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Atypical antipsychotics induce both proinflammatory and adipogenic gene expression in human adipocytes in vitro

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ABSTRACT

Schizophrenia requires lifelong treatment, potentially causing systemic changes in metabolic homeostasis. In the clinical setting, antipsychotic treatment may differentially lead to weight gain among individual patients, although the molecular determinants of such adverse effects are currently unknown. In this study, we investigated changes in the expression levels of critical regulatory genes of adipogenesis, lipid metabolism and proinflammatory genes during the differentiation of primary human adipose-derived stem cells (ADSCs). These cells were isolated from patients with body mass indices <25 and treated with the second-generation antipsychotics olanzapine, ziprasidone, clozapine, quetiapine, aripiprazole and risperidone and the first-generation antipsychotic haloperidol. We found that antipsychotics exhibited a marked effect on key genes involved in the regulation of cell cycle, signal transduction, transcription factors, nuclear receptors, differentiation markers and metabolic enzymes. In particular, we observed an induction of the transcription factor NF-KB1 and NF-KB1 target genes in adipocytes in response to these drugs, including the proinflammatory cytokines TNF-α, IL-1β, IL-8 and MCP-1. In addition, enhanced secretion of both IL8 and MCP-1 was observed in the supernatant of these cell cultures.

In addition to their remarkable stimulatory effects on proinflammatory gene transcription, three of the most frequently prescribed antipsychotic drugs, clozapine, quetiapine and aripiprazole, also induced the expression of essential adipocyte differentiation genes and the adipocyte hormones leptin and adiponectin, suggesting that both glucose and fat metabolism may be affected by these drugs. These data further suggest that antipsychotic treatments in patients alter the gene expression patterns in adipocytes in a coordinated fashion and priming them for a low-level inflammatory state.

1. Introduction

Atypical antipsychotics (AAPs), or second-generation antipsychotics (SGAs), are widely prescribed for the treatment of several psychiatric disorders. However, these drugs are associated with many mild and serious side effects. The major side effects of AAPs are weight gain and its associated metabolic disorders, such as type II diabetes and dyslipidemia [1,2]. The increase in obesity-related adipose tissue mass may derive from both increased adipocyte size due to lipid accumulation in differentiated adipocytes, and increased adipocyte number due to the differentiation of adipose-derived stem cells (ADSCs) present in adipose tissue [3]. Studies in cultured rodent adipocytes suggest that certain AAPs can facilitate lipid storage and stimulate adipogenesis [4,5]. However, there is limited information regarding the effect of AAPs on human preadipocytes [6]. It is not yet known how AAPs affect the differentiation process of resident preadipocytes or the terminally differentiated adipocytes, or whether increased lipid storage could cause a level of cellular stress high enough to trigger a cell death pathway at the gene expression level in adipocytes. Nevertheless, obesity is often associated with a low-grade state of inflammation that is attributed to the production of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1β, and monocyte chemoattractant protein-1 (MCP-1) in adipose tissues. As a consequence of this inflammatory environment, macrophages are recruited to the adipose tissue and, in turn, produce additional inflammatory mediators [7]. The aim of the

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Table 1
Biochemical classification of the studied genes.

Studied genes		
Cell cycle	ANAPC2	Anaphase promoting complex subunit 2
	CDK4	Cyclin-dependent kinase 4
Apoptosis	BCL2	B-cell leukemia/lymphoma 2
	BAX	B-cell leukemia/lymphoma 2
Receptors and transporters	ABCA1	ATP-binding cassette, sub-family A member 1
	LEPR	Leptin receptor
	INSR	Insulin receptor
Signal transduction	GHR	Growth hormone receptor
	IRS1	Insulin receptor substrate 1
	PPARGC1A	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
	SIRT1	Sirtuin 1
Transcription factors	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha
	SREBF1	Sterol regulatory element binding transcription factor 1
	NFκB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells
Nuclear receptors	PPARA	Peroxisome proliferator activated receptor alpha
	PPARG	Peroxisome proliferator-activated receptor gamma
Adipogenic differentiation markers	ADFP	Adipose differentiation related protein
	FABPN	Fatty acid bindin protein
Lipid metabolism enzymes	LPL	Lipoprotein lipase
	ACSL1	Acyl-CoA synthetase long-chain family member 1
Adipokines	ADIPOQ	Adiponectin
	LEP	Leptin
Cytokines and chemokines	TNF	Tumor necrosis factor
	CCL2	Chemokine (C-C motif) ligand 2
	IL1β	Interleukin 1, beta
	IL8	Interleukin 8

