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Olanzapine (LY170053, 2-Methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5] Benzodiazepine), but Not the Novel Atypical Antipsychotic ST2472 (9-Piperazin-1-ylpyrrolo[2,1-*b*][1,3]benzothiazepine), Chronic Administration Induces Weight Gain, Hyperphagia, and Metabolic Dysregulation in Mice

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ABSTRACT

A mouse model of atypical antipsychotic-associated adverse effects was used to compare the liability to induce weight gain, food intake, and metabolic alterations after chronic olanzapine (OL; LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno-[2,3-*b*][1,5] benzodiazepine) and ST2472 (ST; 9-piperazin-1-ylpyrrolo[2,1-*b*][1,3]benzothiazepine) administration. By adding two equipotent doses (3 and 6 mg/kg) of either OL or ST to a high-sweet, high-fat (HS-HF) diet, mice were allowed to self-administer drugs up to 50 days. Body weight and food intake were evaluated daily. Locomotor activity was recorded over 48 h at two different time points. Dyslipidemia was measured by central visceral obesity. Blood serum levels of insulin (IN), glucose (Glu), triglycerides (TGs), nonesterified fatty acids (NEFAs), cholesterol (Ch), and ketone (Ke) bodies were quantified. OL treatment at 3 mg/kg enhanced body weight, whereas at the highest dose, the increase became evident only during the last 10 days of treatment. OL (3 mg/kg) increased HS-HF intake over time, whereas the highest dose reduced intake during the second 10 and final 10 days of administration. Both compounds induced nocturnal hypomotility at the highest dose. In contrast to ST, 3 mg/kg OL elevated serum levels of IN, Glu, TG, NEFA, Ch, and Ke, whereas 6 mg/kg OL elevated those of Glu, TG, and Ch. In contrast, ST did not affect weight gain, food intake, and metabolic markers. Given the similarities between OL-induced obesogenic effects and medical reports, this study further supports the view that ST may represent a new class of agents characterized by a low propensity to induce side effects with promising clinical safety.

One of the major concerns deriving from the clinical use of atypical antipsychotics (AAPs) is their liability to induce weight gain. Thus, in recent years, particular effort has been paid in the investigation of the health-threatening association between AAP medication and increased risk of developing obesity, diabetes mellitus, and lipid abnormalities (Wetterling, 2001). Obesity is an epidemic emergency in Western countries, and AAP-associated weight gain is a relevant clinical problem further exacerbated by the elevated comorbid metabolic alterations and fat deposition observed in the schizophrenic population (Bergman and Ader, 2005). Hence, a better understanding of mechanisms underlying the correlation between AAP therapy and liability to dysmetabolism may also contribute to exploration of the pathological basis linking brain, energy storage, and peripheral satiety-related signals to obesity.

Clozapine (CLZ) and olanzapine (OL; LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5] benzodiazepine) are both greatly implicated in the high incidence of dyslipidemia and weight gain (Baptista et al., 2004; Newcomer, 2005). Increased appetite and food intake is also re-

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ABBREVIATIONS: AAP, atypical antipsychotic; CLZ, clozapine; OL, olanzapine; LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5] benzodiazepine; ST, ST2472, 9-piperazin-1-ylpyrrolo[2,1-*b*][1,3]benzothiazepine; 5-HT, 5-hydroxytryptamine; HS-HF, high-sweet, high fat; IN, insulin; Glu, glucose; TG, triglyceride; Ch, cholesterol; NEFA, nonesterified fatty acid; Ke, ketone; ANOVA, analysis of variance; BW, body weight; L:D, light-dark; SREBP, sterol regulatory element-binding protein; HSD, honest significant differences; NA, noradrenaline.

ported in patients taking AAPs (Casey and Zorn, 2001). This clinical picture received confirmation in various models of male and female rodents (Goudie et al., 2002; Cooper et al., 2005; Cope et al., 2005). However, the observation that CLZ-induced weight gain is inconsistent in rats (Cooper et al., 2008) and that, in comparison with human patients, the use of drugs such as ziprasidone and aripiprazole in rodents led to “false-positive” results, has questioned the predictive validity of these animal models, particularly when the weight gain is modeled alone (Kalinichev et al., 2005). Therefore, the incorporation of additional variables in the study of AAP-associated metabolic effects has been recently recommended (for full discussion, see Cooper et al., 2008). In a recently described model of OL-associated adverse effects (Coccurello et al., 2006), we have found a picture of metabolic dysregulation where the coexistence of hyperglycemia, hyperinsulinemia, increased triglycerides, and adiposity pointed toward the possibility to develop a model of AAP-induced metabolic syndrome in female mice.

