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ORIGINAL INVESTIGATION

Acute effects of orexigenic antipsychotic drugs on lipid and carbohydrate metabolism in rat

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Abstract
Objective This study aims to investigate whether orexigenic antipsychotic drugs may induce dyslipidemia and glucose disturbances in female rats through direct perturbation of metabolically active peripheral tissues, independent of prior weight gain.
Methods In the current study, we examined whether a single intraperitoneal injection of clozapine or olanzapine induced metabolic disturbances in adult female outbred Sprague–Dawley rats. Serum glucose and lipid parameters were measured during time-course experiments up to 48 h. Real-time quantitative PCR was used to measure specific transcriptional alterations in lipid and carbohydrate metabolism in adipose tissue depots or in the liver.
Results Our results demonstrated that acute administration of clozapine or olanzapine induced a rapid, robust elevation of free fatty acids and glucose in serum, followed by hepatic accumulation of lipids evident after 12–24 h. These metabolic disturbances were associated with biphasic patterns of gluconeogenic and lipid-related gene expression in the liver and in white adipose tissue depots.
Conclusion Our results support that clozapine and olanzapine are associated with primary effects on carbohydrate and lipid metabolism associated with

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transcriptional changes in metabolically active peripheral tissues prior to the development of drug-induced weight gain.

Keywords Antipsychotic · Animal model · Clozapine · Energy · Metabolism · Fatty acid · Lipid · Glucose · Diabetes · Obesity · Gene expression

Introduction

Treatment with the atypical antipsychotic drugs clozapine and olanzapine is associated with elevated risk of weight gain and other metabolic disturbances (Allison et al. 1999; Leucht et al. 2009; Lieberman et al. 2005; Rummel-Kluge et al. 2010). Among the mechanisms assumed to underlie these adverse effects are increased appetite and excess food intake, possibly mediated via histamine H1 and serotonin 5HT2C receptor antagonism in the hypothalamus (Kroeze et al. 2003; Nasrallah 2008) as well as alterations in hypothalamic fatty acid metabolism and neuropeptide expression (Ferno et al. 2011; Kim et al. 2007b; Reynolds and Kirk 2010). Altered brown adipose tissue thermogenesis has also been suggested to contribute to antipsychotic-induced weight gain (Ota et al. 2002; Stefanidis et al. 2009). However, recent findings indicate that some antipsychotic-induced metabolic adverse effects, such as dyslipidemia and glucose dysregulation, may occur independently of weight gain (Albaugh et al. 2011; Birkenaes et al. 2008; Nagamine 2008; Newcomer 2005; Procyshyn et al. 2007; Sacher et al. 2008; Tulipano et al. 2007). We have previously demonstrated that at the transcriptional level, several antipsychotic drugs upregulate lipogenesis controlled by the sterol regulatory element-binding protein (SREBP) transcription factors in cultured glial and liver cells (Ferno et al. 2005, 2006). This effect was proposed as a potentially relevant mechanism for drug-induced metabolic adverse effects (Ferno et al. 2005; Raeder et al. 2006) and has been replicated by others in adipocytes (Yang et al. 2007). The SREBP transcription factor exists as two isoforms: SREBP1 mainly controls the expression of fatty acid biosynthesis genes, whereas SREBP2 generally regulates genes involved in cholesterol biosynthesis and uptake (for review, see Shimano 2009).

To explore whether atypical antipsychotic drugs exert direct acute effects on metabolic processes, independent of weight gain, we recently investigated the effect of a single intraperitoneal injection of clozapine on hepatic gene expression and lipid levels in female Sprague–Dawley rats (Ferno et al. 2009). Clozapine administration rapidly induced hepatic upregulation of several SREBP-, PPAR- and LXR-controlled genes as well as hepatic accumulation of triglycerides, phospholipids, and cholesterol, within 48 h. Our results implied that administration of clozapine induces direct transcriptional effects in the liver, potentially facilitating hepatic lipid deposition, independent of food intake and weight gain. Alternatively, the accumulation of lipids in liver may indicate that clozapine exerts a direct effect on white adipose tissue (WAT). It is well-known that WAT is an important metabolic organ, not only in terms of lipid storage but also due to its endocrine role, as a source of hormones and cytokines essential to the regulation of metabolic homeostasis. We have recently proposed that initial defects in WAT expandability and/or function may trigger ectopic fat deposition in other metabolically relevant organs (Virtue and Vidal-Puig 2008, 2010). Thus, in the present study, we investigated the acute effects of clozapine and olanzapine on gene expression in WAT depots and in the liver in rat, as well as their effects on serum levels of glucose, lipids, and metabolically active hormones.

Material and methods

Animal studies

Adult female outbred Sprague–Dawley rats (Mollegaard, Denmark) with body weight from 260 to 275 g were housed three per cage. The light phase lasted from 0800 to 2000 hours, and ambient temperature was 22–24°C. Before drug exposure, food was limited to 15 g/day on average, through which the rats gained weight without developing obesity, and there was free access to water. The experiments were carried out in accordance with the Declaration of Helsinki and the guidelines of the Norwegian Committee for Experiments on Animals. The number of rats was the minimum necessary to obtain significant results and in agreement with the triple R spirit for reduction of the number of animals used. All procedures were performed so that suffering of the animals was minimized. We carried out two separate acute time-course experiments on two different occasions, with rats exposed to a single i.p. injection of either clozapine (25 mg/kg; Sigma, USA) or olanzapine (5 mg/kg; Toronto Research Chemicals, Canada), dissolved in 0.5 ml lactic acid (6 μg/ml), adjusted to pH=5.5. Control animals were injected with corresponding amounts of lactic acid adjusted to body weight. In relative terms, the dose of olanzapine was rather low as compared to the high dose of clozapine. We anticipated that this situation would decrease the size effect of olanzapine outcome parameters as compared to clozapine. In order to obtain enough statistical power, the number of animals exposed to olanzapine (n=9) was therefore larger than those exposed to clozapine (n=5). Following the i.p. injections, food was available ad libitum. In the clozapine experiment, treated and control rats were killed after 0.25, 0.5, 1, 3, 6, 12, 24, or 48 h. In the olanzapine experiment, both treated and control rats were

killed after 0 (untreated), 1, 3, 6, 12, or 24 h. Rats were killed between 0900 and 1200 hours, except for the 6-h time point where the animals were killed at 1400 hours. Biological replicate studies were carried out at selected time points for both clozapine (0.25, 0.5, 12, and 24 h) and olanzapine (3 and 6 h), with $n=5$ animals for each time point. Similar to the first experiment, food was available ad libitum after injection, and all rats were anesthetized one by one by isoflurane gas (Isoba vet; Schering-Plough, Denmark) and decapitated, and truncal blood was collected. Samples were taken from the liver median lobe and from mesenteric, ovarian, and retroperitoneal adipose tissues and freeze-clamped in liquid nitrogen before storage at −80°C.

Measurements of clozapine and olanzapine serum concentrations

Determination of serum clozapine and olanzapine levels was performed with an LC-MS/MS instrument (clozapine) (Waters, USA), or a UPLC-MS/MS instrument (Waters, USA), as previously described for clozapine (Ferno et al. 2009), using promazine (Sigma Aldrich, USA) as an internal standard. The lower quantification threshold of clozapine and olanzapine was 0.5 and 0.10 nM, respectively.