The expression of 26 genes in adipocytes treated with antipsychotics during adipogenic differentiation were measured using qPCR.

present study was to investigate the effect of AAP treatment on differentiated adipocytes. For this purpose, we designed a qPCR array to investigate changes in gene expression in differentiated adipocytes in the presence of AAPs. The PCR array measured the expression of 26 genes (Table 1) encoding important regulators of adipocyte function, signaling molecules involved in energy storage and expenditure, and those related to obesity. For these expression studies, we used six SGAs (olanzapine, ziprasidone, clozapine, quetiapine, aripiprazole and risperidone) and one first-generation antipsychotic (haloperidol). To date, only a few studies have examined the effect of the AAP drugs used for the treatment of psychiatric disorders at the gene expression level [5,6,8–10].

In this study we found a concerted induction of proinflammatory genes and upregulation of inflammatory mediators in response to a versatile group of antipsychotic drugs. Three of the most potent agents, clozapine, quetiapine and aripiprazole, demonstrated a clear propensity to also induce adipogenic genes.

2. Material and methods

2.1. Selection of preadipocyte tissue donors

Preadipocytes were obtained from subcutaneous abdominal adipose tissue of four healthy males aged 45–75 years who underwent a planned surgical treatment (herniotomy). The study protocol was approved by the Ethics Committee of the University of Debrecen, Hungary (No. 3186-2010/DEOEC RKEB/IKEB).

2.2. Preadipocytes were isolated, cultured and differentiated as described previously [11]

2.2.1. Drug treatment

The seven schizophrenia drugs were dissolved in DMSO (Sigma) and used in the following final concentrations: olanzapine 50 ng/mL, ziprasidone 50 ng/mL, clozapine 100 ng/mL, quetiapine 50 ng/mL, aripiprazole 100 ng/mL, haloperidol 10 ng/mL, risperidone 50 ng/mL. Drugs were added on the first day of differentiation of adipocytes and then subsequently every day until day 11. The cell culture media were replaced every third day.

2.3. PCR array

mRNA expressions were determined with CAPH09329 Custom Human RT2 ProfilerTM PCR Arrays (SABiosciences). cDNA synthesis, labeling and hybridization were carried out according to manufacturer's protocol. The fold changes for target genes presented in the figures and Supplementary information were calculated as the ratio of expression levels of the (untreated) control and AAP-treated differentiated adipocytes.

To determine which changes in gene expression were most closely correlated, the fold-changes of relative expression levels were log2-transformed and clustered by complete linkage of Euclidian distances using the Gene Cluster 3.0 software and visualized on heat maps using TreeView (Eisen lab, UC Berkeley).

2.4. Determination of cytokine release

Culture supernatants were harvested during drug treatment and stored for cytokine measurements. Media from the same donor and drug-treated sample were pooled, and the level of IL-8 and MCP-1 was measured using an ELISA DuoSet Kit (R&D). Assays were performed according to the manufacturer's protocols.

For the statistical analyses, a two-tailed paired t-test was applied. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Analysis of gene expression patterns in differentiating adipocytes in the presence of antipsychotic drugs

The homogeneity of ADSCs was characterized by FACS analysis (see Supplementary material), which revealed that more than 90% of the cell population expressed the mesenchymal stem cell-related markers CD73, CD90, CD105 and CD147 (Supplementary Table 1). On day 1 of the differentiation process, we began administering the drugs to the cells at doses comparable to their therapeutic plasma concentrations. After 11 days, there were multiple small lipid droplets in the cytoplasm, detected by laser scanning cytometry, in both treated and untreated adipocytes. Next, we analyzed the expression patterns of 26 genes. The relative expression changes of the 26 genes measured for adipocyte stages are presented in Fig. 1, showing significantly increased expression of the genes associated with adipogenesis and proinflammatory cytokine production.