The goal of this study was to investigate the potential differences in the liability of AAPs to induce metabolic dysregulation by comparing OL with ST2472 (ST), a recently described novel antipsychotic compound (Stasi et al., 2008). ST2472 binds to various receptors. It has affinity (K_i) between 0.01 and 0.09 nM for $NA\alpha_{1B}$ receptors; between 0.1 and 0.9 nM for $5\text{-}HT_{2C}$, $NA\alpha_{1A}$, and α_{1B} receptors; between 1 and 9.9 nM for $D_{4.4}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, $5\text{-}HT_6$, $5\text{-}HT_7$, $NA\alpha_{1D}$, $NA\alpha_{2B}$, $NA\alpha_{2C}$, and H_1 receptors; between 10 and 99.9 nM for D_1, D_2, D_3, D_5, $5\text{-}HT_{5A}$, $NA\alpha_{2A}$, M_1, M_4, and M_5 receptors and NA transporter; between 100 and 999 nM for $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_{1D}$, $5\text{-}HT_3$, M_2, M_3, and H_2 receptors; >1 μM for adenosine A_1, A_{2A}, A_{2B}, A_3, $NA_{\beta 1}$, benzodiazepine$_c$, benzodiazepine$_p$, cannabinoid$_1$, cannabinoid$_2$, $GABA_A$, $GABA_B$, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, kainate, *N*-methyl-D-aspartate, Gly, NK_1, NK_2, NK_3, κ, μ, δ, NY, NT, ML_1, and ML_2 receptors, for L-type (DHP, diltiazem, and verapamil sites) and N-type Ca^{2+}, K^+_{ATP}, SK^+_{Ca}, K^+_V, Na^+, and Cl^- channels, and for 5-HT, GABA, DA, and choline transporter (Stasi et al., 2006). After being proven for the efficacy of ST as an antipsychotic and its very low propensity to elicit catalepsy, extrapyramidal and cardiovascular side effects, and hyperprolactinemia (Stasi et al., 2008), the present study assessed to what extent the chronic use of this compound, as compared with OL, could produce significant weight gain and AAP-associated metabolic dysregulation. OL and ST were demonstrated to be equipotent in test, predictive of antipsychotic effects such as the conditioned avoidance response ED_{50}: ST = 1.4 mg/kg and OL = 1.5 mg/kg (Stasi et al., 2008). Thus, we sought to characterize at potentially therapeutical doses the impact of a prolonged oral administration (up to 50 days) of OL and ST on body weight, food intake, and visceral fat accumulation. Additionally, the association between these second generation antipsychotics and disturbances in glucose, insulin, and lipid metabolism was explored. Following other reports (i.e., Albaugh et al., 2006) in which, to reduce stressogenic procedures, animals were allowed to self-administer drugs, OL and ST were mixed with a special high-sweet, high-fat (HS-HF) palatable wet mash diet. Finally, 48-h locomotor activity was recorded twice: between days 21 and 24 and between days 43 and 46.

Materials and Methods

Subjects. Mice, weighing 28 ± 0.5 g on arrival, were purchased at approximately 10 weeks of age from Charles River Italia (Calco, Italy). Animals were individually housed in a controlled 12-h light/dark cycle (7:00 AM–7:00 PM), under constant temperature and humidity. Mice had free access to standard laboratory dry food pellets (4RF21; Mucedola s.r.l., Milan, Italy) and water in their home cages (26.7 × 20.7 × 14 cm). Forty female CD-1 mice were used. Animal maintenance and experiments were conducted in accordance with the Council Directive of the European Community (86/EEC), Italian D.L. 116 (January 27, 1992), approved by the Company veterinarian and the Italian Government.

Pharmaceuticals. Olanzapine (Zyprexa-Velotab; Eli Lilly & Co., Indianapolis, IN) in oral, rapidly dissolving 10-mg tablets, was purchased on the market. Sigma-Tau’s Chemistry Department synthesized ST2472. Both compounds were dissolved in 0.5% carboxymethylcellulose sodium salt with 0.1% HCl and brought up to volume with sterile isotonic saline (0.9%). Vehicle solution consisted of 0.5% carboxymethylcellulose, 0.1% HCl, and sterile isotonic saline (0.9%). Mice were randomly assigned to each treatment group, and both OL and ST were orally self-administered by means of a wet mash diet (see below) up to 50 days of chronic treatment. Each group consisted of eight animals, and the following doses were used: 0 (vehicle), 3 (OL and ST), and 6 (OL and ST) mg/kg/day.

