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Effects of second-generation antipsychotics on human subcutaneous adipose tissue metabolism

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ABSTRACT

Objective: Metabolic syndrome is prevalent in up to 50% of schizophrenia patients, which reduces their quality of life and their compliance with the treatment. It is unclear whether metabolic adverse effects of these agents are due to their direct effect on insulin-sensitive tissues or are secondary to increased adiposity. The study aimed to investigate the direct effects of the second-generation antipsychotics olanzapine and aripiprazole on human subcutaneous adipose tissue and isolated adipocyte metabolism.

Methods: Abdominal subcutaneous adipose tissue needle biopsies were taken from 72 healthy subjects (49 F/ 23 M; age: 19–78 yr; BMI: 20.0–35.6 kg/m^2). Isolated adipocytes or adipose tissue were respectively pre-incubated short- (30 min) and long-term (24 h, 72 h) with or without olanzapine (0.004 μM – 20 μM) and aripiprazole (0.002 μM – 100 μM). Pre-incubated adipose tissue was then snap-frozen for mRNA expression analysis of adipokines genes and genes involved in inflammation, adipogenesis, and mitochondrial function. Isolated adipocytes were used to measure basal and insulin-stimulated glucose uptake and lipolysis.

Results: Acute treatment with a therapeutic concentration of olanzapine decreases basal lipolysis in isolated adipocytes; this effect was not observed after long-term incubation with the drug. Supra-therapeutic concentration of aripiprazole reduced basal and insulin-stimulated glucose uptake after short- and long-term pre-incubation. Both drugs at supra-therapeutic concentrations downregulated the expression of the pro-inflammatory cytokines *IL6* and *IL1B* genes after 72 h incubation. Similarly, supra-therapeutic concentrations of both drugs and therapeutic concentration of olanzapine, reduced the expression of *PPARGC1A*, *PDK4*, and *CPT1B* genes involved in the regulation of mitochondrial functions. Neither of the antipsychotics affected the expression of the main adipokines *LEP* and *ADIPOQ*, genes involved in the regulation of lipid metabolism, *LPL* and *FASN*, nor the master adipogenesis regulator, *PPARG*.

Conclusion: Therapeutic concentrations of olanzapine and aripiprazole have a moderate direct effect on adipocyte lipid and glucose metabolism, respectively. At supra-therapeutic concentrations, both of the antipsychotics seem to act as anti-inflammatory agents and mildly suppressed genes involved in the regulation of mitochondrial functions, which could potentially contribute to metabolic adverse effects. Alternatively, second-generation antipsychotics could induce metabolic side effects via acting on other insulin-sensitive tissues and central nervous system.

Abbreviations: ADIPOQ, adiponectin; ARI, aripiprazole; BMI, body mass index; BSA, bovine serum albumin; CPT1B, Carnitine Palmitoyltransferase 1B; DEXA, dexamethasone; DMEM, Dulbecco's modified Eagle medium; FABP4, Fatty Acid Binding Protein 4; FASN, Fatty Acid Synthase; HDL, high-density lipoprotein; HOMAIR, homeostatic model assessment of insulin resistance; IL, interleukin; ISO, isoproterenol; KRH, Krebs-Ringer bicarbonate buffer; LDL, low-density lipoprotein; LEP, leptin; LPL, Lipoprotein lipase; MetS, metabolic syndrome; OLA, olanzapine; PBMC, peripheral blood mononuclear cells; PDK4, Pyruvate dehydrogenase lipoamide kinase isozyme 4; PEST, penicillin-streptomycin; PPARG, Peroxisome Proliferator-Activated Receptor Gamma; PPARGC1A, PPARG Coactivator 1 α; RIT, ritonavir; SGA, second-generation antipsychotic; sIL2R, – soluble IL-2 receptor; T2DM, Type 2 Diabetes Mellitus; TFAM, Mitochondrial transcription factor A; TNF, tumor necrosis factor

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1. Introduction

Schizophrenia is a severe mental condition that affects more than 23 million people worldwide (WHO, 2018). Patients with schizophrenia are estimated to have 10–20 years shorter life-expectancy than the general population (Laursen, 2011), with cardiovascular diseases being among the most common causes of death (Hennekens, 2007).

Antipsychotic drugs are the first-line treatment of schizophrenia. More recently developed second-generation (SGAs) or atypical antipsychotics are effective in the treatment of negative and cognitive components of schizophrenia and cause less motor side effects compared to first-generation antipsychotics (Nasrallah, 2008). However, despite their efficacy, SGAs are associated with induction of insulin resistance and substantial weight gain (Nasrallah, 2008). Although patients with schizophrenia tend to have a higher risk of developing obesity, type 2 diabetes (T2DM), and metabolic syndrome (MetS) due to their lifestyle and dietary habits (Mitchell et al., 2013), patients taking SGAs have a significantly higher prevalence of MetS compared to drug-naive patients (Mitchell et al., 2013). Clozapine and olanzapine are strongly associated with the development of MetS (in up to 50% patients vs. 20% of drug-naïve patients) (Mitchell et al., 2013), while aripiprazole is considered to be close to metabolically neutral in the clinical setting leading to a much lower weight gain and a significantly lower increase in plasma cholesterol and plasma glucose in comparison to olanzapine (Rummel-Kluge et al., 2010). Therefore, olanzapine and aripiprazole were selected for our study based on their widespread use and good efficacy, but with significantly different metabolic effects.

MetS contributes to the overall reduction in life expectancy, as it increases the risk of cardiovascular diseases by a factor of 1.5 (Galassi et al., 2006). Increased incidence of MetS in schizophrenia patients leads to a reduced quality of life and poor compliance with the treatment and, as a result, higher relapse rate (Ballon et al., 2014).

The ability of SGAs to cause insulin resistance and MetS has not been fully understood. It has been suggested that metabolic side effects are partly due to effects of the drugs on the central nervous system (CNS), since several neurotransmitter networks are involved in appetite and energy expenditure regulation (Nasrallah, 2008; Wysokiski and Koszewska, 2014). According to this view, the observed insulin resistance would develop secondary to increased adiposity. Literature provides some conflicting reports on this matter. For example, administration of olanzapine in healthy individuals resulted in a significant weight gain (Fountaine et al., 2010; Sacher et al., 2008), possibly due to increased food intake (Fountaine et al., 2010). However, olanzapine-induced insulin resistance in insulin-sensitive tissues with no body weight changes in healthy individuals has also been reported (Teff et al., 2013). A meta-analysis indicated that SGAs lead to weight gain and directly induce insulin resistance. The authors also underlined the necessity for future studies to understand the underlying mechanisms (Burghardt et al., 2018).

Some animal studies also indicate possible direct effects of clozapine and olanzapine on insulin-sensitive tissues, since exacerbation of lipid and carbohydrate metabolism, independently of weight gain has been observed (Albaugh et al., 2011a,b; Minet-Ringuet et al., 2007). These processes are compromised in obesity as well as in T2DM (Pereira et al., 2016). Therefore, it was of considerable interest to explore whether SGAs directly influence glucose and lipid metabolism in human adipose tissue.