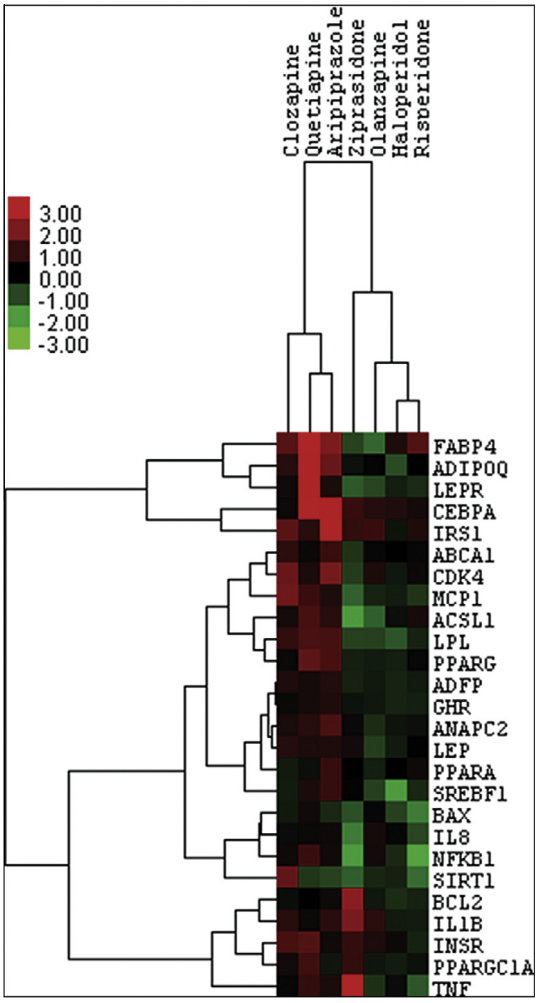

Fig. 1. Heat map of relative gene expression changes induced by antipsychotics in differentiating adipocytes. Hierarchical clustering of the 7 characterized antipsychotic drugs according to their effects on the expression profiles of inflammatory and lipid metabolic genes. The colors in the heat map from green to red indicate the mean relative log-transformed inductions of normalized gene expression in differentiated primary human adipocytes measured by quantitative RT-PCR from 4 independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Transcriptional effects of antipsychotic drugs on proinflammatory cytokine production

NF-KB1 is a transcription factor that plays a key role in the regulation of immune responses, such as the inflammatory response. Compared to controls, NF-KB1 was expressed at an increased level in differentiated adipocytes treated with antipsychotics. Most of the antipsychotics increased the gene expression of NF-KB1: olanzapine 1.8 ± 2.0-fold; clozapine 3.5 ± 5.1-fold; quetiapine 2.8 ± 2.1-fold; aripiprazole 2.7 ± 3.4-fold; and haloperidol 1.8 ± 2.5-fold (Fig. 2A). In line with this, the expression of several proinflammatory genes, including the NF-KB1 target genes TNF-α, IL-1β, IL-8 and MCP-1, was measured in differentiated adipocytes. Increased TNF-α mRNA levels were induced by ziprasidone (3.8 ± 6.0-fold), clozapine (2.3 ± 3.1-fold), quetiapine (1.8 ± 1.0-fold) and haloperidol (1.7 ± 1.7-fold) (Fig. 2B). Of the proinflammatory genes, IL-1β and IL-8 were upregulated the most during treatment with antipsychotics. Almost all of the drugs enhanced IL-1β and IL-8 expression by 1.7–8.6-fold; clozapine caused the highest increase in both IL-1β and IL-8 expression level, by 8.2 ± 11.6-fold and 7.2 ± 12.3-fold, respectively (Fig. 2C and D). Expression of the chemokine monocyte chemotactic protein (MCP-1) was moderately increased by 2.5 ± 2.0-, 1.8 ± 1- and 3.2 ± 4.3-fold in the presence of clozapine, quetiapine and aripiprazole, respectively, in comparison to the untreated control cells (Fig. 2E).

3.3. Cytokine production in culture supernatant of antipsychotic drug-treated differentiating ADSCs

To examine the effect of antipsychotic treatment on proinflammatory cytokine production, the level of TNF-α, IL-1β, IL-8 and MCP-1 in the supernatant of the adipocyte cell cultures was determined via ELISA. The level of TNF-α in the supernatant did not change significantly for any of the antipsychotic treatments except one. Upon ziprasidone treatment, the concentration of TNF-α in the supernatant was significantly higher (22.2 ± 11.5 pg/mL) compared to the untreated samples (16.0 ± 6.7 pg/mL) (data not shown). The level of secreted IL-8 was enhanced during all antipsychotic treatments compared to the control. While the untreated adipocytes produced 19.7 ± 9.8 pg/mL IL-8, clozapine and aripiprazole treatment resulted in significantly higher levels (47.1 ± 24.9 and 47.1 ± 14.6 pg/mL) (Fig. 3A). Control adipocytes secreted MCP-1 (413.2 ± 79.5 pg/mL), the level of which was increased in the presence of clozapine (534.4 ± 200.8 pg/mL), ziprasidone (520.8 ± 187.4 pg/mL) and olanzapine (718.8 ± 389.9 pg/mL) (Fig. 3B).