Hepatic triglyceride, cholesterol, phospholipid, and glycogen measurements

Levels of glucose, triglycerides, phospholipids, and cholesterol in the rat liver were measured enzymatically on the Hitachi 917 system (Roche Diagnostics, Germany) using the Gluco-quant glucose kit (Roche Diagnostics, Germany), the GPO-PAP triglyceride kit (Roche Diagnostics, Germany), the CHOD-PAP cholesterol kit (Roche Diagnostics, Germany), and the PAP 150 phospholipid kit (Diasys Diagnostics System, Germany), respectively, according to the manufacturers' instructions. Liver lipids were extracted by the method of Bligh & Dyer (Bligh and Dyer 1959), evaporated under N_2 and re-dissolved in isopropanol before analysis. Hepatic glycogen measurements were carried out using a colometric assay kit (cat. no. K-646-110, BioVision, USA), with standard curves as recommended by the manufacturer. Ten milligrams of liver tissue was homogenized in 200 μl distilled H_2O. Ten-microliter homogenate was then diluted 1:10, and measurements were carried out on 5-μl diluted homogenate.

Leptin, adiponectin, insulin, and glucagon measurements

Truncal vein blood was collected in serum tubes, left on ice for 30 min and centrifuged at 3,000 rpm for 10 min. Serum was transferred to pre-cooled Eppendorf tubes immediately after centrifugation and stored at −20°C. Serum insulin levels were measured. Serum leptin, adiponectin, insulin, and glucagon levels were all assessed by means of double-antibody radioimmunoassays provided by Linco Research (Linco Research, USA) (Caminos et al. 2005). All samples were assayed in duplicate within one assay, and the results were expressed in terms of the leptin, adiponectin, insulin, or glucagon standards. For leptin, the limit of assay sensitivity was 0.5 ng/ml with intra- and inter-assay variations at 2.0% and 5.7%, respectively. For adiponectin, the limit of assay sensitivity was 1 ng/ml, and the intra- and inter-assay variations were 4.1% and 6.6%, respectively. For insulin, the limit of assay sensitivity was 0.1 ng/ml, and the intra- and inter-assay variations were 1.4% and 9.1%, respectively. For glucagon, the limit of assay sensitivity was 20 pg/ml, and the intra- and inter-assay variations were 4.9% and 11.7%, respectively.

RNA extraction, cDNA synthesis, and gene expression analysis

Tissue was homogenized by adding pieces of liver or fat (about 20 and 100 mg each, respectively) to round-bottomed 2-ml Eppendorf tubes with 5-mm stainless steel beads (Qiagen, cat no 69989) and 600 μl RNA lysis buffer (Applied Biosystems, USA). Homogenization was carried out using a Beadmill TissueLyser (Qiagen). RNA extraction was performed on the ABI Prism™ 6100 Nucleic Acid PrepStation (Applied Biosystems) for the liver samples and the RNeasy Lipid Tissue Mini Kit (Qiagen) for the adipose tissues. Quantity and quality of the RNA were measured on the NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, USA). Real-time PCR was carried out on an ABI Prism 7900HT sequence detector system (Applied Biosystems) using cDNA synthesized by the TaqMan Reverse Transcription reagents (Applied Biosystems) as template. Each sample was run in triplicate as previously described (Ferno et al. 2005). Relative gene expression levels were determined by the comparative C_t method (Winer et al. 1999). Expression levels were normalized relative to three endogenous controls, acidic ribosomal phosphoprotein (*Arbp*/*P0*), β-actin (*Actb*), and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), with similar results. Mean fold change (drug vs. vehicle) was calculated at all time points in the experiment.

Statistical analysis

Statistical significance was determined by the unpaired Student's t test. The p values for the differences in normalized expression levels between antipsychotic- and vehicle-treated animals were calculated at all time points. All statistical tests were conducted with Excel 2003 (Microsoft Corporation, USA).

Results

Clozapine and olanzapine serum concentrations peak within the first hour post-intraperitoneal administration

Serum drug concentrations were measured at several time points in female rats exposed to a single intraperitoneal dose of clozapine (25 mg/kg) or olanzapine (5 mg/kg) in two separate time-course experiments, up to 48 and 24 h, respectively. The serum level (mean ± SD) of clozapine peaked at 15 min (4.4±0.3 μM), the first measured time point after the injection, and remained high at 1 h (3.7±0.2 μM) (Ferno et al. 2009). For olanzapine, the highest serum level was observed after 1 h (1.3±0.5 μM), which was the first time point in this experiment (Table 1). In agreement with the short half-life of antipsychotic drugs in rats, the initial peak levels were followed by a rapid decline for both drugs, and serum concentrations of clozapine (Ferno et al. 2009) and olanzapine (Table 1) were close to zero after 12 h.

Clozapine and olanzapine acutely increase serum lipid levels and induce hepatic lipid accumulation

To investigate whether clozapine and olanzapine could have direct effects on lipid-related parameters in serum, independent of weight gain, we measured free fatty acids, triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, phospholipids, leptin, and adiponectin after a single dose of the antipsychotic drugs. The most pronounced change was a rapid and persistent elevation of serum free fatty acids, evident as early as 15 min after clozapine injection, peaking (322±50%; mean ± SD, $P<0.001$) after 30 min (Fig. 1a). Olanzapine also induced a peak level (205 ±74%, $P<0.01$) of free fatty acids within 1 h of its administration (Fig. 1b). Independent biological experiments confirmed the early increase in free fatty acids for both drugs (Supplementary Table 1).

Table 1 Olanzapine serum concentrations after single injection in female SD rats

Time	Concentration (nM)
1 h	1,333±448
3 h	536±119
6 h	149±59
12 h	5±3
24 h	0.5±0.4

Olanzapine serum concentration (nanomolars; mean ± SD) measured after a single intraperitoneal injection (5 mg/kg). Measurements were done at 1, 3, 6, 12, and 24 h after the olanzapine injection. The data are representative of $n=9$ animals

Similarly, a moderate, but statistically significant increase in serum triglycerides was observed at 1 h for both clozapine (151±34%, $P<0.05$) and olanzapine (140±34%, $P<0.05$) relative to vehicle-treated rats, followed by a trend toward reduced levels at later time points (Table 2). With respect to total, HDL, and LDL cholesterol, there was a statistically significant reduction or trend toward reduced cholesterol levels during the first 3 h for both clozapine and olanzapine, followed by gradual return to the levels observed in vehicle-treated rats (Table 2). A similar tendency was observed for phospholipids (Table 2). To check for possible gender-specific effects, male Sprague–Dawley rats were included in the clozapine study (3 h only) (see separate column in Table 2). With the exception of triglyceride levels, changes in serum lipids corresponded well in male and female rats and were particularly evident for free fatty acids (Table 2). We found that changes in lipid parameters were only associated with minor, non-significant changes in serum levels of leptin and adiponectin in clozapine-exposed rats (Table 2). Since the magnitude of changes in the other serum parameters was most pronounced for clozapine, leptin, and adiponectin levels, these were not further investigated in olanzapine-exposed rats. Similar to what we previously observed for clozapine (Ferno et al. 2009), olanzapine induced a statistically significant accumulation of lipids in the liver at the later time points, most pronounced for triglycerides at 12 h (Supplementary Table 2).

Antipsychotic-induced changes in carbohydrate metabolism

In addition to their effects on lipid metabolism, antipsychotic agents are associated with increased risk of carbohydrate dysregulation in patients. In our study, single doses of both clozapine and olanzapine induced rapid and marked transient elevation of serum glucose levels, with maximum increase after 30 min (197±29%, $P<0.001$) for clozapine and after 1 h (115±9%, $P<0.001$) for olanzapine (Fig. 2a, b). The rapidly increased glucose levels were replicated in an independent biological experiment with clozapine (Supplementary Table 1). Glucagon and insulin are key regulators of carbohydrate homeostasis, with opposite effects on glucose metabolism. Interestingly, the clozapine-induced increase in glucose levels was associated with elevated serum glucagon levels in two independent experiments, whereas the effect on serum insulin levels was inconsistent (Table 2 and Supplementary Table 1), as could be anticipated from the non-standardized nutritional status. The relatively low dose of olanzapine did not induce any statistically significant effects on either serum insulin or glucagon levels (Table 2).