Adipose tissue is a critical player in obesity, and it functions not solely as lipid storage but also as an endocrine organ producing adipokines important for appetite and insulin sensitivity regulation (Cohen and Spiegelman, 2016), e.g., adiponectin and leptin. Only a few studies have reported increased expression of genes regulating lipogenesis and adipogenesis *in vitro*, e.g. *PPARG*, *LPL*, in differentiated human adipocytes upon treatment with supra-therapeutic concentration of olanzapine (Chen et al., 2017; Sertié et al., 2011), while therapeutic concentration of the drug was demonstrated to induce gene expression of pro-inflammatory agents, such as *TNFA* and *IL1B* (Sárvári et al., 2014). It has also been reported that olanzapine did not affect adipocyte differentiation, but significantly increased lipid droplet accumulation in differentiated human adipocytes without affecting gene expression of *SREBF1*, *FASN*, *LEP*, and *ADIPOQ in vitro* (Nimura et al., 2015).

Schizophrenia patients exhibit higher serum levels of pro-inflammatory cytokines, such as IL6, TNFα, IFNγ, and sIL2R in comparison to healthy controls (Martínez-Gras et al., 2012). Animal studies suggest that olanzapine can induce adipose tissue inflammation and macrophage infiltration (Victoriano et al., 2010), but direct effects on human adipose tissue have not been studied previously.

Several animal studies have demonstrated that stimulation or blockade of histaminergic (H1), muscarinic (M3) and serotonergic (5HT2) receptors in different cell types induce or downregulate mRNA expression of proteins involved in the regulation of mitochondrial function and fatty acid transport, such as PGC1α, PDK4, CPT1B and aP2 (Gautam et al., 2006; Harmon et al., 2016; Zeng et al., 2007). PGC1α is a marker of mitochondrial biogenesis, while PDK4 stimulates fatty acid oxidation via inhibition of pyruvate dehydrogenase (Rowe et al., 2010). CPT1B and aP2 (or FABP4) regulate the transfer of free fatty acids to the inner mitochondrial membrane (Furuhashi et al., 2014; Rowe et al., 2010). Since SGAs target H1, M3, and 5HT2 receptors, they could theoretically modulate the expression of these genes and thereby regulate lipid metabolism.

This study aimed to investigate the direct effects of SGAs on human white adipose tissue glucose and lipid metabolism, which has not been studied previously to our knowledge. We also aimed to study their ability to induce adipose tissue inflammation and affect mRNA expression of genes regulating mitochondrial functions. Based on their propensity for MetS induction in patients, we chose olanzapine and aripiprazole as our study drugs.

2. Materials and methods

2.1. Subjects

Needle biopsies were obtained from abdominal subcutaneous adipose tissue from 72 healthy individuals (49 women and 23 men; age: 19–78 y.o.; BMI: 20.0–35.6 kg/m^2) after local dermal anesthesia with lidocaine (Xylocain; AstraZeneca, Södertälje, Sweden). Due to the limited amount of the tissue, not all the experiments were performed on tissue from the same subjects, however, individual experiments were conducted in the samples from the same subject and paired statistical analyses were performed. Fasting blood samples were collected for biochemical analysis of study participants (Table 1) at the Department of Clinical Chemistry, Uppsala University Hospital. Subjects with Type 2 Diabetes, endocrine disorders, cancer or other major illnesses and patients taking antipsychotic, antidepressant, or neuroleptic medications were excluded from the study. The Regional Ethics Review Boards in Uppsala approved the study. All participants gave their written informed consent.

2.2. Isolation of human adipocytes for glucose uptake and lipolysis

Adipocytes were isolated from adipose tissue needle biopsies via digestion with 1 mg/ml collagenase A (from *Clostridium histolyticum*, Roche, Manheim, Germany) in Medium 199 (Gibco, Life Technologies, Paisley, UK) supplemented with 6 mM glucose, 4% bovine serum albumin (BSA, Sigma, MO, USA), 150 nM adenosine (Sigma, MO, USA), pH = 7.4 for 60 min in 37 °C water bath shaking at 105 rpm. Cells were filtered through 250 μm nylon mesh, and the floating adipocytes fraction was collected and washed four times with Medium 199. For lipolysis measurement, isolated mature adipocytes were diluted with Medium 199 to 3–5% lipocrit. For glucose uptake experiments, isolated mature adipocytes were subsequently washed four times with 5 min interval with glucose-free Krebs-Ringer bicarbonate medium (KRH)

supplemented with 4% BSA, 150 nM adenosine, pH = 7.4. Cells were then subsequently diluted to 6–7% lipocrit with KRH.

2.3. Incubation of adipose tissue or isolated adipocytes with SGAs

To replicate the clinical conditions more accurately, the concentrations of the drugs for our study were chosen based on the reported therapeutic steady-state plasma concentration: olanzapine: 0.2 μM (at dose 25 mg/day), aripiprazole 1.0 μM (at dose 30 mg/d) (Citrome et al., 2009; Kirschbaum et al., 2008; Mallikaarjun et al., 2004). We also used a concentration 10 times higher (olanzapine 2.0 μM and aripiprazole 10 μM) to ensure we cover all relevant therapeutic concentrations with some margin.

Short-term: Following dilution, the isolated adipocytes were short-term pre-incubated without (Control) or with olanzapine (0.004–20 μM) (n = 11–13) and aripiprazole (0.002–100 μM) (n = 11–13) for 30 min shaking at 37 °C and 65 rpm (n = 26). Protease inhibitors (PI) were shown to reduce glucose uptake in adipocytes after short-term pre-incubation (Hresko and Hruz, 2011); therefore, 20 μM concentration of ritonavir was used as positive control. After that, the pre-incubated adipocytes were used to measure glucose uptake and lipolysis. Short-term pre-incubation time (30 min) was selected to study if the drugs have any direct effect on adipocyte metabolism, which usually requires rapid cell signalling.

Long-term: The long-term pre-incubation times (24 h and 72 h) were selected because both drugs have a very long half-life: olanzapine – 33 h, aripiprazole – 75 h (Mccormack and Wiseman, 2004; McIntyre et al., 2011), additionally, schizophrenia patients are chronically taking these medications; therefore, the effect of the drugs on adipose tissue might require long-term exposure to the agents. Furthermore, to study the effects of SGA on gene expression we used adipose tissue instead of adipocytes, since maintaining the interaction between the different cell types in the adipose tissue may be critical for its function during long-term incubation. For instance, *in vitro* incubation for 24 h of mature rat adipocytes has shown a significant decrease in the glucose transporter GLUT4 mRNA expression (Gerrits et al., 1993). Adipose tissue collected by subcutaneous adipose tissue needle biopsies was long-term incubated in DMEM with 6 mM glucose (Gibco, Life Technologies, Paisley, UK), 10% fetal bovine serum (FBS, Gibco, Life Technologies, Paisley, UK) and 1% penicillin-streptomycin (PEST, Invitrogen) in the absence and presence of olanzapine (0.2 μM and 2.0 μM) and aripiprazole (1.0 μM and 10 μM), and dexamethasone (0.3 μM) for 24 h (n = 27) and 72 h (n = 33). Synthetic glucocorticoid dexamethasone (DEXA) reduces glucose uptake in adipose tissue (Sidibeh et al., 2018). Therefore, dexamethasone was used as the positive control for the long-term incubation (24 h and 72 h). Part of the incubated adipose tissue was washed with ice-cold phosphate-buffer saline (PBS) (Medicago, Uppsala, Sweden) and thereafter snap-frozen and transferred to −80 °C for further gene expression analysis (n = 12–21). After pre-incubation of adipose tissue without (control) or with SGAs for 24 h and 72 h, adipocytes were isolated with collagenase A as previously reported, for glucose uptake (24 h, n = 8; 72 h, n = 4–6) and lipolysis (n = 7) measurements.