3.4. Effects of antipsychotic drugs on the gene expression patterns of cell cycle, apoptosis and adipogenesis regulators of differentiating ADSCs

The heat map of gene expression data of individual antipsychotic drugs revealed that one of the most effective antipsychotic drugs in terms of the modulation of gene expression was clozapine. Fig. 4 shows the mean fold changes in the expression level of non-inflammatory genes measured in differentiating ADSCs. The expression of cell cycle and apoptosis-related genes changed by 2.8–7.2-fold (Fig. 4A). The expression of signal transduction components, receptors and transcription factors showed a 1.4–9.9-fold increase (Fig. 4B). The expression level of adipogenic differentiation markers increased by 1.9–6.4-fold (Fig. 4C). The transcriptional effects of other drugs are shown in Supplementary Figs. 1–4. Quetiapine and aripiprazole enhanced the mRNA level of ANAPC2 (2.1 ± 2.4-fold and 2.7 ± 3.3-fold respectively), olanzapine, quetiapine and aripiprazole increased CDK4 expression by 1.58 ± 1.56-, 1.76 ± 1.61-, and 2.97 ± 2.37-fold, respectively.

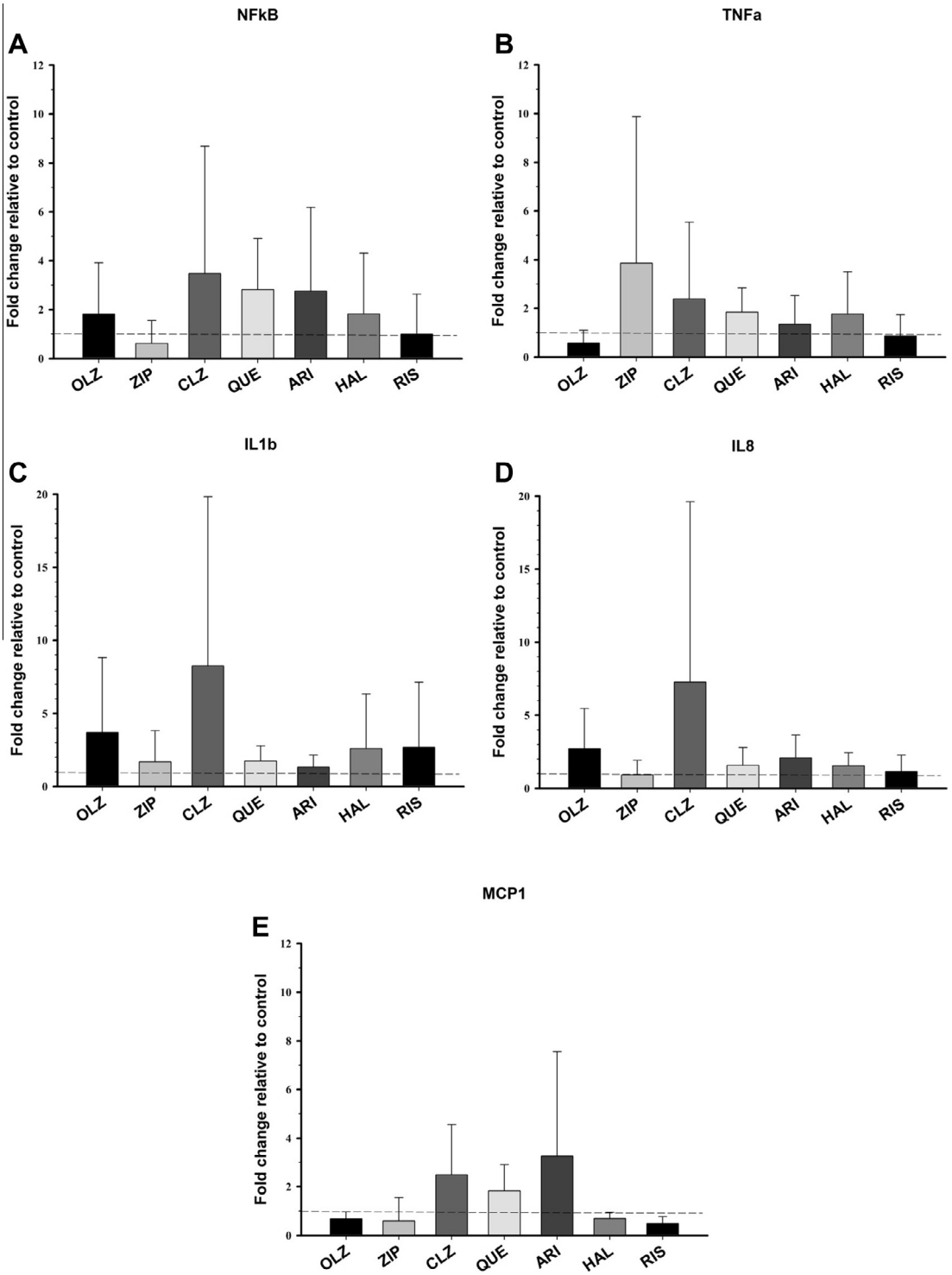