Based on the increased serum glucose and glucagon levels, we investigated transcriptional changes of key

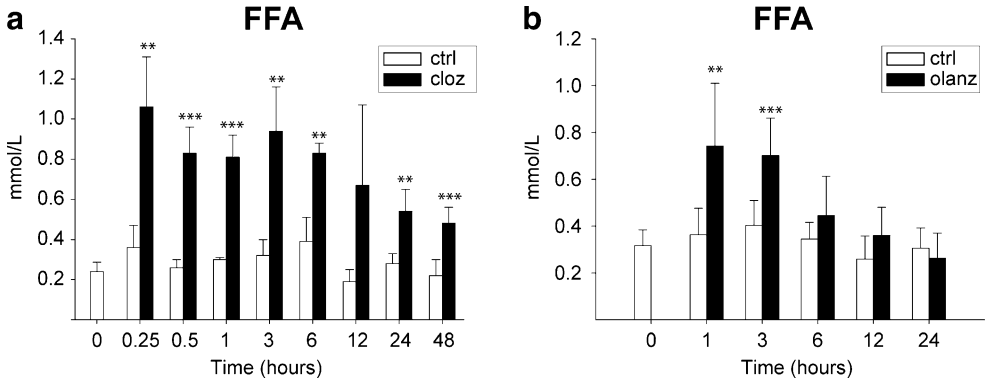

Fig. 1 The effect of clozapine and olanzapine on serum free fatty acid (*FFA*) levels. The serum levels of FFAs were measured in rats exposed to **a** clozapine (25 m/kg; *dark bars*) or vehicle (lactic acid; 6 μg/ml; *light bars*) or **b** olanzapine (5 m/kg; *dark bars*) or vehicle (lactic acid; 6 μg/ml; *light bars*). The levels were measured at 0.25, 0.5, 1, 3, 6, 12, 24, and 48 h in the clozapine experiment (n=5) and at 1, 3, 6, 12, and 24 h in the olanzapine experiment (n=9). **p<0.01; ***p<0.001

gluconeogenic genes in the liver. In clozapine-exposed rats, phosphoenolpyruvate carboxykinase (*Pepck*) was upregulated in parallel with peaking drug concentrations at early time points, with a maximum expression level after 1 h (2.69±0.30, P<0.01). The early upregulation was followed by a non-significant trend toward downregulation at later time points, as serum drug levels declined (Fig. 3a). Another important modulator of gluconeogenesis in the liver, the peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*Pgc1a*), also displayed maximum

Table 2 Serum levels of metabolic parameters following clozapine and olanzapine exposure to rats

	15 min	30 min	1 h	3 h	3 h (male)	6 h	12 h	24 h	48 h
Clozapine									
TG	94±16	123±20	151±34*	127±12	82±20	73±14	82±26	102±15	102±27
Total cholesterol	79±5*	85±10	75±10	85±07	83±12*	95±18	111±7	99±9	102±26
HDL cholesterol	82±6	88±9	80±8*	89±9	90±12	104±19	105±7	90±6	104±27
LDL cholesterol	120±25	104±8	89±17	89±13	80±33	106±26	155±35*	103±24	108±38
PL	81±9	88±12	75±14	91±7	87±7*	98±15	114±12	112±10	104±16
FFA	F	F	F	F	3.60±0.79***	F	F	F	F
Adiponectin	–	78±25	–	98±30	–	–	79±27	87±31	81±14
Leptin	62±47	88±53	96±27	163±42	103±39	158±40	94±32	67±12	69±14
Glucagon	128±102	130±24*	–	110±15	–	76±15	–	–	–
Insulin	11±8***	66±40	47±38	44±14	38±23*	43±21*	67±27	56±18	51±39
Olanzapine									
TG			140±34**	110±36		84±8	71±25	84±22	
Total cholesterol			97±17	85±9*		97±12	100±19	100±11	
HDL cholesterol			97±17	87±10		99±12	102±17	101±10	
LDL cholesterol			102±27	70±14*		97±20	87±22	104±21	
PL			96±12	88±9*		96±9	100±18	97±9	
Glucagon			114±28	131±122		–	–	–	
Insulin			121±42	106±46		98±32	121±48	113±50	

The serum levels (mean ± SEM) of metabolic parameters during time-course experiments measured in rats exposed to clozapine (25 mg/kg) at 0.25, 0.5, 1, 3, 6, 12, 24, and 48 h (n=5). The levels of TG, total cholesterol, HDL cholesterol, LDL cholesterol and PL, FFAs, and insulin were measured at all time points for both treatment, whereas glucagon was measured only at selected time points. Adiponectin and leptin were measured in the clozapine-exposed rats only. The levels are given relative to vehicle-exposed rats that were defined as 100%

TG triglycerides, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *PL* phospholipids, *FFAs* free fatty acids, *F* data available in Fig. 1a

*p<0.05; **p<0.01; ***p<0.001

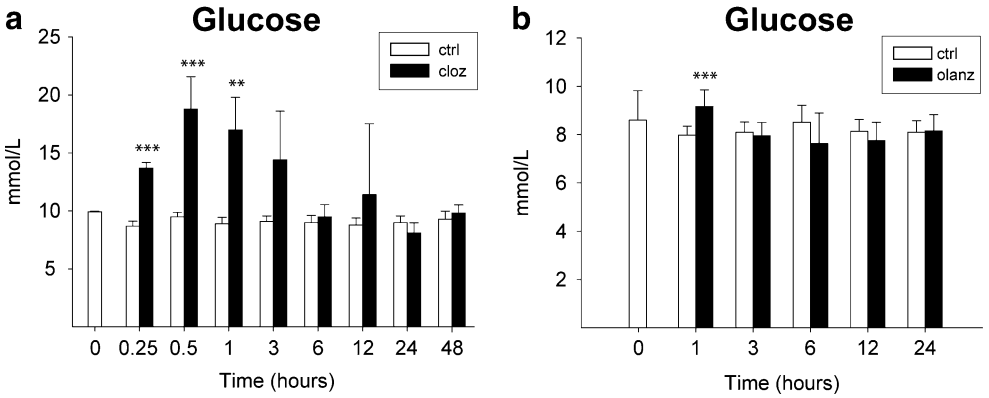

Fig. 2 The effect of clozapine and olanzapine on serum glucose level. The serum levels of glucose were measured in rats exposed to **a** clozapine (25 m/kg; *dark bars*) or vehicle (lactic acid; 6 μg/ml; *light bars*) or **b** olanzapine (5 m/kg; *dark bars*) or vehicle (lactic acid; 6 μg/ml; *light bars*). The levels were measured at 0.25, 0.5, 1, 3, 6, 12, 24, and 48 h in the clozapine experiment (n=5) and at 1, 3, 6, 12, and 24 h in the olanzapine experiment (n=9). **p<0.01; ***p<0.001

hepatic expression levels after 1 h (5.07±1.28, P<0.05) in clozapine-exposed rats (Fig. 3b). We also measured the expression levels of hepatic insulin receptor substrate 2 (*Irs2*), an essential component of the insulin-signaling pathway in liver typically downregulated in steatosis-associated hepatic insulin resistance (Shimomura et al. 2000). A striking upregulation of *Irs2* was evident after 30 min (6.51±0.87, P<0.01) in clozapine-exposed rats, with subsequent downregulation to the level in rats exposed to vehicle (Fig. 3c). A comparable, but less pronounced, transcriptional pattern was observed in olanzapine-exposed rats for all the abovementioned genes (Fig. 3d–f). In WAT, the expression of the insulin-responsive glucose transporter 4 (*Glut4*) was markedly reduced by both clozapine and olanzapine at the later time points in all adipose tissue depots (Table 3 and Supplementary Tables 3, 4, and 5).

