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Review

# Adipose tissue as a target for second-generation (atypical) antipsychotics: A molecular view

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ABSTRACT

Schizophrenia is a neuropsychiatric disorder that chronically affects 21 million people worldwide. Second-generation antipsychotics (SGAs) are the cornerstone in the management of schizophrenia. However, despite their efficacy in counteracting both positive and negative symptomatology of schizophrenia, recent clinical observations have described an increase in the prevalence of metabolic disturbances in patients treated with SGAs, including abnormal weight gain, hyperglycemia and dyslipidemia. While the molecular mechanisms responsible for these side-effects remain poorly understood, increasing evidence points to a link between SGAs and adipose tissue depots of white, brown and beige adipocytes. In this review, we survey the present knowledge in this area, with a particular focus on the molecular aspects of adipocyte biology including differentiation, lipid metabolism, thermogenic function and the browning/beiging process.

## 1. Introduction

### 1.1. Etiology and pathophysiology of schizophrenia and its treatment with antipsychotic agents

Schizophrenia is a chronic, severe mental disorder that affects about 21 million people worldwide. The disease typically manifests in late adolescence/early adulthood and usually involves positive symptoms that reflect an excess or distortion of normal functions, resulting in behavior problems such as delusions, hallucinations, trouble thinking and concentrating; and/or negative symptoms related to withdrawal or lack of normal cognitive functions, including apathy, avolition, alogia and anhedonia [1,2]. Often, the symptoms are associated with psychotic episodes that disrupt the stability and quality of life of patients, denying them a normal life in society. Fortunately, pharmacological interventions are effective in suppressing the symptomatology sufficiently, restoring a productive life and allowing the integration of patients into society [3]. According to current guidelines, antipsychotic agents are the first line of treatment in schizophrenia [4,5]. Because discontinuation of treatment is associated with an exponential risk of relapse as compared with maintenance therapy, patients generally continue the same treatment that was effective in the acute phase for as long as it is well tolerated.

Since the introduction of chlorpromazine in 1952, the first-generation antipsychotics (FGAs) have changed psychiatric care dramatically, allowing many patients with debilitating and severe mental illnesses (schizophrenia, bipolar mania and acute agitation, among other conditions) to reintegrate into society. FGAs predominantly counteract the positive symptoms of schizophrenia through mechanisms that remain unknown. The most accepted hypothesis of FGAs action relates to the dopaminergic theory of schizophrenia, which posits that the positive symptomatology is caused by the increased subcortical release of

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*Abbreviations:* SGAs, second-generation (atypical) antipsychotics; FGAs, first-generation (typical) antipsychotics; D2, dopamine-2; L-DOPA, l-3,4-dihydroxyphenylalanine; WAT, white adipose tissue; TG, triglyceride; BAT, brown adipose tissue; UCP-1, uncoupling protein-1; SREBP, sterol regulatory element-binding protein; FAS, fatty acid synthase; C/EBP, CCAAT/enhancer binding protein; SCD1, stearoyl-CoA desaturase-1; ASC, adipose-derived stem cells; CNS, central nervous system; Insig, insulin-induced gene; SCAP, SREBP cleavage-activating protein; ER, endoplasmic reticulum; PPAR-γ, peroxisome proliferator-activated receptor gamma; LPL, lipoprotein lipase; PKC, protein kinase C; MSCs, mesenchymal stem cells; PLIN, perilipin; ATGL, adipose tissue triglyceride lipase; FFA, free fatty acids; NF-κB, nuclear factor-κB; TNF, tumor necrosis factor; IL, interleukin; AMPK, 5′ adenosine monophosphate-activated protein kinase; HSL, hormone-sensitive lipase; ACC, acetyl-CoA carboxylase; IBMX, 3-isobutyl-1-methylxanthine; TSPO, translocator protein; GLUT4, glucose transporter-4; BMI, body mass-index; S100B, calcium binding-protein B; ERK, extracellular signal-regulated kinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; AP2, adipocyte protein 2