Fig. 2. Proinflammatory gene expression in differentiated adipocytes in response to antipsychotic treatment. Confluent ADSCs were differentiated into adipocytes in vitro in the presence of antipsychotics. Gene expression of NF-KB1 (A) and its target genes TNF-α (B), IL1β (C), IL8 (D) and MCP-1 (E) were analyzed by qRT-PCR after 11 days of drug treatment. All data shown are means of fold changes and standard deviations calculated from relative changes in normalized expression levels measured in cells from 4 individuals. *Abbreviations:* OLZ, olanzapine; ZIP, ziprasidone; QUE, quetiapine; ARI, aripiprazole; HAL, haloperidol; RIS, risperidone.

Whereas the expression of the anti-apoptotic gene BCL2 increased 2.1 ± 2.7- and 2.3 ± 2.6-fold in the presence of ziprasidone and aripiprazole, respectively, BAX proapoptotic gene expression decreased in the presence of almost all AAPs compared with the control, suggesting that these AAPs support cell survival (Supplementary Fig. 1). The expression of ABCA1, INSR, IRS increased after treatment with olanzapine and aripiprazole (ABCA1: 2.5 ± 3.5-fold, 2.2 ± 1.2-fold; INSR 2.5 ± 1.1-fold, 3.1 ± 3.6-fold; IRS: 2.6 ± 1.5-fold, 4.5 ± 3.3-fold, respectively). While the expression of LEPR was increased by 3.8 ± 5.7-fold and 2.8 ± 2.6-fold due quetiapine and aripiprazole treatment, respectively, GHR expression was less enhanced (1.5 ± 1.0-fold and 2.3 ± 1.4-fold)