Glycogen breakdown in the liver is another mechanism that could potentially contribute to the observed increase in serum glucose levels. Hepatic glycogen levels were not significantly altered at the early time points (data not shown), but a significant reduction was observed at 24 h for both clozapine (50±11%, P<0.05) and olanzapine (56±6%, P<0.05), relative to vehicle-treated rats. Hepatic expression levels of glycogen phosphorylase (*Pygl*), encoding the rate-limiting enzyme of glycogen catabolism in the liver, were examined in clozapine-treated animals, but no significant changes were found at any time point (data not shown).

Clozapine and olanzapine induce transcriptional changes of lipid-related genes in liver and in WAT depots

The marked elevation of serum free fatty acids and the accumulation of hepatic lipids observed after a single dose of clozapine or olanzapine were accompanied by immediate drug-induced transcriptional activation of Srebp-controlled lipogenic genes in the liver for both clozapine (Ferno et al. 2009) and olanzapine (Supplementary Table 6). The initial upregulation was followed by downregulation at later time points, corresponding with rapidly declining serum drug concentrations. For clozapine, such biphasic patterns of Srebp-controlled gene expression were also observed in the WAT depots (mesenteric fat, ovarian fat, and retroperitoneal fat), with most pronounced effects in mesenteric WAT. Analogous to the situation in liver, the mesenteric WAT displayed rapid upregulation for genes involved in fatty acid biosynthesis (e.g., *Srebp1c* and *Fasn*, an Srebp1 target gene) and adipogenesis (e.g., *Pparg*) and for Srebp2 target genes involved in cholesterol biosynthesis (e.g., *Hmgcr*) (Fig. 4 and Table 3). Similar effects were observed in ovarian WAT (Supplementary Table 3), whereas the initial effect in retroperitoneal WAT was opposite, with a trend toward downregulation at the early time points (Supplementary Table 4). In mesenteric WAT from olanzapine-exposed rats, we observed no statistically significant upregulation of lipid-related genes, although a statistically significant downregulation was observed for *Pparg* after 3 and 6 h (Supplementary Table 5). As an indication of drug-induced increase of lipogenesis at early time points, hepatic expression of the cholesterol esterification gene *Soat1* was highly upregulated, both by clozapine (Ferno et al. 2009) and by olanzapine (Supplementary Table 6). Clozapine also induced a consistent, rapid upregulation of *Soat1* in all WAT depots (Table 3 and Supplementary Table 3 and 4). A comparable effect on *Soat1* was induced by olanzapine in mesenteric WAT (Supplementary Table 5).

Immediate drug-induced effects on lipolytic activity in adipose tissues could be of relevance for the elevated serum lipid levels. We therefore measured the expression levels of several lipase-encoding genes, including hormone-sensitive

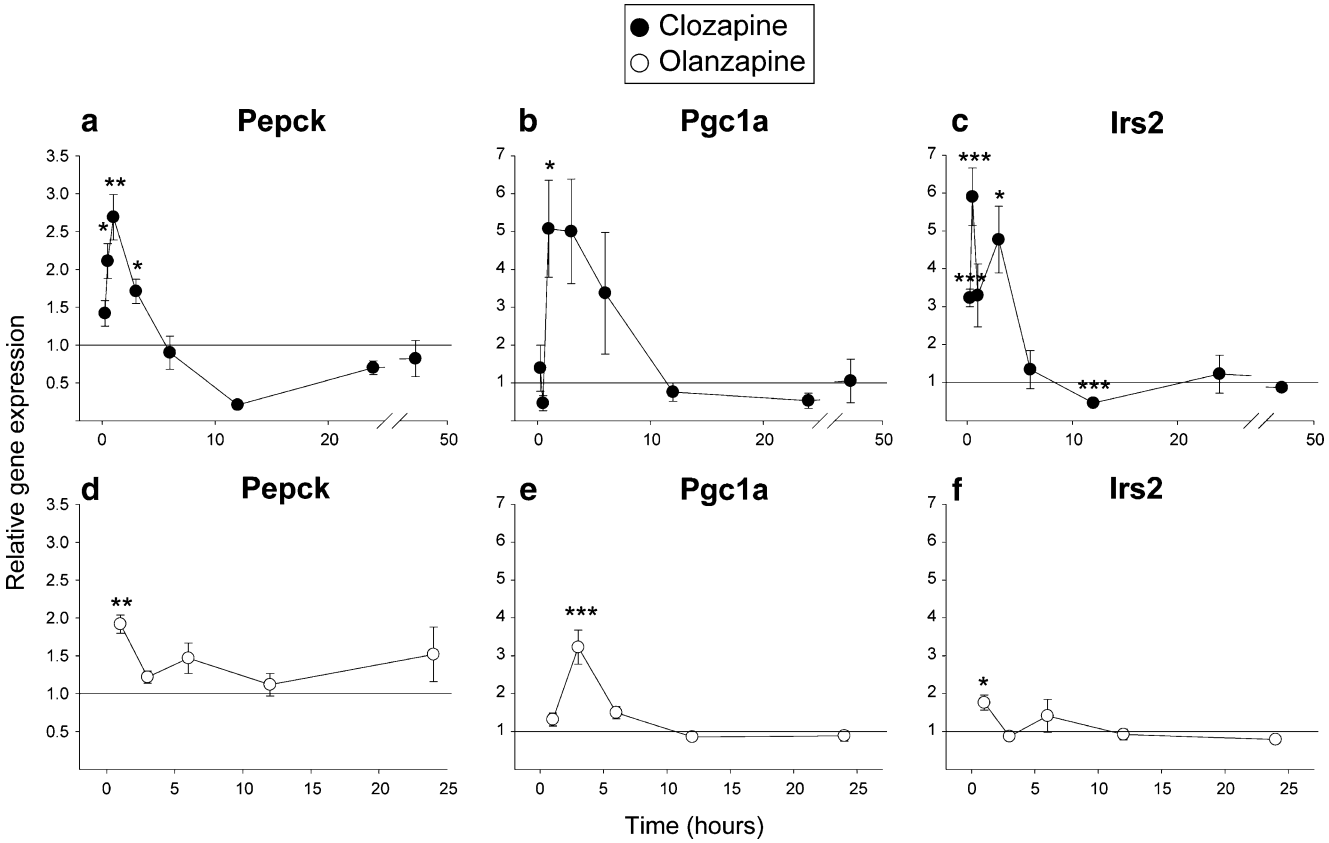

Fig. 3 The effect of clozapine and olanzapine on genes involved in gluconeogenesis and insulin signaling. Relative gene expression of **a** *Pepck*, **b** *Pgc1a*, and **c** *Irs2* in the liver from rats exposed to clozapine (25 m/kg) and **d** *Pepck*, **e** *Pgc1a*, and **f** *Irs2* in the liver from rats exposed to olanzapine (5 mg/kg), measured as fold change (mean ± SEM) relative to vehicle-exposed rats. The expression levels were normalized relative to the expression of the ribosomal gene P0. The relative levels were measured at 0.25, 0.5, 1, 3, 6, 12, 24, and 48 h in the clozapine experiment (n=5) and at 1, 3, 6, 12, and 24 h in the olanzapine experiment (n=9). *p<0.05; **p<0.01; ***p<0.001