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dopamine and the subsequent enhanced activation of dopamine-2 (D2) receptors [6,7], likely derived from disturbances in the cortical pathway through the nucleus accumbens. Conversely, the negative symptoms appear to be caused by blunted dopaminergic signaling through reduced dopamine-1 receptor activity in the prefrontal cortex and diminished nucleus caudatus activity [7–9]. Alterations in the expression and activity of dopamine-3 receptors have also been associated with the negative symptoms of schizophrenia [10]. Some studies have corroborated the dopaminergic theory of schizophrenia, showing that D2 antagonists such as FGAs act on different dopaminergic pathways (mesolimbic, mesocortical, nigrostriatal and tuberoinfundibular) to control schizophrenia symptomatology. By contrast, D2 agonists including l-3,4-dihydroxyphenylalanine (L-DOPA), cocaine and amphetamines, trigger psychomimetic effects in individuals that are not schizophrenic *per se*. Nevertheless, FGAs are associated with extrapyramidal side-effects such as dyskinesia and dystonia [11]. In an attempt to counteract this, a variety of new agents were investigated in the 1990s, leading to the approval of second-generation (atypical) antipsychotics (SGAs), which are now the mainstay for patients with schizophrenia and other psychotic disorders [4]. Beyond the interaction of SGAs with dopaminergic receptors, as for FGAs, they also have the ability to block serotonergic (5-HT$_{2A}$ and 5-HT$_{2C}$) receptors, with a higher affinity than that for D2 receptors [8,11], suggesting that alterations in serotoninergic pathways could also play a role in schizophrenia development. More importantly, the neurological side-effects associated with FGAs are not as evident in patients on SGAs, which can counteract both positive and negative symptoms of the disease [4]. However, clinical observations have revealed a variety of discrete metabolic dysfunctions in a relevant proportion of patients on SGAs, such as abnormal body weight gain, hyperglycemia and dyslipidemia [12–14]. These side-effects (Table 1) suggest that the use of SGAs has indirect effects on different peripheral tissues and systems, including fat depots, liver and immune cells.

### *1.2. Adipose tissue: a hub for energy balance and endocrine signaling*

Abnormal body weight gain is a major side-effect of therapy with SGAs [11] and is associated with an increase in fat (adipose) depots [3,14,15]. Adipose tissue is a highly specialized organ regulating energy homeostasis and metabolism, and is comprised mainly of three general classes of adipocytes in mammals – white, brown and beige adipose cells – which have distinct developmental origins, morphologies, lipid droplet distribution, mitochondrial networks and gene expression patterns [16].

Functionally, white adipose tissue (WAT) is the predominant store of surplus energy in the body, in the form of triglycerides (TGs), and contains adipocytes with a large unilocular lipid droplet and few mitochondria. WAT accounts for 5–50% of the total body weight in humans and includes both visceral (within the abdominal cavity) and subcutaneous (underneath the skin) depots, with important ontogenetic and metabolic differences between the two [16]. Under a state of positive energy balance, adipose tissue expands *via* hypertrophy of existing adipose cells and *via* hyperplasia, with *de novo* formation of adipocytes. Beyond its classical role as an energy storage and release unit, WAT also functions to protect other organs (liver and muscle) from lipid-associated toxicity (lipotoxicity) [17] and is a key player in endocrine signaling [18].

Brown adipose tissue (BAT) is much less abundant than WAT and is located mainly in subcutaneous interscapular regions. BAT is characterized by the presence of small multiocular adipocytes that were initially perceived as skeletal muscle-like lineage cells, but more recently their adipocyte origin has been suggested [19,20]. BAT is abundant in mitochondria enriched in uncoupled protein-1 (UCP-1) in the inner membrane, which functions to dissipate chemical energy in the form of heat by uncoupling fuel oxidation from ATP synthesis [21–23]. In humans, BAT was previously believed to exist only in newborn infants, but it has recently been identified in adults in the lower neck area [24–26], where its functions and characteristics are currently under extensive study.