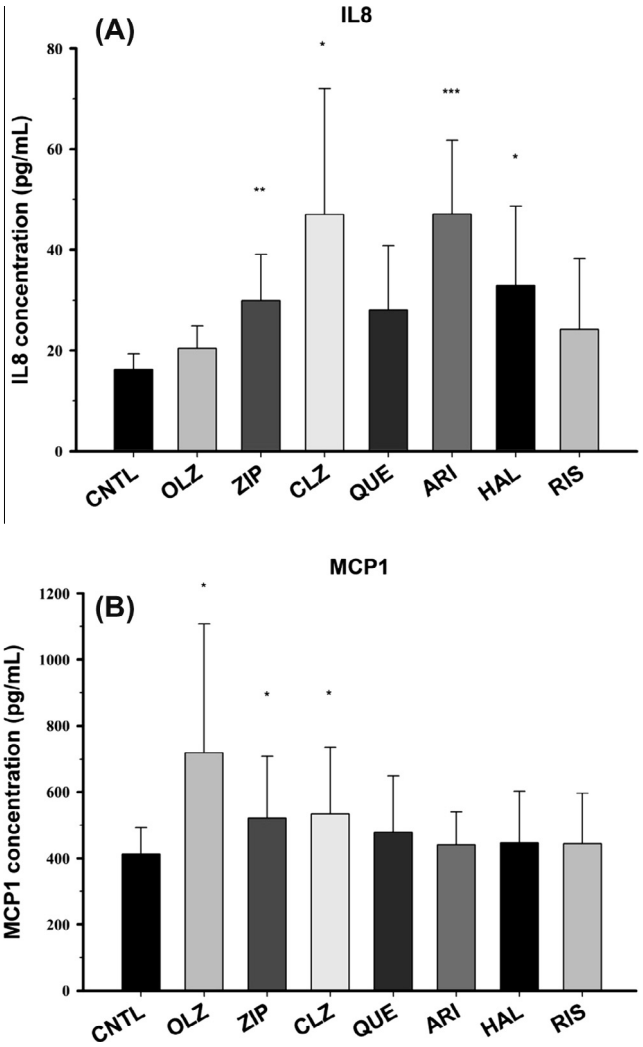

Fig. 3. IL-8 and MCP-1 secretion of antipsychotic drug treated differentiating adipocytes. Differentiating adipocytes were treated with the antipsychotic drugs every day for 11 days. Supernatant was collected every third day and stored for ELISA measurement. We pooled the supernatants from each sample and used them for the ELISA measurements (A) IL-8, (B) MCP-1. *Abbreviations:* CNLT, control; OLZ, olanzapine; ZIP, ziprasidone; QUE, quetiapine; ARI, aripiprazole; HAL, haloperidol; RIS, risperidone.

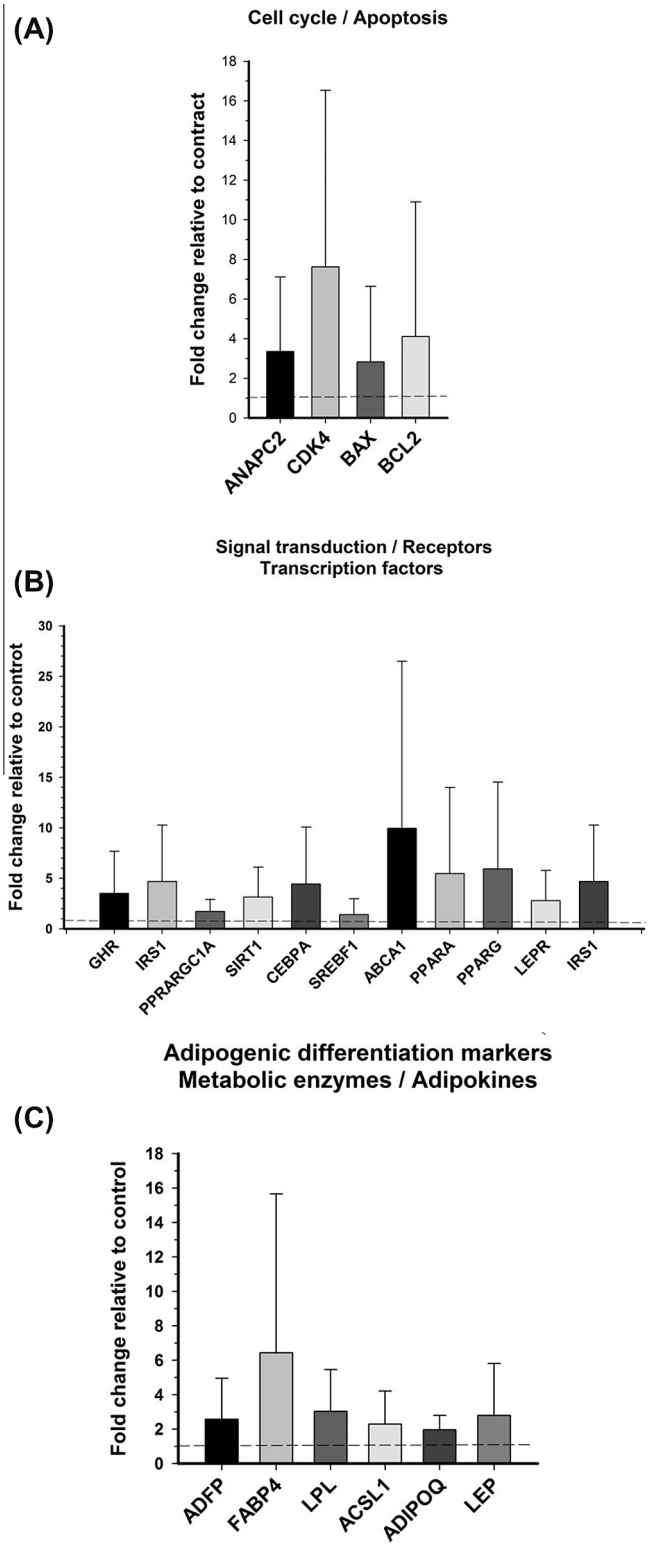

Fig. 4. Clozapine-induced gene expression changes in differentiating adipocytes. Differentiating ADSCs were treated with clozapine for 11 days. The expression of cell cycle/apoptosis genes (A), signal transduction genes/receptors/transcription factors (B), and adipogenic differentiation markers/metabolic enzymes/adipokines (C) were analyzed by qRT-PCR. All data shown are means of fold changes and standard deviations calculated from relative changes in normalized expression levels measured in cells from 4 individuals.