Diet and Habituation Procedure. Before starting OL and ST self-administration, mice were habituated for 7 days to an HS-HF palatable diet (HF-HS wet mash). During this phase, the wet mash was available only for 2 h/day to prevent significant weight gain before the start of the experimental phase. Wet mash was made up of a mixture of 1 part ground standard dry powdered food pellets in sweetened distilled water (HS 10% sucrose solution) and 30% fat [pure animal lard (HF)].

Body Weight and Food Intake. Body weight (grams) was measured daily throughout chronic OL and ST treatment. The intake of HS-HF diet was also measured daily for each animal from day 2 up to the day on which mice were sacrificed, namely, 1 day after the end of treatment (day 51). HS-HF intake was calculated as the difference between the weight of wet mash just before the meal presentation and remaining food collected the following day.

Locomotor Activity. Locomotor activity was recorded for 48 h at two specific time points, as follows: between days 21 and 24 and then between days 43 and 46. Home cage (35.5 × 23.5 × 19 h cm) locomotor activity scores of individually housed mice were sampled every 20 min in a soundproof environment by the use of an infrared photobeam frame system (Activity Scope apparatus; NewBehavior AG, Zurich, Switzerland). Recording sessions always started immediately before lights out (7:00 PM).

Biochemical Analysis. Blood samples were taken by trunk bleeding and separated by centrifugation (at 4000 rpm for 30 min, corresponding to 1255*g*) to obtain the serum required for determination of IN, Glu, TG, Ch, NEFA, and Ke. Biochemical analyses for Glu, TG, Ch, NEFA, and ketone bodies were performed by the AutoAnalyzer Cobas Mira (Roche Applied Science, Indianapolis, IN). Insulin was measured by radioimmunoassay using rat insulin standards, which showed 100% cross-reaction with mouse insulin (Biotrack RPA-547; GE Healthcare, Chalfont St. Giles, UK).

Periuterine Fat Mass Quantification. At least 6 h after removal of food pellets (on day 51), mice were sacrificed by decapitation. Then periuterine fat pads were surgically removed and weighed (weight approximated to 0.001 g).

Data Analysis. Body weight was analyzed by two-way ANOVA with treatment (five doses) and six periods (one time point for baseline and five time points of 10 days each for treatment) as within-subject variable. Repeated-measures ANOVA was used to analyze the amount of HS-HF wet mash intake consumed by the experimental groups for each of the five 10-day periods of chronic administration. Two-way ANOVA was used to compare the same numbers of locomotor samplings (20-min sampling of activity counts) during 48 h of continuous recording (24-h light/24-h dark phases) during days 21 to 24 and 43 to 46, respectively. Periuterine fat masses and IN, Glu, TG, Ch, NEFA, and Ke serum levels were all analyzed by one-way ANOVA. All post hoc analyses, when allowed, were carried out by Tukey HSD test.

Results

Body Weight. Body weight (BW) values are reported for each day of observation, from days −4 to 0 (baseline period) up to day 50 of treatment. Repeated-measures ANOVA was performed taking into account BW development across time, including six time points of distribution, as follows: one time point for baseline (5 days) and five time points of treatment of 10 days each (five 10-day periods), with treatment (five groups) and time (six time points) as factors. Statistical analysis evidenced a general increase of body weight due to the chronic treatment: both a significant main effect of treatment ($F_{4,20} = 4.59$; $p < 0.001$) and a significant treatment × time interaction ($F_{20,175} = 7.75$; $p < 0.001$) were found. Post hoc analysis revealed that no differences among groups occurred either during baseline (days −4 to 0) or throughout the first 10 days (days 1–10) of treatment, whatever the drug, compared with controls. Post hoc analysis evidenced the progressive enhancement of body weight, with both doses of OL inducing a significant increase in body weight. As illustrated (Fig. 1), OL (3 mg/kg) induced a significant BW increase that started during the second 10 days of treatment and remained significantly higher than other groups for the duration of treatment (Tukey HSD test, $p < 0.05$). A significant weight gain was also evidenced for the OL-administered group at 6 mg/kg during the last 10 days of treatment (days 41–50; Tukey HSD, $p < 0.05$). In contrast, ST treatment did not affect BW during the course of the 50-day treatment.

HS-HF Food Intake. The analysis on HS-HF food intake for five different time points of 10 days each (five 10-day periods) revealed a significant main effect of the treatment ($F_{4,16} = 200.33$; $p < 0.001$) and a significant treatment × time interaction ($F_{4,16} = 23.35$; $p < 0.001$). Post hoc analysis further revealed that the increase of HF-HS wet mash intake was attributable to the lowest doses of OL administered, which significantly enhanced HS-HF wet mash intake from the second 10 up to the last 10 days of treatment (Fig. 2; Tukey HSD test, $p < 0.05$). On the contrary, at the highest dose, OL significantly decreased food intake between days 11 and 20 and between days 41 and 50 (second and fifth 10-day periods, respectively). As illustrated (Fig. 2), in contrast to OL-mediated effects, ST treatment never affected food intake, whatever the dose administered (Tukey HSD, N.S.).