Beige adipocytes are mainly present in subcutaneous white depots and in restricted amounts of visceral WAT [27]. Beige adipocytes are considered brown-like thermogenic adipose cells with a similar multilocular lipid droplet morphology and UCP-1 expression, and are developed from the so-called *beiging* or *browning* of WAT. This differentiation phenomenon is induced by cold stress or agonists that can mimic this effect, such as β3-adrenergic agonists [28,29]. The origin of beige adipocytes is, however, not completely understood and several processes seem to be involved; for instance, they can be derived from a beige progenitor lineage [19,27,30], but can also transdifferentiate from mature white adipocytes [31,32], or even differentiate from other origins [16].

As an endocrine organ, adipose tissue actively participates in inflammatory processes by producing and secreting a wide variety of bioactive peptides including cytokines and the so-called adipokines such as leptin and adiponectin. These peptides have both local and distant actions related to the modulation of lipid and glucose metabolism and energy balance [16].

Given the evident metabolic dysregulation in some patients on SGAs, a better understanding of the effects of these drugs on adipocytes will be important for elucidating the molecular mechanisms underlying these dysfunctions in patients. Accordingly, this review will examine recent advances in our understanding of the impact of SGAs on the mechanisms of adipocyte differentiation and function.

## 2. Effect of second-generation antipsychotics on white adipose tissue

WAT is organized into discrete depots – mainly visceral and

**Table 1**

List of first generation and second-generation antipsychotics mentioned in this review and their side-effect profiles.

| Antipsychotics mentioned in this Review | Extrapyramidal side effects in Humans | | | Metabolic side effects in Humans | | |
|---|---|---|---|---|---|---|
| | Sedation | Cognitive impairment | Tardive dyskinesia | Diabetes | Increased Lipids/dislipidemia | Hyperprolactinemia |
| Chlorpromazine | + + | + + | + + | + + + | + + + | + |
| Haloperidol | + | 0 | + + | 0/+ | 0/+ | + + |
| Pimozide | 0/+ | + | + + + | 0/+ | 0/+ | + + |
| Clozapine | + + + | + + + | 0 | + + + | + + | + |
| Olanzapine | +/+ + | + + | 0/+ | + + + | + + + | + |
| Aripiprazole | 0/+ | 0 | 0/+ | 0/+ | 0/+ | 0 |
| Quetiapine | + + | +/+ + | 0/+ | + + | + + | 0 |
| Risperidone | + | 0 | 0/+ | + | + | + + + |
| Ziprasidone | + | 0 | 0/+ | 0/+ | 0/+ | + |
| Blonanserin | 0/+ | 0 | + | 0/+ | + | + |

0: none; 0/+ sporadic; +: occasionally; + +: recurrent; + + + very often.

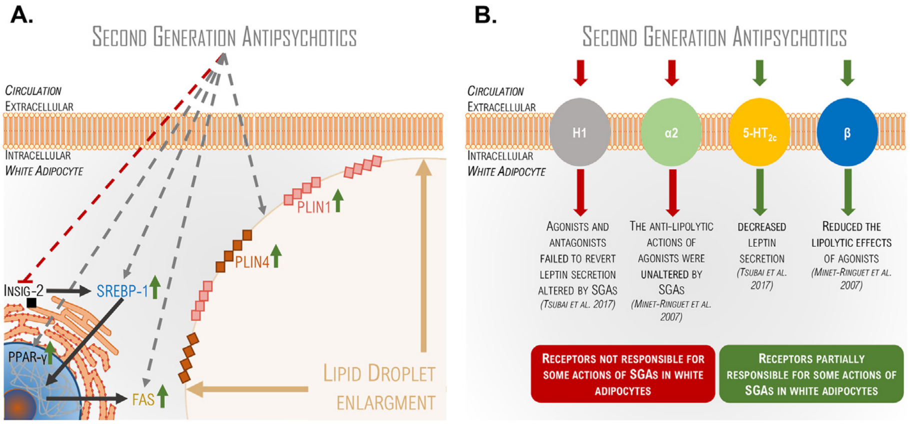

**Fig. 1.** Impact of SGAs in white adipocytes. A. Scheme of the molecular effects of SGAs in white adipocytes focusing on lipid-related proteins and enlargement of the lipid droplets. B. List of the receptors through which SGAs can impact white adipocytes.

subcutaneous – that are differentially associated with insulin resistance and the risk of metabolic syndrome. While the accumulation of visceral WAT is considered deleterious, due to its promotion of inflammation, subcutaneous WAT seems to be protective against the development of metabolic diseases in mice and humans [33,34].