lipase (Hsl; *Lipe*), lipoprotein lipase (*Lpl*), and adipose triglyceride lipase (Atgl; *Pnpla2*) in all three WAT depots. Indeed, in mesenteric WAT from clozapine-exposed rats, we found statistically significant upregulation of *Hsl* (1.43± 0.12, P=0.04) at 3 h, followed by downregulation at later time points (Table 3). Similar patterns were observed for *Lpl* and *Atgl*, although the initial upregulation did not reach statistical significance (Table 3). A biphasic pattern equivalent to that observed for lipogenic genes was also found in ovarian WAT from clozapine-exposed rats (Supplementary Table 3), whereas in retroperitoneal WAT we observed an opposite immediate effect for all measured lipase genes, with downregulation at early time points (Supplementary Table 4). Olanzapine did not affect the expression levels of any of the investigated lipase genes in mesenteric WAT (Supplementary Table 5). Taken together, these results indicated that the drug-induced initial effects on lipid-related gene expression were comparable across various metabolically active tissues, whereas the transcriptional changes at the later time points, occurring in parallel with declining drug serum concentrations, were less uniform.

The late phase transcriptional downregulation—a rebound effect

The initial upregulation observed for several genes in this study paralleled the serum drug concentration peaks and was evident across several metabolically active tissues, but most pronounced in the liver both for clozapine (Ferno et al. 2009) and for olanzapine (Supplementary Table 6). The subsequent downregulation corresponded with declining serum drug concentrations, with the degree of downregulation varying across different tissues and for the different genes within each tissue. To investigate whether the downregulation was a delayed direct effect of the drugs, or alternatively, a compensatory feedback mechanism in response to the early upregulation, we explored whether clozapine could also stimulate transcriptional activation in the late phase of transcriptional downregulation. A second injection of clozapine (25 mg/kg) was therefore administered 11 h after the first dose and the rats were killed 1 h later, i.e., 12 h after the initial injection. We found that the expression levels of *Fasn*, *Soat1*, *Pepck*, and *Irs2*, all of which displayed high initial

Table 3 The effect of clozapine on lipogenesis-related gene expression in mesenteric WAT

	15 min	30 min	1 h	3 h	6 h	12 h	24 h	48 h
Fabp4	0.99±0.07	0.98±0.19	1.55±0.12*	1.08±0.11	1.26±0.18	0.60±0.11	0.47±0.07**	0.96±0.21
Pparg	1.28±0.08	1.19±0.13	1.76±0.22*	1.03±0.11	0.51±0.09	0.90±0.25	0.76±0.07*	0.83±0.19
Dgat1	1.03±0.07	1.28±0.16	1.49±0.13	1.39±0.14	2.23±0.30*	0.50±0.08*	0.65±0.09*	1.04±0.24
Dgat2	1.34±0.27	1.23±0.16	1.78±0.23	1.03±0.12	0.92±0.17	0.34±0.05*	0.44±0.06**	0.64±0.17
Gpam	1.34±0.34	0.99±0.08	0.86±0.20	1.21±0.25	1.36±0.43	0.28±0.02	0.28±0.06*	0.50±0.16
Hmgcs1	1.52±0.39	1.22±0.15	1.16±0.22	0.75±0.20	0.93±0.18	0.70±0.09	0.57±0.11*	1.01±0.17
Ldlr	1.76±0.20*	0.91±0.27	1.95±0.17**	2.49±0.54	2.09±0.35*	1.20±0.14	0.97±0.17	0.91±0.24
Abca1	1.01±0.13	0.70±0.19	1.25±0.06	1.33±0.31	1.01±0.21	0.70±0.14	1.00±0.15	1.19±0.17
Acox1	1.35±0.26	1.28±0.11	1.58±0.21	1.63±0.22	1.55±0.13	0.61±0.16	0.45±0.03***	0.77±0.17
Soat	1.03±0.09	1.51±0.18*	2.08±0.27*	1.39±0.13*	1.75±0.31	1.05±0.20	1.57±0.30	1.05±0.09
Pnpla2	1.23±0.08	1.04±0.15	1.19±0.10	1.12±0.06	1.21±0.14	0.71±0.16	0.50±0.10**	1.01±0.25
Lipe	1.66±0.09	1.18±0.16	1.40±0.15	1.43±0.12*	1.48±0.11	0.70±0.10	0.53±0.11**	0.92±0.19
Lpl	0.87±0.08	0.91±0.28	1.54±0.21	1.51±0.19	1.56±0.19	0.80±0.20	0.50±0.04	0.53±0.15
Adipoq	1.00±0.12	1.21±0.15	1.20±0.13	1.24±0.20	1.05±0.08	0.66±0.14	0.40±0.04*	0.93±0.21
Glut4	1.86±0.57	1.06±0.14	0.65±0.18	1.09±0.10	1.72±0.45	0.14±0.02*	0.16±0.05*	0.35±0.11*

Relative expression in mesenteric WAT of several genes involved in lipid homeostasis, measured as fold change (mean ± SEM) in clozapine- (25 mg/kg) relative to vehicle-exposed rats. Genes involved in adipogenesis (*Fabp4* and *Pparg*), lipid biosynthesis and transport (*Dgat1*, *Dgat2*, *Gpam*, *Hmgcs1*, *Ldlr*, and *Abca1*), fatty acid β-oxidation (*Acox1*), cholesterol esterification (*Soat1*), lipolysis (*Pnpla2*, *Lipe*, and *Lpl*), adipokine production (*Adipoq*), and glucose transport (*Glut4*) were examined. The expression levels were normalized relative to the expression of the ribosomal gene *P0*. The relative levels were measured at 0.25, 0.5, 1, 3, 6, 12, 24, and 48 h with each data is representative of $n=5$ animals for each gene at each condition at each time point

*$p<0.05$; **$p<0.01$; ***$p<0.001$

fold change, were significantly upregulated or demonstrated a trend toward upregulation 1 h after the second dose of clozapine administered during the rebound phase, although to a lesser extent than by the first dose (Fig. 5).