Locomotor Activity. Two-way repeated measures ANOVA with treatment (five levels) and activity, with time points (days 21–24 and 41–43) and day phase [light-dark (L:D)] as factors, was run on a 48-h cumulative activity count sampled every 20 min. The analysis did not evidence a significant main effect of treatment ($F_{4,35} = 1.93$; N.S.), with only a significant L:D phase effect emerging ($F_{1,35} = 265.19$; $p < 0.001$). However, a significant time point × L:D phase interaction was evidenced ($F_{1,35} = 27.19$; $p < 0.001$). As illustrated (Fig. 3), post hoc comparison further revealed a significant decrease of locomotor activity that occurred for both OL and ST at the highest dose tested during the dark phase of the second time point (Tukey's HSD test, $p < 0.05$).

Periuterine Fat Mass. Periuterine adipose depots were evaluated by one-way ANOVA analysis with treatment (groups) and fat masses as main factors. Fifty days of OL administration induced a statistically significant main effect of treatment ($F_{4,35} = 11.32$; $p < 0.001$), with major enhancement of the periuterine adipose masses found in animals chronically treated with OL at the lowest dose (3 mg/kg) administered (Fig. 4; Tukey HSD test, $p < 0.05$). In contrast, neither the highest dose of OL nor ST treatment (whatever the dose considered) significantly affected adipose mass accumulation.

Biochemical Analysis. One-way ANOVA was carried out for each metabolic parameter analyzed for treatment (groups), with IN, Glu, TG, Ch, NEFA, and Ke values as main factors. Because the value of one serum sample (a vehicle-treated animal) was not detectable, for the determination of the effects of drug treatment on IN concentration, 39, instead

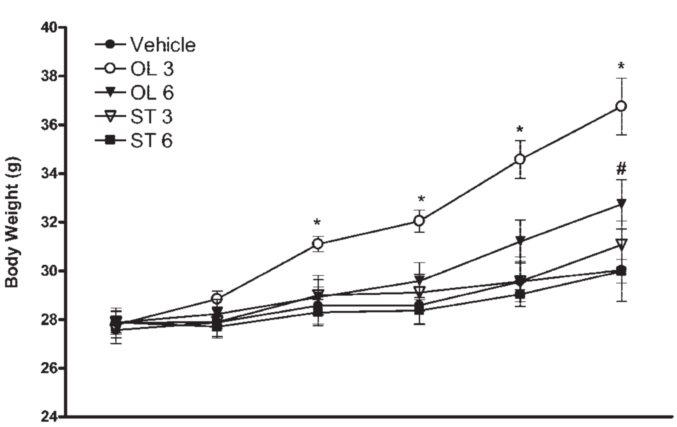

Fig. 1. Body weight (grams) was monitored daily over a 50-day chronic OL and ST self-administration. The figure reports body weight evolution and the significant dose-dependent weight gain induced by OL. In contrast, ST treatment did not affect weight gain. Data are represented as mean ± S.E.M. *, $p < 0.05$ compared with vehicle group. #, $p < 0.05$ compared with vehicle group (Tukey HSD test).

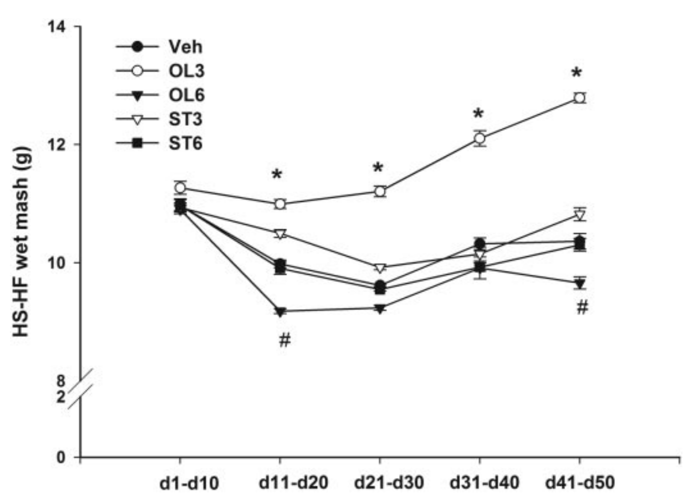

Fig. 2. HS-HF wet mash intake (grams) was monitored daily over a 50-day chronic OL and ST self-administration. The figure reports food intake evolution and the significant increase induced by 3 mg/kg OL. Intake was significantly reduced during the second and fifth 10-day periods by the highest dose (6 mg/kg) of OL. In contrast, ST treatment did not affect HS-HF wet mash intake. Data are represented as mean ± S.E.M. *, $p < 0.05$ compared with vehicle group. #, $p < 0.05$ compared with vehicle group (Tukey HSD test).