It has been described that patients with schizophrenia have significantly more visceral WAT than healthy individuals [15], and this could explain their susceptibility to develop insulin resistance and more severe metabolic dysfunctions such as type 2 diabetes mellitus [35]. As mentioned, SGAs therapy induces an increase of both subcutaneous and visceral fat in some patients [15], and also alters the mechanisms of differentiation and response to physiological stimulus, as described below.

### *2.1. Effect of second-generation antipsychotics on the differentiation of white adipocytes*

A possible explanation for weight gain associated with SGAs is their positive influence on the differentiation of preadipocytes to mature adipocytes. In relation to adipocyte differentiation, the sterol regulatory element-binding protein (SREBP) family of transcription factors, constituted by the SREBP-1a and -1c isoforms (encoded by the SREBF1 gene) and SREBP-2 (encoded by SREBF2) [36], play a major regulatory role. Yang et al. showed that treatment of differentiating murine 3T3-L1 preadipocytes with the SGA olanzapine (10 or 50 μM for 24 h) increases TG accumulation and activates SREBP-1, accompanied by the overexpression of fatty acid synthase (FAS), an enzymatic complex of long-chain fatty acid synthesis [36]. They also found that SREBP-1, but not the transcription factor CCAAT/enhancer binding protein α (C/EBP-α), is overexpressed and activated in olanzapine-stimulated 3T3-L1 cells. Similarly, olanzapine (100 μM for 3 h) increases lipogenesis and reduces lipolysis [37]. Likewise, clozapine (10 μM) induces stearoyl-CoA desaturase-1 (SCD1) and SREBP-1 expression levels at both early (day 3) and late (day 7) stages of human adipose-derived stem cells (ASC) differentiation [38]. Overall, these studies show that some SGAs increase the expression of SREBP-1 and its downstream lipogenic targets, although to different levels. Indeed, it has been suggested that the effects of SGAs on lipid droplet formation (clozapine > olanzapine > risperidone) depend on the nuclear translocation of SREBP-1 and the subsequent modulation of adipogenesis [38]. Moreover, in the human hepatic HepG2 cell line, treatment with olanzapine (10 and 50 μM for 24 h) increases SREBP-1 response element activity in a dose-dependent manner [39]. However, these effects are not so evident in other hepatic cell types treated with olanzapine, haloperidol or mirtazapine (25 μM for 24 h), as reported by Raeder and co-workers [40]. Given the aforementioned results in adipocyte culture, we speculate that in addition to acting on the central nervous system (CNS) leading to weight gain and lipid abnormalities [41,42], SGAs function in a cell-autonomous manner by increasing lipogenesis.