2.4. Glucose uptake

Pre-incubated isolated adipocytes were stimulated with or without physiological and supra-physiological concentrations of insulin (25 and 1000 μU/ml) for 15 min. After that, the cells were incubated with 0.26 mCi/l (0.86 μM) of ^{14}C-glucose (Perkin Elmer, Boston, MA, USA) for 45 min. The reaction was stopped via cell suspension transfer to pre-cooled vials, and the cell pellet was separated via centrifugation through 1 ml of Silicone fluid 100 cS (VWR Chemicals, Leuven, Belgium), and the glucose uptake rate was calculated as:

$$\text{Cell clearance of medium glucose} = \frac{\text{cell-associated radioactivity} \times \text{volume}}{\text{radioactivity of the medium} \times \text{cell number} \times \text{time}}$$

Cell-associated radioactivity was measured using Liquid Scintillation Analyser (Perkin Elmer, MA, USA). Cell size was measured as a mean diameter (μm) of consecutively measured 100 adipocytes from the same individual (Lundgren et al., 2007). Data were calculated as a fold change to respective control in each condition for further statistical analysis.

Table 1
Anthropometric and fasting biochemical characteristics of subjects involved in the study.

Variables	
Sex (male/female, n)	23/49
Age (years)	41 ± 18
Body mass index (kg/m^2)	26.4 ± 3.3
Waist-hip ratio (WHR)	0.88 ± 0.09
Systolic blood pressure (mmHg)	125 ± 16
Diastolic blood pressure (mmHg)	78 ± 9
HbA_{1c} (mmol/mol)	33 ± 7
Plasma glucose (mmol/l)	5.4 ± 1.2
Serum insulin (mU/l)	8.7 ± 4.4
HOMA IR	2.2 ± 1.2
Body fat mass (%)	31.1 ± 9.9
Plasma triglycerides (mmol/l)	1.3 ± 1.1
Plasma total cholesterol (mmol/l)	5.3 ± 4.4
Plasma LDL-cholesterol (mmol/l)	3.2 ± 1.1
Plasma HDL-cholesterol (mmol/l)	1.4 ± 0.6

Data are mean ± SD; HbA1c, glycosylated hemoglobin; HOMA IR, homeostatic model assessment of insulin resistance index (fasting blood glucose x fasting insulin/22.5); LDL, low-density lipoprotein; HDL high-density lipoprotein.

2.5. Lipolysis

Following short-term or long-term pre-incubation with the SGAs, as previously described, the adipocytes were stimulated with 0–100 IU/ml insulin for 10 min at 37°C 65 rpm (n = 11). Cells were thereafter incubated with or without 0.5 μM β-receptor agonist isoproterenol for 2 h at 37°C and 65 rpm. The reaction was terminated by transferring the incubation vials to ice.

Glycerol release into the medium was used as an indicator of lipolysis and was measured by colorimetric absorbance at 540 nm with Tecan Infinite M200PRO (Tecan Trading AG, Mannerdorf, Switzerland) plate reader. Data were calculated as a fold change to respective control in each condition for further statistical analysis.

2.6. Adipose tissue gene expression

After the long-term incubation with olanzapine and aripiprazole (see 2.3) stored adipose tissue was used for assessment of mRNA expression of genes involved in inflammation (*IL6, IL1B, TNFA, IL18*), regulation of mitochondrial functions and lipid transfer (*PDK4*, *PPARGC1A*, *CPT1B*, *TFAM*, *FABP4*), adipogenesis and lipogenesis (*PPARG*, *FASN*, *LPL*), and adipokines genes (*ADIPOQ*, *LEP*). RNA was isolated using RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) and High-Capacity cDNA Reverse Transcription Kit (Applied

Table 2
List of Taqman probes used for gene expression analysis.

Gene name	Gene symbol	Taqman probe
Adiponectin	*ADIPOQ*	Hs00605917_m1
Fatty acid binding protein 4	*FABP4*	Hs01086177_m1
Fatty acid synthase	*FASN*	Hs00165653_m1
Interleukin 18	*IL18*	Hs01038788_m1
Interleukin 1 beta	*IL1B*	Hs01555410_m1
Interleukin 6	*IL6*	Hs00985639_m1
Lipoprotein lipase	*LPL*	Hs00173425_m1
Leptin	*LEP*	Hs00174877_m1
Pyruvate dehydrogenase kinase 4	*PDK4*	Hs01037712_m1
Peroxisome proliferator-activated receptor gamma	*PPARG*	Hs01115513_m1
PPARG coactivator 1 alpha	*PPARGC1A*	Hs00173304_m1
Transcription factor A, mitochondrial	*TFAM*	Hs00273372_m1
Tumour necrosis factor alpha	*TNFA*	Hs00174128_m1

Biosystems, Thermo Fisher Scientific, CA, USA) according to the manufacturer's protocol. The gene expression measurement was performed with the QuantStudio 3 System (Thermo Fisher Scientific, MA, USA) using TaqMan assay probes listed in Table 2 (Thermo Fisher Scientific, MA, USA). Relative gene expression was normalised to the expression of *18S* (for *IL6*, *IL1B, TNFA, IL18*). Relative gene expression of all the other genes was normalised to the expression of *GUSB*.

2.7. Cell viability assay

Following incubation of adipose tissue or isolated adipocytes with antipsychotics a cell viability assay was performed using WST-1 reagent (Roche, Mannheim, Germany) according to the manufacturer's protocol. Cells were diluted to 6–7% lipocrit with Hank's medium and incubated with medium supplemented with 10% WST-1 reagent (according to manufacturer's protocol) for 2 h at 37°C. The absorbance of the medium was measured at 450 nm with Tecan Infinite M200PRO (Tecan Trading AG, Mannerdorf, Switzerland) plate reader. The optical density was further used to measure % of live cells normalized to the Control.

2.8. Statistical analysis

IBM SPSS Statistics 25 software was used to analyze the data. Data were initially tested for distribution using the Shapiro-Wilk test. In the present study, several parameters in the glucose uptake, lipolysis, and gene expression where not normally distributed and therefore the Friedman's test was used when several stimuli were compared to basal. For a paired analysis, Wilcoxon signed-rank test was used. In multiple comparisons, the p-values were corrected and also reported with Bonferroni correction. A p-value of < 0.05 was considered to be statistically significant.

3. Results

3.1. Effects of olanzapine and aripiprazole on glucose uptake

The effect of SGAs on adipose tissue glucose metabolism was evaluated by measuring glucose uptake after short- and long-term incubation of isolated adipocytes and adipose tissue, respectively. Short-term (Fig. 1A) or long-term (24 h, not shown; 72 h, Fig. 1B) incubation of adipocytes and adipose tissue, respectively, with both therapeutic (0.2 μM) and supra-therapeutic (2.0 μM) concentrations of olanzapine did not affect basal or insulin-stimulated glucose uptake in these cells.