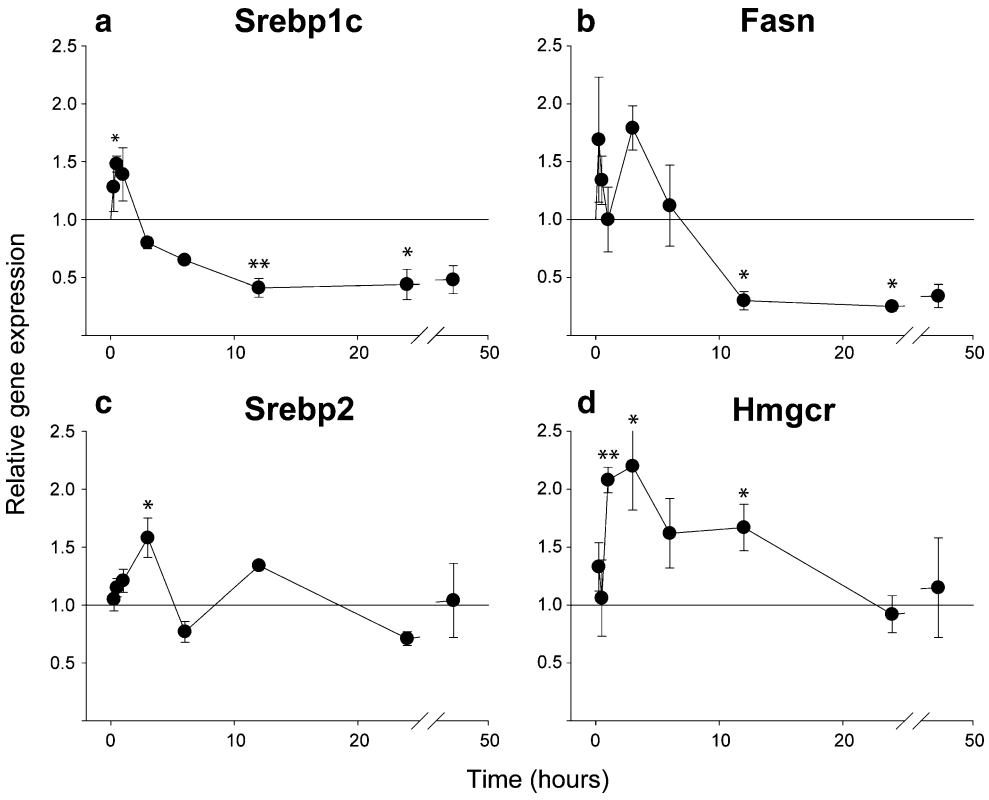

Fig. 4 The effect of clozapine on lipogenic genes in mesenteric WAT. Relative gene expression in mesenteric fat of **a** *Srebp1c*, **b** *Fasn*, **c** *Srebp2*, and **d** *Hmgcr*, measured as fold change (mean ± SEM) in clozapine- (25 m/kg) relative to vehicle-exposed rats. The expression levels were normalized relative to the expression of the ribosomal gene P0. The relative levels were measured at 0.25, 0.5, 1, 3, 6, 12, 24, and 48 h, with each data point representative of $n=5$ animals. *$p<0.05$; **$p<0.01$

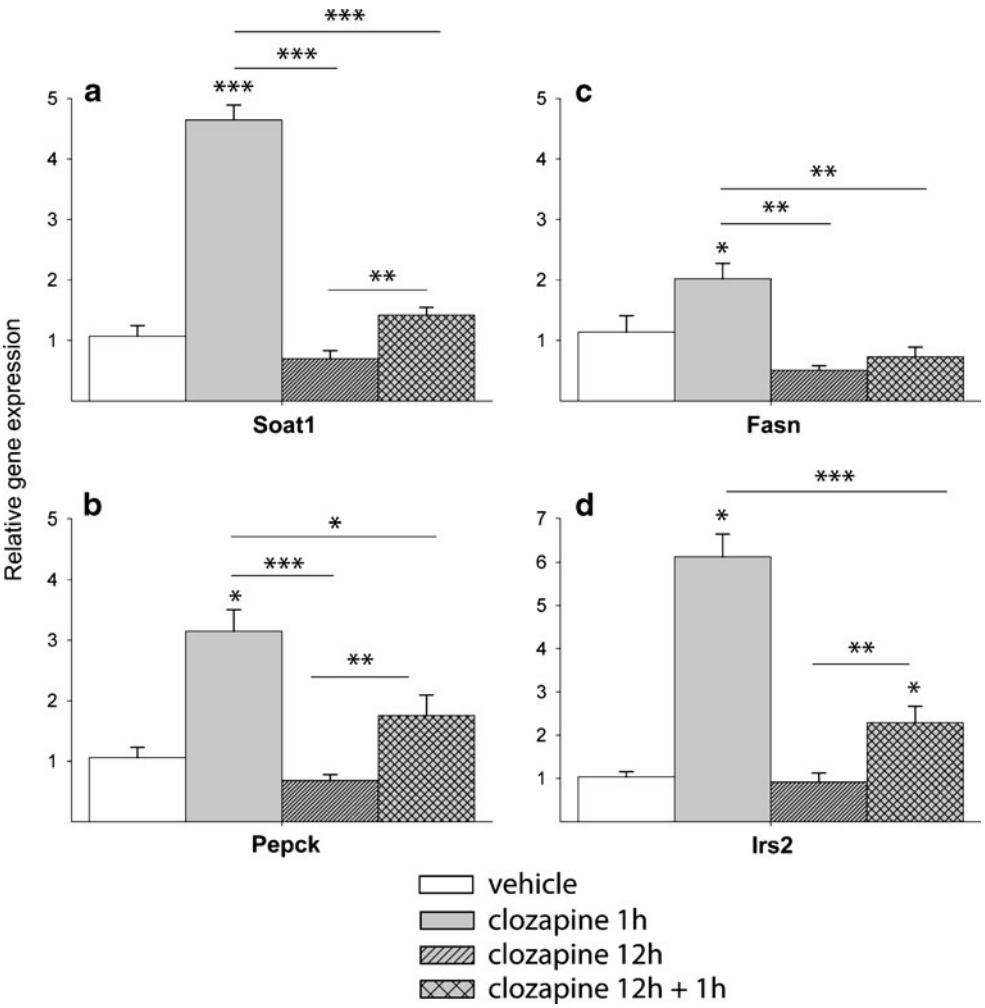

Fig. 5 Transcriptional effects in liver of subsequent injections with clozapine. Relative expression levels of **a** *Soat1*, **b** *Fasn*, **c** *Pepck*, and **d** *Irs2*, following two subsequent injections, containing either vehicle (lactic acid; 6 μg/ml) or clozapine (25 mg/kg) in various combinations. The injections were administered 12 and 1 h before killing, in the following combinations: Ctrl (vehicle 12 h/vehicle 1 h), clozapine 1 h (vehicle 12 h/clozapine 1 h), clozapine 12 h (clozapine 12 h/vehicle 1 h), and clozapine 12+1 h (clozapine 12 h/clozapine 1 h). The expression levels were normalized relative to the expression of the ribosomal gene P0. Each data point representative of $n=5$ animals. *$p<0.05$; **$p<0.01$; ***$p<0.001$

Discussion

In this study, we demonstrated that administration of a single dose of clozapine or olanzapine to non-fasted female rats rapidly increased serum levels of glucose, glucagon, and free fatty acids and led to accumulation of hepatic lipids at late time points. These metabolic changes were associated with a biphasic pattern of lipid-related gene expression in both liver and WAT depots, generally displaying a rapid upregulation and, in parallel with declining drug serum concentrations, a subsequent normalization or downregulation. Genes involved in gluconeogenesis also displayed marked initial upregulation, coinciding with increased levels of serum glucagon but not insulin.

Antipsychotic-induced elevation of serum free fatty acids and associated lipid-related gene expression

One plausible mechanism for the rapid elevation of serum free fatty acids is instant, lipase-mediated degradation of triglycerides in WAT. This is supported by the rapidly increased expression of lipase genes, such as *Hsl*, *Lpl*, and *Atgl*, both in mesenteric and ovarian WAT. Although it is unlikely that rapid effects on metabolic serum parameters are mediated via transcriptional events, the observed mRNA changes may reflect initial alterations at the protein and enzymatic activity level. Instant lipolytic activity in WAT with subsequent free fatty acid elevation in serum may involve a stress-like increase in sympathetic nervous activity and catecholamine release (Bartness and Song 2007; Romijn and Fliers 2005). In this sense, it is well established that both clozapine and olanzapine block the antilipolytic α2-adrenoceptors (Langin 2006; Roth et al. 2004).