The insulin-induced gene (Insig) family proteins are important negative regulators of SREBP function and lipogenesis. Insigs are SREBP-chaperone proteins that form a complex with SREBP and SREBP cleavage-activating protein (SCAP) in the endoplasmic reticulum (ER). When sterol levels are low, SREBPs are activated by proteolytic cleavage and the N-terminal domain is released from the ER and translocates to the nucleus where it acts as a transcription factor for multiple lipid biosynthesis genes [43]. The Insig family is composed of two isoforms: Insig-1, a target of nuclear SREBPs whose mRNA levels are directly associated with their presence in the nucleus, and Insig-2, which is negatively regulated by insulin in a SREBP-independent manner [36]. In sterol-replete conditions, Insig-2 can retain the SCAP/SREBP complex in the ER [44]. Of interest, a relationship between SREBP-induced lipid biosynthesis, Insig-2 blockade and SGA-associated weight gain was described by Chen et al., showing that clozapine (10 μM) significantly suppresses Insig-2 expression during adipogenesis of human ASCs, at both early and induction (late) phases. Moreover, after treatment, the levels of Insig-2 negatively correlated with the expression of SREBP-1 and lipid-biosynthesis genes. Of note, the authors found that on the third day of the differentiation, all of the SGAs tested – clozapine (10 μM), olanzapine (1 μM) and risperidone (0.4 μM) – decreased Insig-2 expression; however, only clozapine significantly suppressed Insig-2 at day 7 of differentiation and, consequently, SREBP-1 activity was increased [38]. Moreover, even though olanzapine treatment did not maintain the suppression of Insig-2 expression on the last day of differentiation, it increased SREBP-1 expression. Conversely, Insig-2 overexpression during human ASC differentiation results in a suppression of SREBP-1 expression, leading to the inhibition of SGA-induced lipid biosynthesis [38]. This study implicates Insig-2 in the pathogenesis of the metabolic dysfunctions developed by patients under SGAs treatment. Despite the fact that the interactions between the two Insig isoforms are related to SGA-induced metabolic syndrome, Insig-1 is reported not to correlate with these metabolic side effects *per se* [45] (Fig. 1A).

In mammalian cells, peroxisome proliferator-activated receptor gamma (PPAR-γ) is critical not only for adipogenic differentiation, but also for the maintenance of the mature adipocyte phenotype [46,47] and, together with C/EBPs, is considered a master regulator of adipogenesis [48]. Sertie et al. reported the impact of SGAs, particularly clozapine (used at 20–30 μM) and olanzapine (40–100 μM), in the enhancement of both early (day 3) C/EBP-β and PPAR-γ expression, and late (day 14) PPAR-γ and lipoprotein lipase (LPL) levels during adipogenic differentiation of human ASCs [49]. Consistent with this, Hemmrich et al. differentiated human ASCs in the presence of clozapine (5 and 50 μM) for the first 5 days and then in its absence for 9 additional days (14 days of differentiation in total), finding increased activity of glycerol 3-phophatase dehydrogenase, another key marker of adipogenic differentiation. They also found that clozapine increases the percentage of differentiated cells when compared with untreated controls [50]. These results provide a plausible explanation as to why clozapine treatment is associated with a higher risk of weight gain in relation to other SGAs [51,52]. Likewise, other agents, particularly pimozide (10 μM), is reported to promote adipogenesis of 3T3-L1 preadipocytes up to day 6 of differentiation by inhibiting fatty acid binding protein 4 and upregulating PPAR-γ protein levels of the cells [53]. Similarly, treatment of human ASCs for 3 days with clozapine, olanzapine, quetiapine or risperidone (all used at 250 nM) increases total lipid production, an effect blocked by the addition of a protein kinase C (PKC)-β inhibitor [51]. At the molecular level, this study shows that treatment of ASCs with SGAs, particularly clozapine, promotes the translocation of PKC-β to the plasma membrane and its activation. Importantly, adipogenesis of skeletal muscle-derived stem cells is also boosted by SGAs, suggesting that the weight gain associated with SGAs also involves the commitment of adipogenic features of other stem cell pools [54].

