, FAT: +7.0 ± 3.5 cm³; P = 0.65 between groups) and subcutaneous truncal depots (OLZ: +9.8 ± 1.5 cm³; FAT: +7.8 ± 3.2 cm³; OLZ vs. FAT: P = 0.60). Consistent with matched adiposity, the decrement in SLclamp after fat feeding (~8.9 ± 4.1 dl·min⁻¹·kg⁻¹/[μU/ml]) was of similar magnitude to that induced by OLZ (OLZ vs. FAT: P = 0.63). Insulin resistance due to fat feeding was associated with an impressive threefold compensatory increase in β-cell sensitivity to glucose (Fig. 7A). This robust upregulation was observed in all animals, demonstrating an average increase in β-cell slope from 0.74 ± 0.21 to 2.18 ± 0.57 μU·ml⁻¹·mg⁻¹, dl⁻¹ (P = 0.01). This contrasts sharply with OLZ-treated dogs, in which no measurable change in β-cell sensitivity to glucose was observed (Fig. 6B). Remarkably, despite matched changes in adiposity and insulin sensitivity, the β-cell compensation for resistance seen with fat feeding (14.4 ± 2.4 vs. 32.7 ± 9.2; P = 0.053) was not observed during OLZ (35.7 ± 4.2 vs. 24.8 ± 6.6; P = 0.222; Fig. 7B). This disparate effect of treatment on DI was significant (P = 0.02).

DISCUSSION

Recently, the use of atypical antipsychotics has been clouded by reports of drug-associated development of pronounced weight gain and diabetes. Increased incidence of diabetes suggests these agents may also impair pancreatic function, as β-cell defects are considered requisite for pathogenesis of the disease. However, no prospective mechanistic studies to examine possible drug effects on β-cell function, glucose metabolism, and body composition in the absence of underlying psychiatric disease have been published to date. The present studies not only reveal differential effects of two atypical antipsychotics on adiposity and hepatic insulin resistance but also show for the first time a substantial effect of one antipsychotic to impair pancreatic β-cell function.

Differential changes in body weight observed in this dog study were consistent with observations in patients (rev. in 39). OLZ-treated animals exhibited greater (+5.9% of pre-drug weight) and more consistent weight gain than more modest (+3.9%) weight gain observed with RIS. Although drug-induced weight gain is often attributed to stimulatory effects of drug on food intake or appetite (20,40), in the current study, observed body weight changes are not explained solely by alterations in food intake. OLZ-induced weight gain may reflect increased consumption of calories, but such increases mirrored those observed in control animals (Fig. 2A and C), suggesting no additional effect of drugs beyond that of placebo-treated dogs. In contrast, modest weight gain induced by RIS over the entire treatment period occurred in the

FIG. 5. Effect of treatment on hepatic insulin sensitivity. Units of sensitivity are dl·min⁻¹·kg⁻¹/[μU/ml]. **P = 0.009 vs. pre-drug.
Moreover, the time courses of changes in body weight and food intake during both OLZ and RIS treatment were discordant. For both drugs, early weight loss was observed within 1 week of treatment despite increased (OLZ) or stable (RIS) consumption of calories, an observation not apparent in control animals. These data suggest that weight gain with antipsychotics reflects treatment effects on both caloric intake and energy expenditure (20,41). Further studies are required to determine the specific drug effects on the components of energy balance.

Measures of body weight alone may not reveal differential effects of agents on adiposity. Despite similar changes in weight between OLZ versus placebo and similar caloric intake between groups, MRI analyses revealed that OLZ-treated dogs developed substantial adiposity that greatly exceeded observed changes in control animals, indicating OLZ caused preferential deposition of energy into adipose tissue. Total trunk fat stores nearly doubled with OLZ, reflecting proportional increases in subcutaneous and visceral depots. RIS caused more modest increases. While the role of specific receptor subtypes (e.g., H1 receptors) have been implicated in antipsychotic-induced weight gain (42), the mechanism for this substantial difference in adiposity between tested medications remains to be determined.

Although published literature is replete with reports of weight gain with antipsychotic treatment, few studies have quantified adiposity. Eder et al. (19) report a small (16%) increase in adiposity, measured by bioelectric impedance, in schizophrenics on OLZ monotherapy for 8 weeks, although such methodology yields a questionable measure of adiposity (43). Moreover, this study used a minimal (3-day) washout period before OLZ treatment for the large number of subjects with prior treatment history with other antipsychotics. Induction of central obesity by atypical antipsychotics has also been inferred from cross-sectional studies (44,45) reporting waist-to-hip ratios. Clearly, the present studies are unique in that they provide the first accurate measures of adiposity during antipsychotic treatment and support the idea that adiposity may be increased considerably more than is reflected in body weight alone.

The present study is the first to prospectively examine the effects of OLZ and RIS on the discrete actions of insulin on $R_d$ and HGO in the absence of prevailing disease. Insulin stimulation of $R_d$ was unaffected by either drug or placebo. In sharp contrast, dogs treated with OLZ exhibited severe hepatic insulin resistance (75%). This differs with the more modest declines with RIS or placebo. While OLZ-induced hepatic insulin resistance may reflect a primary hepatic defect, other sites of action are also plausible. Given that visceral adipose depot was greatly enlarged by OLZ, and this depot is insulin resistant (46), it is possible that portal FFAs draining from visceral stores