Clozapine and olanzapine elevate serum glucose and glucagon and markedly induce hepatic gluconeogenic gene expression

The rapid increase in serum glucose levels induced by clozapine and olanzapine and the parallel upregulation of the gluconeogenic genes *Pepck* and *Pgc1α* in the liver are consistent with recent findings of antipsychotic-induced hepatic glucose production in rats (Albaugh et al. 2011;

Boyda et al. 2010; Chintoh et al. 2009; Smith et al. 2008). In our study, these effects coincided with elevated serum glucagon levels rather than altered insulin levels, in line with findings of others that increased hepatic glucose production is mediated through a rise in serum glucagon levels (Smith et al. 2008; Smith et al. 2009). Glycogenolysis could also potentially contribute to elevated serum glucose, but in our experiments, hepatic glycogen levels were unaltered at the early time points, when serum glucose levels were peaking. Others have proposed that antipsychotic-induced hepatic glucose production results from suppressed insulin secretion from β-cells as well as from reduced hepatic insulin sensitivity (Chintoh et al. 2009). The rapid, marked increase in hepatic *Irs2* expression in our study is suggestive of intact hepatic insulin signaling at the early time points, indicating that drug-induced insulin resistance in the liver did not markedly contribute to the elevated serum glucose. However, it should be kept in mind that several pathological mechanisms may coincide, and our data do not exclude the possibility that primary drug-induced disturbances on lipid homeostasis may reduce hepatic insulin sensitivity beyond the initial stage. The reduced levels of hepatic glycogen observed at 24 h are compatible with such a scenario. Antipsychotic-induced hepatic insulin resistance has been suggested to be mediated via the hypothalamic–pituitary–adrenal axis, involving increased corticosteroids levels (Martins et al. 2010; Tulipano et al. 2007). In line with this, antipsychotic-induced glucose and corticosteroid responses have been suggested to be causally related (Assie et al. 2008). Corticosteroids are known to stimulate lipolysis in WAT (Duclos et al. 2005; Hauner and Pfeiffer 1989), and drug-induced elevation of corticosteroids may thus also be relevant for the observed antipsychotic-induced elevation of free fatty acids in our study.

Biphasic gene expression—direct effects followed by feedback mechanisms?

The biphasic gene expression pattern, with initial upregulation and subsequent downregulation, was consistent both in liver and in WAT depots. This pattern was particularly evident in clozapine-treated animals, possibly related to the relatively high clozapine dose chosen to challenge the system and to compensate for the rapid half-life of antipsychotic drugs in rats. The biphasic expression pattern paralleled serum drug concentrations, with transcriptional decrease at later time points likely aggravated by compensatory rebound effects occurring in response to the initial, drug-induced, non-physiological activation. A second dose of clozapine, administered during this rebound phase, induced a blunted but still robust transcriptional reactivation of genes involved in both lipid (*Soat1* and *Fasn*) and carbohydrate (*Pepck* and *Irs2*) metabolism, strongly suggesting that the late phase downregulation is indeed a feedback effect and not a direct effect of the drugs.

The early upregulation of Srepb-controlled gene expression confirms our finding from cell cultures of a direct antipsychotic drug effect on lipogenic gene expression. The subsequent downregulation of Srebp-controlled gene expression observed both in liver and in adipose tissue from clozapine-exposed rats appeared paradoxical in light of the concomitant hepatic lipid accumulation. However, in the liver, the reduced transcription may be explained by the abovementioned feedback mechanisms that are probably, at least in part, triggered by increased serum lipid load from the elevated serum free fatty acids. With respect to adipose tissue, reduced expression of lipogenic/adipogenic genes may in fact be compatible with hepatic lipid accumulation, since it indicates that WAT storage capacity is temporarily reduced, which may contribute to elevation of blood lipids and lipid accumulation in non-adipose tissues (Frayn 2002; Kim et al. 2007a; Lazar 2005; Martinez de Morentin et al. 2010; Virtue and Vidal-Puig 2008). Thus, the reduced lipogenic gene expression in WAT depots at late time points may represent an additional mechanism contributing to the sustained elevation of serum free fatty acids and accumulation of hepatic lipids in rat. The downregulation of the insulin-responsive *Srebp1c* and its target genes in WAT at the late time points suggests a state of reduced insulin sensitivity (Deng et al. 2007; Ranganathan et al. 2006). This is in agreement with the concomitant reduction of glycogen levels in the liver, indicative of hepatic glycogenolysis. A state of reduced insulin signaling in WAT is also supported by the downregulation of the insulin sensitivity marker *Glut4* (Shepherd and Kahn 1999).

In conclusion, we here demonstrate that acute administration of clozapine or olanzapine rapidly increases serum free fatty acids and glucose levels, associated with hepatic lipid accumulation and immediate, drug-induced transcriptional activation of lipid homeostasis genes in WAT depots and in the liver. The antipsychotic-induced elevation of serum glucose levels appears to be related to transcriptional activation of hepatic gluconeogenesis and to changes in serum glucagon rather than serum insulin levels. Clozapine administration elicits a more potent metabolic response than olanzapine, but this is likely attributable to the clozapine dose. The similar findings in clozapine- and olanzapine-treated rats demonstrate that these antipsychotic drugs are able to exert direct drug effects on peripheral metabolically active tissues, independent of increased food intake and weight gain, and that such effects may be relevant for the metabolic disturbances associated with atypical antipsychotic drugs.

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Conflict of interest The authors declare that there is no conflict of interest.

References

Albaugh VL, Judson JG, She P, Lang CH, Maresca KP, Joyal JL, Lynch CJ (2011) Olanzapine promotes fat accumulation in male rats by decreasing physical activity, repartitioning energy and increasing adipose tissue lipogenesis while impairing lipolysis. Mol Psychiatry 16:569–581

Allison DB, Mentore JL, Heo M, Chandler LP, Cappelleri JC, Infante MC, Weiden PJ (1999) Antipsychotic-induced weight gain: a comprehensive research synthesis. Am J Psychiat 156:1686–1696

Assie MB, Carilla-Durand E, Bardin L, Maraval M, Aliaga M, Malfetes N, Barbara M, Newman-Tancredi A (2008) The antipsychotics clozapine and olanzapine increase plasma glucose and corticosterone levels in rats: comparison with aripiprazole, ziprasidone, bifeprunox and F15063. Eur J Pharmacol 592:160–166

Bartness TJ, Song CK (2007) Brain-adipose tissue neural crosstalk. Physiol Behav 91:343–351

Birkenaes AB, Birkeland KI, Engh JA, Faerden A, Jonsdottir H, Ringen PA, Friis S, Opjordsmoen S, Andreassen OA (2008) Dyslipidemia independent of body mass in antipsychotic-treated patients under real-life conditions. J Clin Psychopharmacol 28:132–137

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Boyda HN, Tse L, Procyshyn RM, Wong D, Wu TK, Pang CC, Barr AM (2010) A parametric study of the acute effects of antipsychotic drugs on glucose sensitivity in an animal model. Prog Neuropsychopharmacol Biol Psychiatry 34:945–954

Caminos JE, Nogueiras R, Gallego R, Bravo S, Tovar S, Garcia-Caballero T, Casanueva FF, Dieguez C (2005) Expression and regulation of adiponectin and receptor in human and rat placenta. J Clin Endocrinol Metab 90:4276–4286

Chintoh AF, Mann SW, Lam L, Giacca A, Fletcher P, Nobrega J, Remington G (2009) Insulin resistance and secretion in vivo: effects of different antipsychotics in an animal model. Schizophr Res 108:127–133

Deng X, Yellaturu C, Cagen L, Wilcox HG, Park EA, Raghow R, Elam MB (2007) Expression of the rat sterol regulatory element-binding protein-1c gene in response to insulin is mediated by increased transactivating capacity of specificity protein 1 (Sp1). J Biol Chem 282:17517–17529

Duclos M, Bouchet M, Vettier A, Richard D (2005) Genetic differences in hypothalamic–pituitary–adrenal axis activity and food restriction-induced hyperactivity in three inbred strains of rats. J Neuroendocrinol 17:740–752