The increase in body weight associated with SGAs treatment can also result from alterations in adipocyte lipid storage (hypertrophy). For instance, differentiation of rat ASCs in the presence of clozapine (10 μM) increases the formation of lipid droplets, whereas the addition of olanzapine (1 μM) or risperidone (0.4 μM) induces only a slight enhancement at day 7 [38]. Likewise, in adipocytes differentiated from human mesenchymal stem cells (MSCs), olanzapine treatment (5–100 μM) up to 12 days increases lipid accumulation in a dose-dependent manner [52]. Further proteomic studies revealed that olanzapine upregulates the levels of perilipin-4 (PLIN4) and other enzymes related to lipid metabolism including FAS. Indeed, MSCs differentiated in the presence of olanzapine show enlarged lipid droplets coated with PLIN1, 2 and 4. However, only PLIN2 protein levels are upregulated by olanzapine at early stages of differentiation and decrease from day 12, whereas PLIN4 and PLIN1 increase at later stages. While it is known that PLIN proteins are regulated by PPAR-γ, the authors failed to find alterations in its mRNA levels by olanzapine. However, C/EBP-α mRNA was decreased at day 9–16 in the presence of olanzapine, which could downregulate its target protein fatty acid translocase/CD36 in late stages of differentiation [55]. Nonetheless, the relevance of this downregulation is controversial as the deletion of fatty acid translocase/CD36 in mice was found to confer protection from high-fat diet-induced adipose tissue deposition [56]. Of note, other genes encoding relevant proteins of lipid metabolism such as SREBP-1c, FAS, LPL, leptin and adiponectin, were unaltered by the treatment. The aforementioned study also showed that the expression of adipose tissue triglyceride lipase (ATGL) is increased by olanzapine. In adipocytes, ATGL is localized on large PLIN1-positive lipid droplets and is liberated upon PLIN1 phosphorylation, leading the authors to postulate that ATGL expression increases in olanzapine-treated MSCs concomitant with the accumulation of lipid droplets (Fig. 1, panel A). Moreover, changes in gene expression and protein levels of PLIN family members by olanzapine might suggest a possible downstream mechanism for the increased adiposity in patients undergoing treatment with this drug [55].

Dyslipidemia is another metabolic side-effect of SGAs therapy and is directly related to the ability of adipocytes to sequester free fatty acids (FFA). In this regard, a study with human adipocytes showed that clozapine, olanzapine, quetiapine or risperidone (all tested at 50 or 100 μM) failed to inhibit fatty acid transport, concluding that this is likely not the mechanism for dyslipidemia observed in patients treated with SGAs [57]. Despite all these achievements, further studies are required for a better understanding of the impact of SGAs on the early and late events of adipogenesis, as well as on the hypertrophy of the adipose cells.

### *2.2. Second-generation antipsychotics modify the endocrine function of white adipocytes*

Adipose tissue is a main player in systemic metabolism and inflammation, or so-called immunometabolism [16,58], and produces and releases a plethora of biomolecules with paracrine, autocrine and endocrine functions associated with the balance between energy expenditure and intake, regulation of lipid and glucose homeostasis, and also inflammatory processes [16].

Adiponectin is the most abundant of the adipokines and is also one of the most relevant molecules secreted by mature adipocytes, and it is negatively regulated in obesity, insulin resistance and metabolic syndrome [59]. Adiponectin modulates pathways related to carbohydrate and lipid metabolism, and also vascular processes, due to its anti-inflammatory, anti-atherogenic and insulin sensitizing properties [60]. Several studies have investigated the adiponectin response to SGAs. Human ASCs differentiated in the presence of clozapine (100 ng/ml), quetiapine (50 ng/ml) or aripiprazole (100 ng/ml) show elevated adiponectin expression [61] and, similarly, olanzapine (10 μM) treatment induces early adiponectin expression in differentiating 3T3-L1 cells [39]. In another study, short (2 days in mature adipocytes) or long (10 days, including during differentiation) clozapine (10–30 μM) treatment in 3T3-L1 adipocytes was found to decrease adiponectin secretion without altering its mRNA levels [62], and 10 days exposure to blonanserin (0.01–0.1 μM) also reduced adiponectin mRNA levels. Thus, this opposite effect on adiponectin expression/secretion is intriguing and may be dependent on the adipocyte origin or the sensitivity of the cell-based systems to SGAs.