![Graphical representation of data](image-url)
β-cells did not lose their ability to secrete insulin in response to hyperglycemia (Fig. 6A). Sowell et al. (25) examined the effects of OLZ and RIS on insulin secretion in healthy human subjects. After 2 weeks of drug treatment, subjects exhibited weight gain and fasting hyperinsulinemia. We report similar effects of 4–6 weeks of treatment in healthy dogs. The Sowell studies reported no change in the insulin response to hyperglycemia with drug treatment, similar to the findings reported herein. However, those studies did not assess whether the observed β-cell response after drugs was appropriate for the prevailing degree of insulin sensitivity or adiposity. Here, we compared the β-cell response to OLZ-induced adiposity and insulin resistance with that of an independent group of animals that developed a similar degree of obesity induced not by OLZ but by consuming a fat-supplemented diet. These results, illustrated in Figs. 6 and 7, are dramatic. Although sequential steps of hyperglycemia elicited an insulin secretory response after OLZ treatment not different from pre-drug response, it is clear by comparison with fat-fed but unmedicated animals that the insulin response after OLZ was severely blunted. This functional impairment of pancreatic β-cells by OLZ was reflected in the ΔDI (Fig. 7B). The effect of fat feeding versus OLZ on β-cell compensation (ΔDI) was markedly different (fat feeding: positive 18.3 ± 7.3, OLZ: negative 10.9 ± 8.3; \( P = 0.02 \)). That secretion remained constant in OLZ-treated animals in the face of resistance indicates that OLZ completely prevented β-cell compensation. Indeed, failure to account for changes in insulin sensitivity in previous studies (25) may have masked the underlying β-cell compensatory dysfunction.

The mechanisms underlying impairment in β-cell compensation with OLZ are unclear. In vitro data suggest that OLZ does not directly impair β-cell insulin release (47), although treatment duration was brief (3 h). Since insulin secretion can be neurally regulated (48,49), the possibility exists that negative effects of OLZ on β-cell compensation may be mediated by its known central actions as a dopamine antagonist (50), although it is unknown whether a similar dopamine-mediated neural pancreatic axis exists for pancreatic endocrine secretion. It is also possible that impairment of β-cell compensation reflects derangement in the signaling pathway by which the pancreas senses insulin resistance and elicits the appropriate secretory response. The effect of OLZ to completely block β-cell upregulation suggests such signaling to the β-cell may be under neural control, involving parasympathetic and/or sympathetic pathways (48,49) or via known OLZ antagonism of muscarinic receptors (29). In fact, stimulation of vagal efferents emanating from the dorsal motor nucleus increases acetylcholine at the β-cell via muscarinic receptors, opening sodium channels and augmenting intracellular calcium influx during hyperglycemia, leading to hypersecretion of insulin. In addition, several dopamine receptor subtypes are also present in the dorsal vagal complex (51), and their antagonism by OLZ may contribute to impaired insulin secretion during treatment. Finally, OLZ-mediated norepinephrine increase in the prefrontal cortex (52) may cause diminished islet function by inhibiting adenylate cyclase (53).

In contrast to OLZ, RIS treatment did not block β-cell

were elevated in OLZ-treated dogs and hepatic insulin resistance developed.

The present studies revealed a dramatic impairment in β-cell compensation during OLZ. OLZ and RIS induced similar whole-body insulin resistance, yet only RIS responded with compensatory upregulation of β-cell sensitivity. Appropriate compensation was also observed in dogs fed an isocaloric moderate fat diet, attaining adiposity matched to that of OLZ. These results demonstrate a substantial defect of β-cell compensatory function induced by OLZ that was apparent only by comparing pancreatic function under similarly “stressed” conditions of insulin resistance.

It is worth noting that during antipsychotic treatment,
upregulation for insulin resistance (Fig. 6), as evidenced by a stable DI during treatment similar to that observed in placebo-treated dogs. However, while animals receiving placebo developed adiposity of similar magnitude to those receiving RIS, placebo treatment did not induce comparable resistance. Additional studies are warranted to test whether β-cell function during RIS is appropriate for prevailing adiposity and insulin resistance or whether these effects may depend on dose or duration of treatment.

The dog model was particularly well suited to investigate the effects of atypical antipsychotics on glucose metabolism. Twenty-five of 26 dogs tolerated the drugs with no apparent side effects, and sedation, a common problem in treated rodents, was limited to a single dog. No deterioration of hepatocellular integrity was noted, as evidenced by stable plasma liver enzymes alanine aminotransferase and aspartate aminotransferase (data not shown). Feeding behavior, similar to humans, allowed us to test direct effects of drugs on caloric intake; food intake and activity were well controlled, and drug administration was uneventful. While the duration of treatment in the present study is shorter than typically employed in treatment of psychiatric patients, it is striking that such substantial alterations in adiposity, hepatic sensitivity, and β-cell function were observed in that brief period.

In conclusion, this study is the first demonstration of the intrinsic effects of the most widely prescribed atypical antipsychotics on weight, adiposity, insulin sensitivity of the liver and peripheral tissues, and pancreatic β-cell function. There were clear differences in the effects of OLZ and RIS. OLZ caused significant weight gain and marked increases in total trunk adiposity, reflecting marked expansion of both visceral and subcutaneous adipose depots and severe hepatic insulin resistance. RIS had modest effects on adiposity that did not differ from the effects of placebo. Most importantly, the present studies reveal a significant effect of OLZ to impair β-cell compensation for insulin resistance. OLZ completely blocked the compensatory response with obesity and resistance seen with fat feeding, whereas β-cell function during RIS appears intact. The mechanisms by which these actions of antipsychotics occur are not known, but these data suggest that drugs may impede possible neural regulation of β-cell compensation. Failure of β-cell compensation to atypical antipsychotics provides a mechanic basis by which diabetes may develop in the vulnerable psychiatric population treated with these therapeutic agents. These results underscore the importance of examining drug effects in the absence of risk factors common among psychiatric patients. Further studies are needed to determine the mechanisms underlying differential metabolic sequelae of these agents, and the processes which may lead to development of diabetes in this population.

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