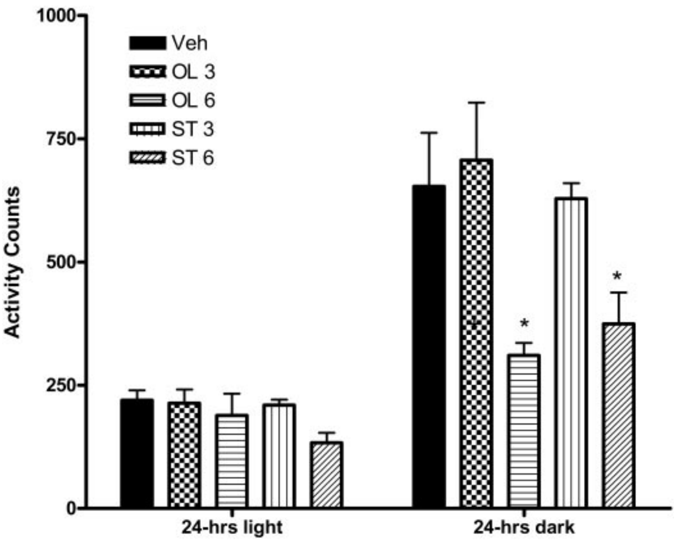

Fig. 3. Spontaneous locomotor activity was monitored twice, at two specific time points, between days 21 and 24 and 43 and 46. The figure depicts activity counts collected during the second time point. It shows that both compounds, at the highest doses, significantly reduced activity during dark phase. Data are represented as mean ± S.E.M. *, $p < 0.05$ compared with vehicle group (Tukey HSD test).

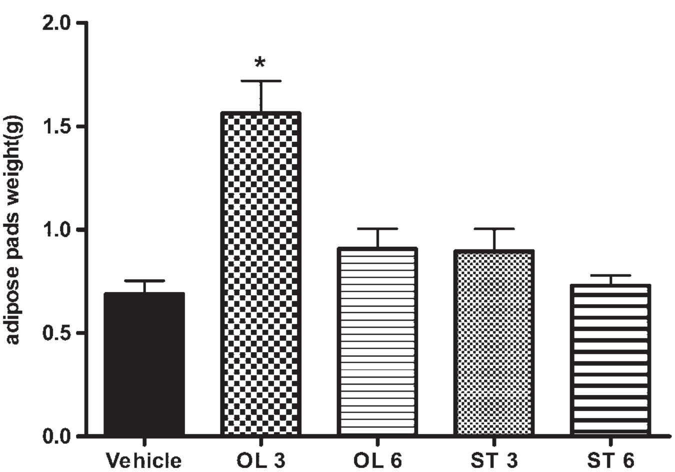

Fig. 4. Effects of chronic OL and ST self-administration on periuterine fat depots accumulation. The figure shows that after 50 days of chronic treatment, only OL (3 mg/kg) significantly enhanced adipose masses. Data are represented as mean ± S.E.M. *, $p < 0.05$ compared with vehicle group (Tukey HSD test).

of 40, samples were processed. The analysis performed on IN serum levels did show a significant main effect of the treatment ($F_{4,34} = 9.55$; $p < 0.001$). Hyperinsulinemia was found only for the lowest dose of OL-treated (3 mg/kg) mice (Tukey HSD test, $p < 0.05$; Fig. 5A). Glu serum levels were significantly higher for both doses of OL administered ($F_{4,35} = 25$; $p < 0.001$; Tukey HSD test, $p < 0.05$). OL-mediated hyperglycemia appeared in a dose-dependent fashion. In fact, a significant difference between OL-treated groups was also found, with the higher Glu serum levels expressed by the animals treated with the lowest dose tested (Tukey HSD, $p < 0.05$; Fig. 5B). TG serum levels were affected by chronic administration ($F_{4,35} = 19.55$; $p < 0.001$), and post hoc analysis further revealed a selective OL-dependent TG enhancement, with a statistically significant difference between OL doses (Tukey HSD, $p < 0.05$; Fig. 5C). Concerning NEFA serum levels, two samples (one vehicle-treated and one 6 mg/kg OL-treated animal) were not quantifiable; therefore, they were excluded from the analysis. Drug treatment significantly affected NEFA levels ($F_{4,33} = 11.04$; $p < 0.001$). Such enhancement of NEFA concentration was due to 3 mg/kg OL treatment (Tukey HSD, $p < 0.05$; Fig. 5D). Ch serum levels were significantly higher after chronic OL administration ($F_{4,35} = 17.11$; $p < 0.001$) at both doses tested. Post hoc analysis also evidenced a major increase of Ch serum concentrations with an OL dose-dependent effect (Tukey HSD, $p < 0.05$; Fig. 5E). Finally, Ke also was affected by OL treatment ($F_{4,35} = 16.74$; $p < 0.001$). OL (3 mg/kg) chronic treatment did induce a significant increase of Ke serum levels, as confirmed by post hoc analysis (Tukey HSD, $p < 0.05$; Fig. 5F). In contrast, none of the metabolic indexes analyzed were affected by ST treatment.