Short-term incubation with therapeutic (1.0 μM) concentration of aripiprazole did not affect basal glucose uptake but reduced insulin-stimulated glucose uptake by about 16% ($p < 0.05$) in isolated adipocytes. Furthermore, the supra-therapeutic concentration of aripiprazole significantly reduced basal and insulin-stimulated adipocyte glucose uptake by 15 and 20%, respectively ($p < 0.05$), in comparison to control (Fig. 1A). After the adjustment of the p-value for multiple testing (data not shown) the fold change observed with aripiprazole 10 μM remained significant ($p = 0.001$), but aripiprazole 1.0 μM at 25 μU/ml became non-significant ($p = 0.814$). Additionally, there seemed to be a dose-dependent inhibition of basal and insulin-stimulated glucose uptake in isolated adipocytes with increasing concentration (1.0–100 μM) of aripiprazole (data not shown). Ritonavir was used as a positive control for short-term incubation and also reduced both basal and insulin-stimulated glucose uptake by 30% ($p < 0.01$), as expected.

Long-term (72 h) incubation with the supra-therapeutic concentration of aripiprazole significantly reduced glucose uptake at maximal insulin stimulation by 20% ($p < 0.05$) (Fig. 1B). The therapeutic concentration of aripiprazole also reduced basal glucose uptake, but this was not significant, while it did not affect the insulin-stimulated glucose uptake. Dexamethasone, which was used as the positive control, reduced glucose uptake in adipocytes by more than 30% ($p < 0.05$) after both 24 h (data not shown) and 72 h incubation period. There were no significant associations between the effects of SGAs on glucose uptake and the age or gender of the subjects (data not shown).

3.2. Effects of olanzapine and aripiprazole on lipolysis

In order to understand, whether SGAs directly affect lipid metabolism in human adipose tissue, lipolysis experiments were performed. Short-term incubation with olanzapine dose-dependently reduced basal lipolysis, with maximal basal lipolysis reduction of about 50% with 20

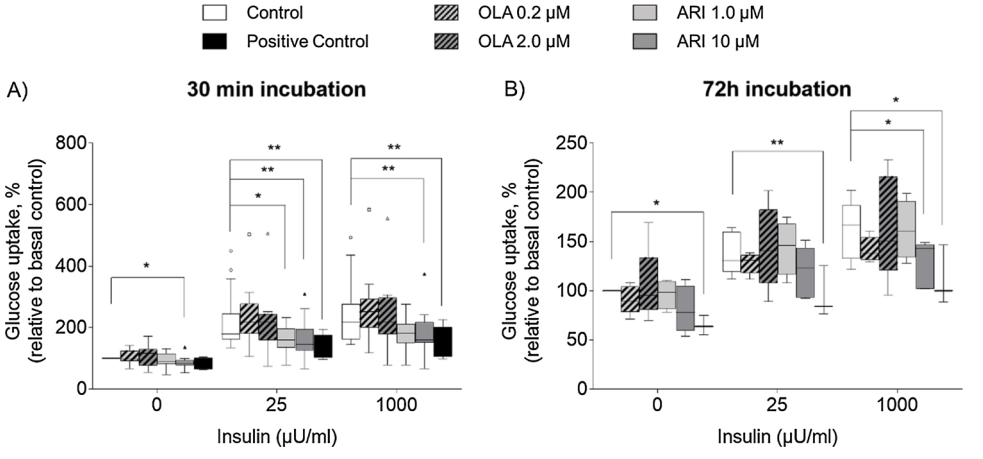

Fig. 1. Effects of SGAs on isolated adipocytes glucose uptake. Box plots represent the ^{14}C-D-glucose uptake by isolated human subcutaneous adipocytes pre-incubated with medium supplemented without (Control) or with olanzapine (OLA) or aripiprazole (ARI) for **A)** short-term, 30 min, and **B)** long-term, 72 h, at basal (0 μU/ml), submaximal (25 μU/ml), and maximal (1000 μU/ml) insulin stimulation. Results are shown as median and interquartile range of A) n = 11–13 independent experiments with ARI and OLA, respectively, RIT (20 μM) – ritonavir, short-term incubation positive control (n = 4) B) n = 4–5 independent experiments with ARI and OLA, respectively, DEXA (0.3 μM) – dexamethasone, long-term incubation positive control (n = 3). Statistical analysis: Wilcoxon signed-rank test for paired analysis was used.

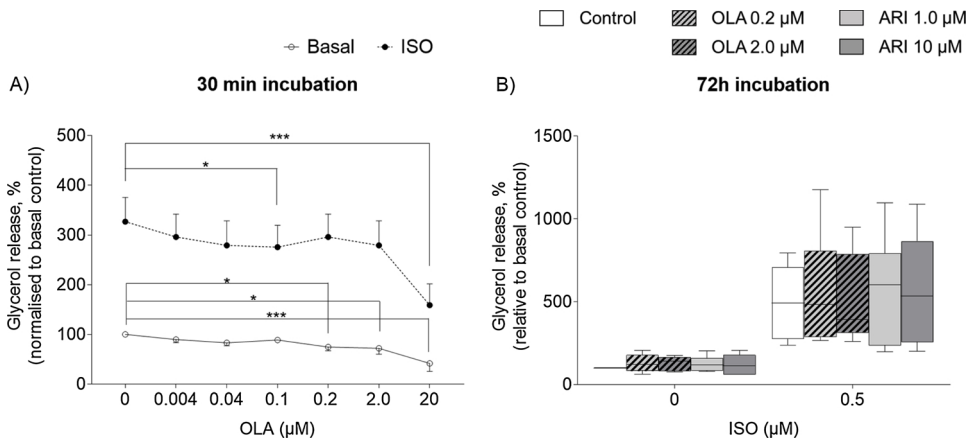

Fig. 2. Effects of SGAs on isolated adipocytes lipolysis. Graph represents the basal and isoproterenol-stimulated (ISO) lipolysis rate measured via glycerol release by isolated human subcutaneous adipocytes pre-incubated with medium supplemented without (Control) or with **A)** 0.004–20 μM olanzapine (OLA) for short-term, 30 min. Results are shown as mean ± SEM of n = 8 independent experiments with OLA and **B)** 0.2 μM and 2.0 μM olanzapine (OLA) or 1.0 μM or 10 μM aripiprazole (ARI) for long-term, 72 h. Results are shown as median and interquartile range of n = 7 independent experiments with OLA and ARI. Statistical analysis: Wilcoxon signed-rank test for paired analysis.

μM olanzapine concentration compared to control ($p < 0.05$) (Fig. 2A). 20 μM olanzapine also significantly reduced isoproterenol-stimulated lipolysis by ca. 50% ($p < 0.001$) (Fig. 2A). Olanzapine at both concentrations, 0.2 and 2.0 μM significantly suppressed basal lipolysis rate by about 25% and 28% ($p < 0.05$), respectively (Fig. 2A). However, the given concentrations of the drug did not affect isoproterenol-stimulated lipolysis rate. After adjustment of the p-value for multiple testing (data not shown), the fold change in basal lipolysis observed with olanzapine 2.0 μM short-term treatment remained significant ($p = 0.037$), but olanzapine 0.2 μM became non-significant ($p = 0.137$).