compared to the control. One of the most significant stimulators of mitochondrial biogenesis is PPARGC1A, which mediates the transcriptional outputs triggered by metabolic sensors such as SIRT1, a NAD+-dependent protein deacetylase. While quetiapine treatment increased the mRNA level of PPARGC1A by 1.8 ± 1.7-fold, SIRT1 expression was upregulated by aripiprazole (1.7 ± 2.5-fold) (Supplementary Fig. 2). Analysis of the pro-adipogenic genes PPAR-γ, PPAR-α, CCAAT/Enhancer Binding Protein (C/EBP-α), and sterol regulatory element-binding transcription factor 1 (SREBF1) showed moderate or high increases in expression levels after treatment with several antipsychotics. PPAR-γ expression increased by 2.7 ± 2.0- and 1.6 ± 1.8-fold, respectively, after treatment with quetiapine and aripiprazole. PPAR-α expression was also substantially higher in cells treated with olanzapine (2.0 ± 1.1-fold), aripiprazole (4.1 ± 4.4-fold) and risperidone (2.6 ± 3.5-fold). In C/EBP-α expression there was a 5.1 ± 7.1-fold increase after quetiapine treatment, and a 3.4 ± 2.9-fold increase with aripiprazole. However, olanzapine (2.0 ± 1.2-fold) and ziprasidone (1.7 ± 1.4-fold) enhanced C/EBP-α expression only moderately. SREBF1 expression increased by 1.9 ± 2.5- and 2.7 ± 2.8-fold in the presence of ziprasidone and aripiprazole, respectively (Supplementary Fig. 3).

The adipogenic differentiation marker, fatty acid binding protein 4 (FABP4), the metabolic enzymes lipoprotein lipase (LPL) involved in the transfer of fatty acids from blood triacylglycerol to triacylglycerol stores of adipocytes, acyl-CoA synthetase-1 (ACSL1), and the adipokine (ADIPOQ) are PPAR target genes. The changes in the expression of FABP4, LPL and ACSL1 were coordinated with that of PPAR-gamma. Upon treatment with olanzapine, quetiapine, aripiprazole, haloperidol and risperidone, the expression of FABP4 mRNA increased by 2.4 ± 2.9-, 5.8 ± 5.3-, 1.7 ± 1.7-, 1.7 ± 1.6- and 1.8 ± 1.7-fold, respectively. Treatment with olanzapine, quetiapine, aripiprazole and risperidone increased LPL expression by approximately 2.0-fold. ACSL1 mRNA expression increased by 1.8 ± 0.3-, 1.7 ± 0.6- and 2.7 ± 2.2-fold in the presence of olanzapine, quetiapine and aripiprazole, respectively. Olanzapine, quetiapine and aripiprazole had moderate effects on ADIPOQ expression (2.2 ± 1.2-, 2.5 ± 2.8- and 1.4 ± 0.6-fold increase, respectively). Leptin expression was increased by 1.7 ± 1.9- and 2.7 ± 0.2-fold respectively in the presence of quetiapine and aripiprazole in differentiated adipocytes. However, aripiprazole was the only AAP that enhanced adipose differentiation-related protein (ADFP) expression slightly, by 1.9 ± 1.5-fold (Supplementary Fig. 4).

4. Discussion

Although AAPs and SGAs are widely prescribed for the treatment of several psychiatric disorders, we have relatively little information about how these drugs affect gene expression in various tissues and whether this varies among individuals. It is also unknown whether changes in peripheral tissues, such as adipose tissue, could manifest as undesirable side effects of SGAs, such as weight gain and metabolic disorders, in which adipose tissue is a crucial site for inflammatory responses and mediators [7,12]. To answer these questions, systematic qPCR analysis was used to measure several candidate genes of adipocyte-derived hormones, receptors and genes related to energy expenditure. One of the most remarkable findings from these gene expression studies was that we observed significant effects of some of these antipsychotics on key genes involved in the regulation of critical adipose biochemical processes, for example, signal transduction, mitochondrial biogenesis, adipogenesis and metabolism. In these subclasses, of the 26 genes measured, clozapine enhanced the expression of 21 genes, aripiprazole 20, quetiapine 18, olanzapine 13, ziprasidone 7 and risperidone 6 genes. In contrast, the first-generation antipsychotic haloperidol induced a minor increase in the expression of only one gene (Supplementary Figs. 1–4). Several studies have shown that aripiprazole treatment may have positive metabolic effects in patients treated with other atypical antipsychotics [13–15]. Our experiments show that in adipocytes aripiprazole induced gene expression of INSR, IRS1, PPARA, LPL, LEP, ADIPOQ and SIRT, which are all key regulators of energy storage, expenditure and mitochondrial biogenesis, and may have a remarkable effect on energy metabolism and lead to a healthier body weight, lower triglyceride levels in the plasma and improved insulin sensitivity. While ziprasidone enhanced the expression of seven genes to a moderate degree, quetiapine induced a greater increase in the expression of 18 genes. It has been reported that patients who switched from quetiapine to ziprasidone showed improvement in clinical symptoms, weight loss and lipid profiles [16]. While ziprasidone decreased GHR expression, quetiapine enhanced it, which may result in the inhibition of insulin-stimulated glucose uptake in adipocytes [17]. One of the most notable related findings is that mice harboring a disrupted GHR gene show extreme insulin sensitivity in the presence of obesity [18]. The expression of 19 genes out of the 26 remained unchanged, consistent with ziprasidone's main advantage in terms of a low propensity to induce weight gain and associated adverse effects [2,19].