Discussion

ST2472 administration in the diet did not cause either incremental effects on body weight and food intake or detrimental consequences on adipose depots and metabolism. In contrast, important metabolic alterations were found in mice chronically administered olanzapine. In agreement with the clinical picture (Newcomer, 2005), the present results show higher serum levels of insulin, glucose, triglyceride, NEFA, cholesterol levels, and ketone bodies in 3 mg/kg OL-treated mice. Although insulin, NEFA, and ketone bodies were not affected by 6 mg/kg OL treatment, there was an increase of glucose, cholesterol, and triglyceride blood serum levels.

The development of hyperglycemia-associated metabolic dysregulation or diabetic ketoacidosis has been described in OL-medicated patients (Ragucci and Wells, 2001; Koller and Doraiswamy, 2002). Despite this, data on AAP-associated hyperglycemia in rodents are less consistent. Although acute or subchronic administration of CLZ has been associated with hyperglycemic responses in male (Murashita et al., 2007) and female (Tulipano et al., 2007) rats and in C57BL/6 mice (Dwyer and Donohoe, 2003), other studies have failed to evidence hyperglycemia in OL-administered female (Fell et al., 2007) and male (Cooper et al., 2007) rats. Together with the possible differences among species- and sex-dependent outcomes, atypical agents significantly interfere with glucose uptake (Dwyer et al., 1999). Indeed, a significant correlation between in vitro inhibition of glucose transporter and propensity to induce hyperglycemia after in vivo antipsychotics challenge has been established (Dwyer and Donohoe, 2003). OL infusion significantly reduced glucose transport rates in cultured adipocytes under insulin stimulation (Vestri et al., 2007). Therefore, the risk of triggering hyperglycemic events could be due to the ability of AAP agents to directly affect insulin metabolism and insulin-mediated glucose transport system in specific targets of insulin action. The long chronic regimen of OL treatment, together with the route of drug administration and the high-fat/high-sweet diet, could have played important roles in the onset of hyperinsulinemia and hyperglycemia observed in this study. Indeed, changing experimental conditions, such as routes of administration, diet, doses, and duration of the treatment, may produce quite contrasting effects (e.g., Minet-Ringuet et al., 2006).

In agreement with other animal studies (Cooper et al., 2005; Albaugh et al., 2006) and clinical reports (Newcomer,