Aripiprazole did not alter adipocyte basal and isoproterenol-stimulated lipolysis at both concentrations (data not shown). Neither SGAs affected the inhibitory effect of insulin on isoproterenol-stimulated lipolysis (data not shown).

72 h incubation with both SGAs did not affect basal or isoproterenol-stimulated adipocyte lipolysis (Fig. 2B), nor did it alter the inhibitory effects of insulin (data not shown). Additionally, there were no significant associations between the effects of SGAs on lipolysis and the age or gender of the subjects (data not shown).

3.3. Effects of olanzapine and aripiprazole on adipocyte viability

To verify that the effects of SGAs on glucose and lipid metabolism are not due to the cell toxicity, viability assays were performed. Short-term incubation with olanzapine and aripiprazole at both therapeutic and supra-therapeutic concentrations did not reduce the cell viability (Fig. 3A). Therefore, the inhibitory effects of the drugs on glucose uptake and basal lipolysis are not likely to be caused by cell death. No significant difference in the percentage of live cells between the tested conditions was observed, which suggests that neither of the SGAs had a toxic effect on the adipose tissue at longer incubation times (Fig. 3B).

3.4. Effects of olanzapine and aripiprazole on mRNA expression of genes involved in inflammation in adipose tissue

Since obesity and T2DM are associated with adipose tissue inflammation, characterised by infiltration of macrophages and elevated pro-inflammatory cytokines expression (Esser et al., 2014; Kohlgruber and Lynch, 2015), we investigated whether SGAs are able to directly induce adipose tissue inflammation. After 24 h neither of the SGAs affected *IL6* and *IL18* gene expression, while olanzapine at a supra-therapeutic concentration (2.0 μM) diminished the expression of *IL1B* by 20% ($p < 0.05$). However, after 72 h of incubation the expression of *IL6, IL1B* and *IL18* was suppressed by the supra-therapeutic concentrations of olanzapine and aripiprazole by about 20–30% ($p < 0.01$) (Table 3). Additionally, *IL18* was downregulated by aripiprazole 1.0 μM (14%, $p < 0.05$) after 72 h incubation. *TNFA* mRNA expression was not affected by any of the SGAs at 24 h and 72 h incubation.

Dexamethasone suppressed mRNA expression of *IL6*, *IL1B,* and *TNFA* by 80%, 85%, and 55%, respectively, as expected ($p < 0.001$). On the contrary, dexamethasone increased the expression of *IL18* at both time points, which has been observed by the group before (data not shown).

These results indicate that 72 -h incubation of adipose tissue with olanzapine and aripiprazole reduced the gene expression of pro-inflammatory cytokines at supra-therapeutic concentrations, while, therapeutic concentrations have a mild effect on the mRNA expression of these genes. After correction for multiple testing, fewer conditions showed a significant fold-change in mRNA expression, with only DEXA significantly up- or downregulated the expression of all measured genes ($p < 0.05$). For 72 h incubation, the significant changes in fold change retained with DEXA condition for *IL6* ($p < 0.01$), and DEXA and ARI 10 μM for *IL1B* ($p < 0.05$), OLA 2.0 for *IL18*.

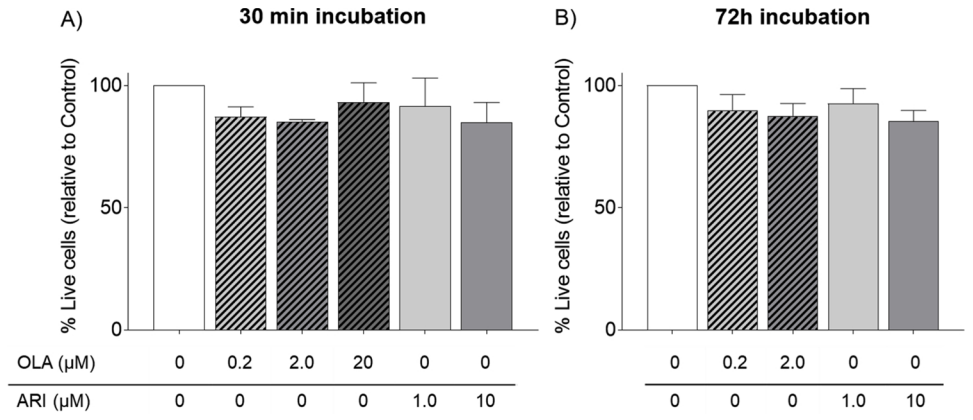

Fig. 3. Effects of SGAs on the adipocytes viability. Data represent adipocytes viability after pre-incubation with medium supplemented without (Control) or with olanzapine (OLA) or aripiprazole (ARI) for **A)** short-term, 30 min, and **B)** long-term, 72 h. Results are shown as mean ± SEM of A) n = 2–8 independent experiments with OLA and ARI, respectively, B) n = 9 independent experiments with both OLA and ARI. Statistical analysis: Wilcoxon signed-rank test for paired analysis.

Table 3
Gene expression in adipose tissue after 24 h and 72 h incubation with olanzapine and aripiprazole.