Inflammatory abnormalities may be involved in the pathophysiology of schizophrenia, although some inflammatory processes may emerge epiphenomenally during treatment. It is also known and widely accepted that macrophages account for almost all obesity-related proinflammatory cytokine production [20]. In olanzapine-treated rats, TNF-α expression increased significantly in adipose tissues with widespread macrophage infiltration, suggesting that macrophages were the source of the overexpression of TNF-α [21]. In our human in vitro differentiated adipocytes treated with antipsychotics we observed a concerted increase in the mRNA levels of the transcription factor NF-KB1 and its target genes, the proinflammatory cytokines TNF-α, IL-1β and IL-8, and the MCP-1. This suggests that chronic treatment with antipsychotics that induce weight gain may cause a low-level proinflammatory state in patients that is initiated by adipocytes. When the adipocytes were treated for up to 34 days, each antipsychotic induced NF-KB1 expression to various extents between day 11 and day 34. This increase in NF-KB1 expression was associated with a coordinated increase in the expression of the proinflammatory cytokines TNF-α, IL-1β and IL-8, and the chemokine MCP-1 was also elevated by day 34 in almost every case (data not shown). An elevated level of MCP-1 could potentially contribute to the infiltration of monocytes/macrophages into adipose tissues, which could further increase the inflammatory properties of adipose tissues. More importantly, high levels of TNF-α have been shown to reduce the function of both IRS1 and glucose transporter 4, and elevated IL-8 expression may inhibit insulin-induced AKT phosphorylation in adipocytes. Together, these changes could cause a critical level of inhibition of insulin activity, leading to insulin resistance and metabolic disorders [22–25]. Clozapine treatment has been associated with elevated weight gain and TNF-α plasma levels [26–28]. According to our clozapine gene induction data and previously published results, although TNF-α mRNA expression was elevated, clozapine concurrently greatly enhanced the expression of both PPARG and adipocyte hormones (Figs. 2 and 4). PPARG is necessary for both adipocyte differentiation and the normal lipid metabolism. Adipocyte hormones such as leptin and adiponectin regulate both glucose and fat metabolism, including food uptake and energy expenditure [10]. While leptin can limit food intake and increase energy expenditure, via which it can regulate the overall body weight, adiponectin plays an important role in insulin sensitization and maintaining energy homeostasis [29,30].

In addition to these biologically important trends, our data revealed a high donor-dependent variability in the effects of antipsychotic treatment, especially in the case of clozapine. This may be explained, in part, by many factors including age, gender, a limited number of donors and the number of neurotransmitter receptors of differentiated ADSCs in cell cultures. The unique feature of this study is the use of primary human cells for these investigations, but this advantage also represents a limitation in data interpretation.

The results obtained in ex-vivo experiments with cultured adipocytes examining the effects of drugs used for the treatment of several psychiatric disorders may be helpful for clarifying the development and the sequence of events that determine the occurrence of weight gain accompanying a low-level inflammatory state during antipsychotic treatment. These data suggest that independently of the primary inflammatory process of the illness, a secondary adipocyte-dependent inflammatory abnormality could develop, which could support the monocyte-macrophage accumulation due to MCP-1 expression, and thus the infiltrating macrophages would be the third source of the proinflammatory cytokine production in adipose tissue, which may further

contribute to the development of metabolic syndrome associated with SGA treatment.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.005.

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