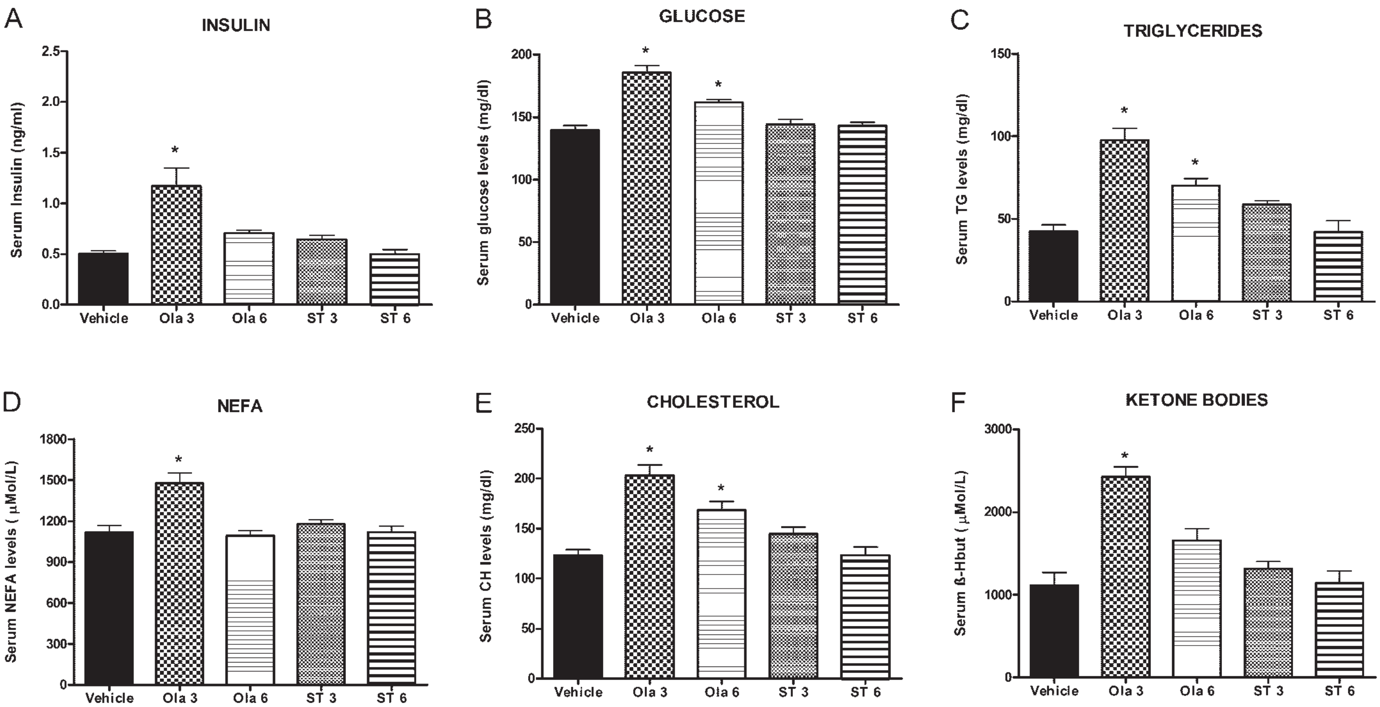

Fig. 5. The figure depicts the effects of OL and ST chronic self-administration on every metabolic marker measured. It shows the differential effects of OL treatment, with the significant impact on blood serum composition (A–F or B, C, and E for 3 and 6 mg/kg OL, respectively). In contrast, whatever the dose administered, ST had no impact on blood serum composition. Data are represented as mean ± S.E.M. *, $p < 0.05$ compared with vehicle group (Tukey HSD test).

2005), chronic self-administration of 3 mg/kg OL gradually increased weight gain from the second 10 days up to the end of treatment period (day 50). In contrast, 6 mg/kg-treated mice showed a rightward shift of the weight gain curve because the onset of significant body weight increase occurred 30 days later as compared with 3 mg/kg-treated animals. However, not only body weight gain was delayed, but in these animals, there was a significant diminution of food intake during the second and last 10-day periods of treatment (day 41–50). A possible reconciliation of this discrepancy may be found in the hypoactivity that, in contrast to days 21 and 24, was evidenced between days 43 and 46. Because of this, a reduction of energy expenditure in 6 mg/kg OL-treated mice may have favored an increase of body weight not sufficiently counteracted by the food intake decrease. Despite the fact that a slight aversion could have decreased food intake and slowed weight gain, the highest dose of OL negatively affected some metabolic indexes. Possible independent effects on weight gain and metabolic alterations, including fat depots, could be produced by OL administration. Indeed, periuterine fat depots were found drastically enlarged, clearly contributing to the final body weight gained by OL-treated animals. This fits with previous results describing enhanced levels of adiponectin or visceral adiposity after OL administration in rats, which may (Cooper et al., 2005; Albaugh et al., 2006) or may not (Cooper et al., 2007) be associated with the increase of body weight. In this regard, adipose tissue may be accounted as a preferential target of AAP-mediated diabetogenic effects. Indeed, convincing evidence of the detrimental effects of various AAPs on lipolysis (i.e., via a reduction of hormone-sensitive lipase and increased fatty acid synthase expression), which favor lipogenesis and adipocyte hypertrophy, have been presented (Minet-Ringuet et al., 2007; Vestri et al., 2007). Accordingly, a recent study (Yang et al., 2007) has identified in the overexpression of a transcription factor involved in the regulation of lipid homeostasis [sterol regulatory element-binding protein (SREBP)-1] a possible candidate mechanism to account for OL-mediated adipogenesis (fatty acid synthase and adiponectin overexpression) in preadipocytes. Interestingly, ziprasidone, which is almost neutral on weight gain and metabolism, displayed a very poor liability to elicit SREBP activation in human liver cells (Raeder et al., 2006). It would be interesting to test in the next future the ST-mediated liability to elicit SREBP expression in liver and in adipocyte cell lines.