Gene symbol	*OLA 0.2 μM* *Fold change*	*OLA 2.0 μM* *Fold change*	*ARI 1.0 μM* *Fold change*	*ARI 10 μM* *Fold change*	*DEXA 0.3 μM* *Fold change*
24 h incubation					
Pro-inflammatory cytokines					
IL6	1.22 ± 0.19	1.13 ± 0.19	0.95 ± 0.13	1.17 ± 0.16	**0.19 ± 0.03****
IL1B	1.07 ± 0.16	**0.78 ± 0.08***	0.90 ± 0.08	0.90 ± 0.14	**0.15 ± 0.03****
TNFA	1.07 ± 0.07	0.96 ± 0.05	0.99 ± 0.07	1.09 ± 0.07	**0.45 ± 0.03****
IL18	1.16 ± 0.14	1.04 ± 0.43	1.04 ± 0.09	1.01 ± 0.10	**1.98 ± 0.27****
Regulation of mitochondrial function					
PDK4	0.87 ± 0.06	0.96 ± 0.05	0.90 ± 0.07	0.89 ± 0.06	**6.27 ± 1.16****
PPARC1A	1.03 ± 0.09	1.05 ± 0.09	0.95 ± 0.03	0.94 ± 0.06	**3.08 ± 0.48****
TFAM	0.89 ± 0.06	0.98 ± 0.04	1.07 ± 0.05	0.96 ± 0.04	**0.75 ± 0.03****
CPT1B	0.96 ± 0.02	0.98 ± 0.03	0.95 ± 0.03	**0.90 ± 0.03***	0.86 ± 0.07
Adipogenesis and lipid metabolism					
FABP4	**0.89 ± 0.05***	0.97 ± 0.06	1.05 ± 0.09	0.94 ± 0.06	0.88 ± 0.08
Adipokines					
LEP	**0.81 ± 0.11***	1.00 ± 0.07	1.11 ± 0.08	0.97 ± 0.06	**2.91 ± 0.73****
ADIPOQ	0.88 ± 0.07	0.99 ± 0.06	1.03 ± 0.15	0.92 ± 0.04	0.91 ± 0.10
72 h incubation					
Pro-inflammatory cytokines					
IL6	1.06 ± 0.11	**0.81 ± 0.05****	1.05 ± 0.10	**0.83 ± 0.05****	**0.14 ± 0.01****
IL1B	1.02 ± 0.08	**0.79 ± 0.07****	0.89 ± 0.06	**0.72 ± 0.08****	**0.13 ± 0.04****
TNFA	1.03 ± 0.06	0.95 ± 0.07	0.93 ± 0.08	1.09 ± 0.09	**0.55 ± 0.07****
IL18	1.06 ± 0.07	**0.79 ± 0.06****	**0.86 ± 0.08***	0.97 ± 0.09	**1.61 ± 0.19****
Regulation of mitochondrial function					
PDK4	**0.84 ± 0.07***	**0.74 ± 0.17****	1.01 ± 0.10	**0.73 ± 0.04****	**2.93 ± 0.49****
PPARC1A	**0.80 ± 0.08***	**0.66 ± 0.05****	0.98 ± 0.09	**0.71 ± 0.04****	**2.44 ± 0.29****
TFAM	0.94 ± 0.06	0.94 ± 0.06	1.01 ± 0.06	**0.88 ± 0.06***	**0.87 ± 0.06***
CPT1B	**0.83 ± 0.04****	**0.82 ± 0.07***	0.93 ± 0.04	**0.78 ± 0.05****	0.94 ± 0.05
Adipogenesis and lipid metabolism					
FABP4	**0.85 ± 0.07***	0.93 ± 0.07	**1.26 ± 0.12***	1.05 ± 0.09	**1.80 ± 0.28***
Adipokines					
LEP	1.00 ± 0.11	1.14 ± 0.12	1.03 ± 0.11	1.13 ± 0.12	**3.84 ± 0.81****
ADIPOQ	1.00 ± 0.13	1.09 ± 0.17	1.16 ± 0.16	1.10 ± 0.13	1.48 ± 0.23

IL6 – Interleukin 6 (n = 21), *IL1B* – Interleukin 1β (n = 21), *IL18* – Interleukin 18 (n = 21), *TNFA* – tumour necrosis factor α (n = 21), *PDK4* – Pyruvate dehydrogenase lipoamide kinase isozyme 4 (n = 12), *PPARGC1A* – PPARG Coactivator 1 α (n = 12), *TFAM* – Mitochondrial transcription factor A (n = 12), *CPT1B* – Carnitine Palmitoyltransferase 1B (n = 12), *FABP4* – Fatty Acid Binding Protein 4 (n = 12), *FASN* – Fatty Acid Synthase (n = 12), *LPL* – Lipoprotein lipase (n = 12), *PPARG* – Peroxisome Proliferator Activated Receptor Gamma (n = 12), *LEP* – leptin (n = 12), *ADIPOQ* – adiponectin (n = 12). Human subcutaneous adipose tissue was incubated with medium supplemented without (control) or with OLA or ARI. DEXA – positive control. *18S* was used as the housekeeping gene for pro-inflammatory cytokines genes. *GUSB* was used as the housekeeping gene for all the rest. Relative expression was calculated as 2^(-ΔΔCt). Results are shown as mean ± SEM. NS - non-significant, * ($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

There was large individual variability in the expression of these genes in response to the drugs, but the subject's gender, BMI, Body fat %, age, HOMA-IR, fasting glucose, waist/hip ratio, systolic and diastolic blood pressure did not explain the observed variability.

3.5. Effects of olanzapine and aripiprazole on mRNA expression of genes involved in mitochondrial function and lipid metabolism in adipose tissue

Adipocyte dysfunction in obesity and diabetes are also linked to mitochondrial dysfunction of white adipose tissue (Cedikova et al., 2016), therefore, we have also tested the effects of SGAs on the expression of several genes regulating mitochondrial function. As it is shown in Table 3, after 24 h incubation neither of the SGAs affected gene expression of *PDK4* and *PPARGC1A* expression. However, 72 h incubation with both therapeutic and supra-therapeutic concentrations of olanzapine significantly reduced the expression of *PDK4, PPARGC1A*, and *CPT1B* by about 20–35% ($p < 0.05$). After 24 h incubation, supra-therapeutic concentration of aripiprazole reduced the *CPT1B* expression by 10% ($p < 0.05$), while 72 h treatment reduced *PDK4*, *PPARC1A*, *TFAM* and *CPT1B* gene expression by about 12–30% ($p < 0.05$).

FABP4 expression was downregulated by the therapeutic concentration of olanzapine by 10% ($p < 0.05$) after 24 h incubation and this effect was enhanced after 72 h, reaching 15% ($p < 0.05$) down-regulation. The therapeutic concentration of aripiprazole, on the contrary, increased the expression of *FABP4* by 26% ($p < 0.05$) in adipose tissue after 72 h incubation.

These results suggest that both SGAs could regulate the expression of genes involved in mitochondrial function and biogenesis and could alter the lipid utilization in adipose tissue.

To test whether the SGAs affect the mitochondrial content in adipocytes, *TFAM* (Mitochondrial transcription factor A) mRNA expression was measured. None of the SGAs at therapeutic concentration affected the expression of *TFAM* after 24 h or 72 h incubation. However, a higher concentration of aripiprazole reduced *TFAM* expression by 12% ($p < 0.05$) (Table 3). With the adjustment of the p-value for multiple testing, fold change in mRNA expression of genes involved in mitochondrial activity regulation, remained significant only with the supra-therapeutic concentrations of the drugs ($p < 0.05$).

We also measured the mRNA expression of *FASN* (fatty acid synthase), *LPL* (lipoprotein lipase), and *PPARG* and found that neither of the SGAs affected these genes (data not shown).

The mRNA expression of adipokines, leptin and adiponectin, also exhibited no changes upon treatment with SGAs, apart from leptin expression being reduced at 24 h with a therapeutic concentration of olanzapine (Table 3).

4. Discussion

In this study of direct effects of SGAs on adipose tissue, we report the following findings: (1) therapeutic concentrations of both olanzapine and aripiprazole did not affect adipocyte glucose uptake. The supra-therapeutic concentration of aripiprazole significantly reduced

both basal and insulin-stimulated glucose uptake in isolated mature adipocytes after short-term incubation of the cells and long-term incubation of the adipose tissue. (2) Short-term exposure to olanzapine reduced basal lipolysis rate in a dose-dependent manner, and this effect was not observed after 72 h incubation, while aripiprazole did not affect lipolysis. (3) Gene expression data indicated that after 72 h, the inflammatory cytokines and genes regulating mitochondrial function were moderately but significantly downregulated by olanzapine and aripiprazole.

Our results suggest that olanzapine and aripiprazole affect adipose tissue metabolism through different mechanisms. While olanzapine did not change glucose uptake, short- and long-term incubation with the supra-therapeutic concentration of aripiprazole significantly reduced both basal and insulin-stimulated glucose uptake by 15–20%. Drug toxicity did not explain the reduction observed with aripiprazole since we observed no significant difference in cell viability.