Ferno J, Raeder MB, Vik-Mo AO, Skrede S, Glambek M, Tronstad KJ, Breilid H, Lovlie R, Berge RK, Stansberg C, Steen VM (2005) Antipsychotic drugs activate SREBP-regulated expression of lipid biosynthetic genes in cultured human glioma cells: a novel mechanism of action? Pharmacogenomics J 5:298–304

Ferno J, Skrede S, Vik-Mo AO, Havik B, Steen VM (2006) Drug-induced activation of SREBP-controlled lipogenic gene expression in CNS-related cell lines: marked differences between various antipsychotic drugs. BMC Neurosci 7:69

Ferno J, Varela S, Skrede S, Vázquez MJ, Nogueiras R, Diéguez C, Vidal-Puig A, Steen VM, López M (2011) Olanzapine-induced hyperphagia and weight gain associate with orexigenic hypothalamic neuropeptide signaling without concomitant AMPK phosphorylation. PLoS ONE 6:e20571

Ferno J, Vik-Mo AO, Jassim G, Havik B, Berge K, Skrede S, Gudbrandsen OA, Waage J, Lunder N, Mork S, Berge RK, Jorgensen HA, Steen VM (2009) Acute clozapine exposure in vivo induces lipid accumulation and marked sequential changes in the expression of SREBP, PPAR, and LXR target genes in rat liver. Psychopharmacology (Berl) 203:73–84

Frayn KN (2002) Adipose tissue as a buffer for daily lipid flux. Diabetologia 45:1201–1210

Hauner H, Pfeiffer EF (1989) Regional differences in glucocorticoid action on rat adipose tissue metabolism. Horm Metab Res 21:581–582

Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T, Durand JL, Li H, Li G, Jelicks LA, Mehler MF, Hui DY, Deshaies Y, Shulman GI, Schwartz GJ, Scherer PE (2007a) Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest 117:2621–2637

Kim SF, Huang AS, Snowman AM, Teuscher C, Snyder SH (2007b) Antipsychotic drug-induced weight gain mediated by histamine H1 receptor-linked activation of hypothalamic AMP-kinase. PNAS 104:3456–3459

Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, Ernsberger P, Jayathilake K, Meltzer HY, Roth BL (2003) H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. Neuropsychopharmacology 28:519–526

Langin D (2006) Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. Pharmacological research: the official journal of the Italian Pharmacological Society 53:482–491

Lazar MA (2005) How obesity causes diabetes: not a tall tale. Science 307:373–375

Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM (2009) Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet 373:31–41

Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Keefe RS, Davis SM, Davis CE, Lebowitz BD, Severe J, Hsiao JK (2005) Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med 353:1209–1223

Martinez de Morentin PB, Varela L, Ferno J, Nogueiras R, Dieguez C, Lopez M (2010) Hypothalamic lipotoxicity and the metabolic syndrome. Biochim Biophys Acta 1801:350–361

Martins PJ, Haas M, Obici S (2010) Central nervous system delivery of the antipsychotic olanzapine induces hepatic insulin resistance. Diabetes 59:2418–2425

Nagamine T (2008) Olanzapine-induced elevation of serum triglyceride levels in a normal weight patient with schizophrenia. Intern Med 47:181–182

Nasrallah HA (2008) Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. Mol Psychiatry 13:27–35

Newcomer JW (2005) Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. CNS Drugs 19(Suppl 1):1–93

Ota M, Mori K, Nakashima A, Kaneko YS, Fujiwara K, Itoh M, Nagasaka A, Ota A (2002) Peripheral injection of risperidone, an atypical antipsychotic, alters the bodyweight gain of rats. Clin Exp Pharmacol Physiol 29:980–989

Procyshyn RM, Wasan KM, Thornton AE, Barr AM, Chen EY, Pomarol-Clotet E, Stip E, Williams R, Macewan GW, Birmingham CL, Honer WG (2007) Changes in serum lipids, independent of weight, are associated with changes in symptoms during long-term clozapine treatment. J Psychiatry Neurosci 32:331–338

Raeder MB, Ferno J, Vik-Mo AO, Steen VM (2006) SREBP activation by antipsychotic- and antidepressant-drugs in cultured human liver cells: relevance for metabolic side-effects? Mol Cell Biochem 289:167–173

Ranganathan G, Unal R, Pokrovskaya I, Yao-Borengasser A, Phanavanh B, Lecka-Czernik B, Rasouli N, Kern PA (2006) The lipogenic enzymes DGAT1, FAS, and LPL in adipose tissue: effects of obesity, insulin resistance, and TZD treatment. J Lipid Res 47:2444–2450

Reynolds GP, Kirk SL (2010) Metabolic side effects of antipsychotic drug treatment—pharmacological mechanisms. Pharmacol Ther 125:169–179

Romijn JA, Fliers E (2005) Sympathetic and parasympathetic innervation of adipose tissue: metabolic implications. Curr Opin Clin Nutr Metab Care 8:440–444

Roth BL, Sheffler DJ, Kroeze WK (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. Nature reviews. Drug discovery 3:353–359

Rummel-Kluge C, Komossa K, Schwarz S, Hunger H, Schmid F, Lobos CA, Kissling W, Davis JM, Leucht S (2010) Head-to-head comparisons of metabolic side effects of second generation antipsychotics in the treatment of schizophrenia: a systematic review and meta-analysis. Schizophr Res 123:225–233

Sacher J, Mossaheb N, Spindelegger C, Klein N, Geiss-Granadia T, Sauermann R, Lackner E, Joukhadar C, Muller M, Kasper S (2008) Effects of olanzapine and ziprasidone on glucose tolerance in healthy volunteers. Neuropsychopharmacology 33:1633–1641

Shepherd PR, Kahn BB (1999) Glucose transporters and insulin action—implications for insulin resistance and diabetes mellitus. N Engl J Med 341:248–257

Shimano H (2009) SREBPs: physiology and pathophysiology of the SREBP family. FEBS J 276:616–621

Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS, Goldstein JL (2000) Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. Mol Cell 6:77–86

Smith GC, Chaussade C, Vickers M, Jensen J, Shepherd PR (2008) Atypical antipsychotic drugs induce derangements in glucose homeostasis by acutely increasing glucagon secretion and hepatic glucose output in the rat. Diabetologia 51:2309–2317

Smith GC, Vickers MH, Cognard E, Shepherd PR (2009) Clozapine and quetiapine acutely reduce glucagon-like peptide-1 production and increase glucagon release in obese rats: implications for glucose metabolism and food choice behaviour. Schizophr Res 115:30–40

Stefanidis A, Verty AN, Allen AM, Owens NC, Cowley MA, Oldfield BJ (2009) The role of thermogenesis in antipsychotic drug-induced weight gain. Obesity 17:16–24

Tulipano G, Rizzetti C, Bianchi I, Fanzani A, Spano P, Cocchi D (2007) Clozapine-induced alteration of glucose homeostasis in the rat: the contribution of hypothalamic–pituitary–adrenal axis activation. Neuroendocrinology 85:61–70

Virtue S, Vidal-Puig A (2008) It's not how fat you are, it's what you do with it that counts. PLoS Biol 6:e237

Virtue S, Vidal-Puig A (2010) Adipose tissue expandability, lipotoxicity and the metabolic syndrome—an allostatic perspective. Biochim Biophys Acta 1801:338–349

Winer J, Jung CK, Shackel I, Williams PM (1999) Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. Anal Biochem 270:41–49

Yang LH, Chen TM, Yu ST, Chen YH (2007) Olanzapine induces SREBP-1-related adipogenesis in 3T3-L1 cells. Pharmacol Res 56:202–208