Although these findings on adipocytes differentiation may help to clarify the contribution of peripheral factors (lipogenesis, glucose transport) in AAP-induced metabolic dysregulation, the central mechanisms by which these agents affect energy metabolism and induce obesity are still poorly understood. Many classes of the receptors targeted by these drugs are accountable for their propensity to induce weight gain. AAPs are nonselective drugs showing antagonistic activity at serotoninergic, noradrenergic, dopaminergic, and histaminergic brain receptors. In various degrees, OL displays a high-affinity ratio for serotonin (particularly 5-HT_{2A} and 5-HT_{2C}), histamine H1, muscarinic (M1–M4), dopamine (D_2 and $D_{2\text{-like}} > D_1$), and α-1 adrenergic receptors (Bymaster et al., 1996; Zhang and Bymaster, 1999). Among the rich sequelae of receptors targeted by AAP drugs, the possible involvement of serotonin 5-HT_{2C} (Tecott et al., 1995), histaminergic H1 (Masaki et al., 2004), and muscarinic receptors (Silvestre and Prous, 2005) in metabolic side effects has been hypothesized. The idea of looking at the positive correlation among receptor occupancy, weight gain, and comorbidity of diabetes mellitus may help to shed light on possible major receptor candidates.

In such framework, particular emphasis was given to the positive correlation between potency to bind the H1 receptor and risk of gaining weight, so that H1 receptor affinity (and, to a lesser degree, α-1 and 5-HT_{2C}) highly correlated with the greatest liability of AAP-induced weight gain (Wirshing et al., 1999; Kroeze et al., 2003). The view that the blockade of H1, 5-HT_{2C}, and muscarinic receptors may be involved in AAP-induced weight gain vulnerability could be evaluated in light of the present data. When compared with OL (K_d = 0.087 nM; Richelson and Souder, 2000), the ST potency at H1, 5-HT_{2A}, and 5-HT_6 was less pronounced (K_i = >1 and <10 nM; Stasi et al., 2006), although it was still relevant. Much greater affinity was showed at 5-HT_{2C} receptors (0.1 ≤ K_i = between 0.1 and 0.9 nM). Despite this, ST was not associated either with weight gain or with glucose and metabolic dysregulation. This seems to support the view of an increased diabetic risk when 5-HT_{2C} receptor blockade is associated with a pre-existing condition of type 2 diabetes mellitus in schizophrenic patients, whereas higher correlations between occupancies of either H1 or muscarinic receptors and weight gain were found regardless comorbid conditions (Matsui-Sakata et al., 2005). Therefore, either the pronounced antagonism at only one of these receptors or their interaction may produce weight gain and diabetes mellitus. Most AAPs are orexigenic, and the stimulation of hypothalamic adenosine monophosphate-activated protein kinase activity seems to underlie this effect, which could be offset by knocking out H1R (Kim et al., 2007). The observation that OL treatment down-regulated H1 expression in hypothalamic nuclei (Han et al., 2008) further supports the importance of H1 receptors in mediating AAP-dependent weight gain. The fact that the nonobesogenic compound ziprasidone also has relatively high H1 affinity (e.g., K_d = 4.6 nM; Richelson and Souder, 2000) apparently conflicts with this view. Hence, the interaction between H1 and other receptors involved in energy homeostasis (e.g., muscarinic-mediated transmission) should not be underestimated. By comparing the binding profile of ziprasidone, ST, and OL, it emerges that, contrary to the latter, both ziprasidone (Bymaster et al., 2003) and ST (Stasi et al., 2006) possess low affinity for muscarinic receptors. OL (but not ziprasidone) can produce peripheral anticholinergic effects such as inhibition of carbachol-stimulated insulin release from pancreatic β-cells (Johnson et al., 2005). Moreover, in comparison with equimolar doses of OL, chronic ST administration did not increase prolactinemia in rats (Stasi et al., 2008). Even though OL is not considered a prolactin-enhancing drug, the lack of ST effect on hyperprolactinemia makes this compound much closer to aripiprazole.

In summary, chronic intermediate doses of OL administration induce overweight, hyperglycemia, hyperinsulinemia, dyslipidemia, ketoacidosis, and visceral fat accumulation, i.e., a picture similar to the metabolic syndrome. It is worth noting that hypertension is constitutively associated to metabolic syndrome and, in contrast, to OL (Patil et al., 2006), and ST was previously shown to not affect blood pressure (Stasi et al., 2008). As a whole, the novel ST2472 turned up as a promising AAP compound, which, after 50 days of (diet-mixed) treatment, did not induce overweight, visceral fat accumulation, and metabolic dysregulation in mice. However, given the potential generation of false negatives and the inconsistency of AAP-induced metabolic dysregulation shown in animal models by compounds (i.e., CLZ) clearly associated with such side effects in clinic, the present results on ST must be taken with caution.

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