Another explanation could be that the supra-therapeutic concentration of aripiprazole interferes with the activation of the insulin signaling pathway, reducing adipocyte glucose uptake. However, this needs to be further investigated.

Our data are in agreement with a previous study showing no effect of therapeutic concentrations of olanzapine on glucose uptake in 3T3-L1 cell line after up to 20 h incubation (Robinson et al., 2006). On the contrary, higher concentrations of the drug (5 μM) decreases glucose transport in 3T3-L1 cells by 30% after 15 h (Vestri et al., 2007). The discrepancy between the findings could be explained by a much higher concentration of the drug used by Vestri et al. (2007). Additionally, 3T3-L1 cells are a murine cell line, which could have a different response to the treatment due to species differences.

Short-term incubation of isolated adipocytes with olanzapine reduced the basal lipolysis in adipocytes, and this effect was dose-dependent. The highest concentration of the drug (20 μM) also reduced isoproterenol-stimulated lipolysis. Treatment of 3T3-L1 cells (Vestri et al., 2007) or rats with olanzapine (Minet-Ringuet et al., 2007) has also been shown to inhibit basal and isoproterenol-stimulated lipolysis rate. Olanzapine seems to interfere with molecular mechanisms regulating lipolysis. An inhibitory effect on isoproterenol-stimulated lipolysis at high concentration of the drug is unlikely to be via interference with β-adrenergic signaling since a reduced IBMX-stimulated lipolysis independent of β-signaling was also observed (Minet-Ringuet et al., 2007). This implies that olanzapine seems to act via a different mechanism, which needs further investigation. Reduced lipolysis would lead to the diminished release of nonesterified fatty acids into circulation and treatment with olanzapine was demonstrated to reduce serum free fatty acid concentrations in healthy volunteers (Albaugh et al., 2011a,b; Vidarsdottir et al., 2010), which is in accordance with our findings. Interestingly this effect disappeared after 72 h incubation of adipose tissue with olanzapine, and both basal and isoproterenol-stimulated lipolysis rates were not altered. The reason for such a change in response to antipsychotic treatment is unclear and could be due to induction of some compensatory mechanisms that would allow the adipose tissue to adapt and maintain homeostasis; however, this needs further investigation. To our knowledge, there are no lipolysis studies with long-term incubation of human adipose tissue with SGAs *in vitro*.

Both short- and long-term treatment of adipocytes and adipose tissue with aripiprazole did not affect lipolysis in isolated adipocytes. In animal models, long-term administration of aripiprazole did not alter the plasma concentration of FFAs in rats (Skrede et al., 2012), which implies that aripiprazole does not seem to interfere with lipid metabolism in adipose tissue.

It is thought that olanzapine is more metabolically detrimental than aripiprazole, and more strongly associated with insulin resistance, dyslipidemia and weight gain (Rummel-Kluge et al., 2010). However, in our *in vitro* model, olanzapine did not affect glucose transport in human adipocytes, while aripiprazole could inhibit glucose uptake at supra-therapeutic concentrations. Our glucose uptake results do not explain why olanzapine is metabolically more detrimental than aripiprazole in the clinical setting. On the other hand, olanzapine dose-dependently impaired lipolysis, which suggests that olanzapine affects adipocyte metabolism to favour lipid storage into adipocytes, thereby increasing adiposity. However, these data need to be interpreted with caution since we mostly observed effects at supra-therapeutic concentrations.

Additionally, it is important to consider the potential effect of the drugs on other insulin-sensitive tissues, such as liver and muscle. Previous *in vitro* studies have shown that olanzapine significantly increases *de novo* lipogenesis and cholesterol synthesis in primary rat hepatocytes (Lauressergues et al., 2010). Olanzapine was also reported to reduce glycogen synthesis and impair insulin signaling in L6 muscle cell line (Engl et al., 2005), while it was shown to induce ER stress and apoptosis in hamster pancreatic β cell line (Ozasa et al., 2013). Both aripiprazole and olanzapine have been shown to act as anti-inflammatory agents, reducing the mRNA expression of pro-inflammatory cytokines upon *ex vivo* treatment of peripheral blood monocytes (Stapel et al., 2018). Therefore, more comprehensive *in vivo* studies on the effects of the drugs on insulin-sensitive tissues, such as liver or muscle, are in demand.

Adipocyte hypertrophy is associated with a higher risk of metabolic disorders (Laforest et al., 2015). However, neither of the SGAs changed adipocyte size after 72 h incubation.

We measured mRNA expression of genes regulating mitochondrial function, namely *PPARGC1A* and its downstream targets, such as *PDK4* and *CPT1B*. We observed that after 72 h, *PDK4*, *PPARGC1A*, and *CPT1B* were downregulated by both concentrations of olanzapine and supra-therapeutic concentration of aripiprazole. This suggests that olanzapine at therapeutic concentrations and higher concentrations of aripiprazole could theoretically interfere with mitochondrial function in adipose tissue, potentially reducing fatty acid oxidation and energy expenditure. To understand whether the drugs could lead to a reduction in mitochondrial content of adipose tissue, *TFAM* expression was measured. *TFAM* is also downstream of *PPARGC1A*, and our analysis indicated that aripiprazole at therapeutic concentration did not affect *TFAM* expression, while the supra-therapeutic concentration of aripiprazole reduced it after 72 h incubation. This correlates with *PPARGC1A* expression profile observed with aripiprazole treatments. However, olanzapine at both concentrations did not change *TFAM* expression, despite reduced *PPARGC1A* expression at both concentrations, implying that mitochondrial content is not altered and potential changes in mitochondrial functions would be explained by altered expression of mitochondrial activity regulators. Future studies to measure the effects of olanzapine and aripiprazole on mitochondria biogenesis and its activity are warranted.

Another gene of interest was *FABP4*, which encodes acid-binding protein 2 (aP2), responsible for transporting free fatty acids and regulation of lipolysis (Furuhashi et al., 2014). The expression of *FABP4* was downregulated by therapeutic concentrations of olanzapine after 24 h and 72 h treatment, highlighting reduced fatty acid uptake into adipocytes by gene downregulation as a plausible mechanism for the observed reduction in lipolysis. On the contrary, the therapeutic concentration of aripiprazole induced *FABP4* expression after 72 h incubation. The different effects of the SGAs on *FABP4* expression could be due to the difference in receptor profile of two drugs. It has been previously shown that stimulation of histaminergic H1 receptors in 3T3-L1 cells results in upregulation of *FABP4* expression (Zeng et al., 2007), therefore, blocking this receptor would potentially reduce the expression of this gene. Olanzapine has a much higher affinity for the H1 receptor than aripiprazole (Nasrallah, 2008), which could explain its higher potency for downregulating *FABP4* expression. Since individual SGAs target different receptors, their specific receptor profile could contribute to understanding their ability to induce metabolic complications. Affinity for H1 receptors significantly correlates with weight gain (Newcomer, 2005), additionally, antagonism of 5HT2C, 5HT1A, and α2 receptors could also contribute to this process

(Nasrallah, 2008). Olanzapine has a much higher affinity for these receptors in comparison to aripiprazole, which could explain its higher potency in inducing weight gain. M3 receptors are found on beta cells in the pancreas and are involved in acetylcholine-regulated insulin production (Nasrallah, 2008). Therefore, blockade of these receptors could potentially interfere with insulin production, disturbing both glucose and lipid metabolism. Future studies should explore the role of receptors for the SGAs on glucose and lipid metabolism in adipose tissue.

We observed that olanzapine and aripiprazole at therapeutic concentrations do not affect the expression of pro-inflammatory cytokines after 24 h and 72 h. However, surprisingly, we saw a downregulation of these genes by supra-therapeutic concentrations of both drugs. Interestingly, we observed a great individual subject variability in the expression of pro-inflammatory cytokines genes that did not significantly correlate with any of the anthropometric measurements. This could be due to differences in pharmacogenetic factors, as there is a large individual difference between patients in terms of both responses to the medications as well as the occurrence of side effects, including metabolic disturbances (Brandl et al., 2014). Our data are inconsistent with results from Sárvári et al (2014). They report that 11-day treatment with olanzapine and aripiprazole induced pro-inflammatory genes *TNFA*, *IL8*, *IL1B*, and *CCL2* in human differentiated adipocytes (Sárvári et al., 2014). Differences in cellular models and incubation times could explain the discrepancy in results. Both olanzapine and aripiprazole have long half-lives (33 h (Mccormack and Wiseman, 2004) and 75 h (McIntyre et al., 2011), respectively) and require around 8 (Mccormack and Wiseman, 2004) and 14 days (McIntyre et al., 2011) to reach steady-state plasma concentrations, respectively and, therefore, it is possible that effects on pro-inflammatory cytokines require longer treatment times. Our data are in agreement with meta-studies that reported anti-inflammatory functions of antipsychotic treatment in schizophrenia patients, as they reduce plasma concentrations of pro-inflammatory cytokines as well as cytokine release by PBMCs *in vitro* (Drzyzga et al., 2006). Pro-inflammatory cytokines, such as TNFα, stimulate leptin production via adipocytes (Finck and Johnson, 2000) and reduce food intake, partly by acting in the hypothalamus to regulate feeding behavior (Fonseka et al., 2016). Reduction in the cytokines level by SGAs could interfere with this process and could also induce hyperphagia and increased adiposity, potentially contributing to the development of MetS (Fonseka et al., 2016).

We observed that both drugs did not affect the expression of the genes involved in adipogenesis and lipid metabolism regulation, i.e., *FASN*, *PPARG*, and *LPL*. Previous reports have shown inconsistent results, with some suggesting no effects (Sárvári et al., 2014; Sertié et al., 2011) or increased (Sárvári et al., 2014; Sertié et al., 2011; Yang et al., 2007) expression of *PPARG*, *LPL,* and *FASN*. As discussed previously, there are differences in the experimental settings, such as drug concentrations, incubation period, and cellular model, which could account for the difference in results.

We also measured the gene expression of main adipose tissue hormones – leptin and adiponectin. We observed that aripiprazole did not change the mRNA expression of *LEP* or *ADIPOQ*. However, *LEP* expression was downregulated by the therapeutic concentration of olanzapine after 24 h incubation, and this effect disappeared after 72 h. Aripiprazole and olanzapine induced *LEP* and *ADIPOQ* expression in differentiated human adipocytes (Sárvári et al., 2014). However, the difference in the results could be explained by the reasons mentioned previously, such as differences in experimental settings. Patient studies also indicate that both aripiprazole and olanzapine led to an increased plasma concentration of leptin (Pérez-Iglesias et al., 2014) and reduced plasma levels of adiponectin (Wampers et al., 2012). Plasma leptin levels exhibit a strong positive correlation to BMI, which suggests that this process is secondary to increased adiposity (Jin et al., 2008).

There were several limitations to our study. One of the limitations is the sample size. Also, longer incubation times are not feasible in our setting due to the possibility of compromising adipose tissue viability and metabolic stability. Additionally, we have not investigated the regulation of protein expression by the drugs. Protein expression could follow a different trend and would provide a closer insight into the mechanisms of SGA-induced metabolic side-effects. Furthermore, we tested the effect of the drugs in subcutaneous adipose tissue and not in the visceral depot, which is more associated with MetS and T2DM.

Elimination of aripiprazole is mainly hepatic via the cytochrome P450 (CYP) 3A4 and 2D6, which transform aripiprazole to its metabolite dehydroaripiprazole (Swainston Harrison and Perry, 2004). At steady-state, about 40% of plasma aripiprazole concentration is represented by dehydroaripiprazole (Caccia, 2011). According to the FDA assessment, drug activity is mainly due to the parent compound, aripiprazole (Otsuka Pharmaceutical, 2005) and to a lesser extent to dehydroaripiprazole. However, dehydroaripiprazole has similar affinity to D2 receptors and longer half-life, as the parent drug (Otsuka Pharmaceutical, 2005; Swainston Harrison and Perry, 2004), and therefore dehydroaripiprazole may be relevant for the clinical effect of aripiprazole treatment.

We observed moderate direct effects of SGAs on human adipose tissue metabolism and gene expression, mostly at supra-therapeutic concentrations. However, the clinical relevance of the findings observed with supra-therapeutic drug concentrations is questionable. Animal studies have suggested that tissue concentrations of SGAs would be much higher than plasma concentrations *in vivo* (Aravagiri et al., 1999; Weigmann et al., 1999). However, is has been reported that the concentration of olanzapine in adipose tissue from treated rats is similar to its plasma concentration (Aravagiri et al., 1999).

To the best of our knowledge, no studies have explored the impact of SGAs in the adipose tissue in observational studies. Therefore, future studies should include adipose tissue biopsies before and after drug therapy to explore the effects on adipose tissue metabolism. Specifically, the impact on adipose tissue glucose metabolism, lipid storage, inflammation, and mitochondria biogenesis and activity are interesting outcome variables. Additionally, the effects of the SGAs on the more metabolically active omental adipose tissue should be explored.

5. Conclusion

In conclusion, our data suggest that aripiprazole and olanzapine at therapeutic concentrations have a minute direct effect on adipocyte glucose uptake and lipolysis. Olanzapine acutely reduced lipolysis in human subcutaneous primary adipocytes, while aripiprazole at higher concentrations reduced glucose uptake, indicating that these two drugs are likely to affect energy metabolism via different mechanisms.

We also observed a modest but significant downregulation of several genes involved in mitochondrial function regulation and lipid metabolism after 72 h incubation. Hence, SGAs could impair the utilization of fatty acids as an energy source and inhibit β-oxidation, therefore, potentially contributing to the induction of MetS. Neither of the drugs at therapeutic concentrations directly induced mRNA expression of pro-inflammatory cytokines but rather acted as anti-inflammatory agents at higher concentrations.

Taken together, our data indicate that these drugs have mild direct effects on adipose tissue metabolism. This may suggest that SGAs can induce metabolic adverse effects by acting on other insulin-sensitive tissues, such as muscle or liver. Alternatively, and most likely, SGAs mainly interfere with CNS pathways that are critical for regulation of energy intake and expenditure and of glucose handling in peripheral tissues.

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Conflict of interest

All authors declare no conflict of interest.

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