In contrast to adiponectin, leptin is a secreted adipokine that is positively related to the amount of body fat, and modulates food intake and energy expenditure [63]; accordingly, leptin is typically elevated in the serum of obese patients [64]. Tsubai et al. reported that exposure of 3T3-L1 adipocytes to clozapine (10–30 μM) for 2 or 10 days decreases leptin mRNA levels and also its secretion into the culture medium [62]. Of interest, this work tested the role of serotonergic 5-HT$_{2c}$ and histaminergic H$_1$ receptors on the impact of clozapine in leptin expression and secretion, as they are ubiquitously expressed in the brain and peripheral tissues [65,66], and play a role in alterations/disorders of eating patterns that lead to dysregulated lipid metabolism [67–69]. The authors used histamine (H$_1$ receptor agonist) and diphenhydramine (H$_1$ receptor antagonist) as well as serotonin (5-HT$_{2c}$ receptor agonist) and SB242084 (5-HT$_{2c}$ receptor antagonist). Short-term treatment of 3T3-L1 cells with histamine or diphenhydramine failed to modify leptin secretion, suggesting that this process is not mediated through H$_1$ receptors. However, treatment with serotonin decreased leptin secretion when compared to control-treated cells, but no synergistic or additive effect was found when it was combined with clozapine (30 μM). Interestingly, SB242084 also decreased leptin secretion in 3T3-L1 cells and further decreased secretion when combined with clozapine (30 and 50 μM) in a dose-dependent manner. Nevertheless, this relationship seems to be more complex because serotonin failed to reverse this effect by increasing leptin secretion (Fig. 1, panel B) [62]. By contrast, in the study performed in human ASCs, treatment with SGAs increased leptin expression [61]. Thus, more studies are required to fully understand the mechanisms governing the interactions between SGAs and serotonergic receptors in adipocyte hypertrophy and adipokine secretion.

Adipocytes secrete cytokines with pro-inflammatory properties, which are the main drivers of the chronic low-grade inflammation associated with obesity-related metabolic abnormalities [70]. In the aforementioned study of Sarvari et al. [58], *in vitro* treatment of human ASCs with olanzapine, ziprasidone, clozapine, quetiapine, aripiprazole, risperidone or haloperidol induced the expression of the transcription factor nuclear factor-κB (NF-κB), a key component of the inflammatory response, and this was accompanied by an increase in the expression of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8 and MCP-1 and the release of IL-8 and MCP-1 into the culture medium. These results suggest that antipsychotic treatments might “prime” patients for developing a low-grade chronic pro-inflammatory state. Indeed, exposure of 3T3-L1 adipocytes to clozapine (30 μM) for 10 days increases MCP-1 and IL-6 expression [62]. This increase in the production of pro-inflammatory molecules in turn promotes insulin resistance and exacerbates metabolic dysfunction [71,72]. Of note, adiponectin can block local pro-inflammatory cytokine production by antagonizing toll-like receptors (TLRs) [73] and inhibiting NF-κB [74,75]. Thus, the hypoadiponectinemia associated with hyperleptinemia following SGAs treatments is accompanied by the increased expression and secretion of pro-inflammatory cytokines [61,62]. The altered pattern of pro-inflammatory cytokine secretion in adipocytes and the “shotgun” affinity of SGAs for several receptors including histaminergic $H_1$, serotonergic 5-$HT_{2c}$, and adrenergic receptors [35], is likely the cause of the development of insulin resistance and type 2 diabetes mellitus associated to the treatment with theses drugs.

### 2.3. Second-generation antipsychotics modulate insulin sensitivity and glucose uptake in white adipocytes

In addition to the control of whole body energetic balance, adipose tissue also regulates glucose and lipid metabolism [16,58,76]. Glucose transport resulting from activation of insulin signaling is highly relevant because it provides both fatty acids and glycerol for TG synthesis [77,78]. Vestri et al. compared the effects of clozapine, olanzapine, risperidone and quetiapine (1–500 μM) with the FGAs butyrophenone and trifluoperazine for insulin-induced glucose transport and lipogenesis/lipolysis in 3T3-L1 cells and rat primary adipocytes. In both systems, olanzapine and clozapine at concentrations as low as 5 μM strongly reduced insulin-induced glucose transport, whereas the FGAs failed to modify this response. Moreover, all of the antipsychotics (tested at 100 μM) increased basal and insulin-induced glucose oxidation rates [37]. Interestingly, another study showed that 3T3-L1 cells treated with haloperidol, quetiapine or clozapine at 10 μM, but not 1 μM, present reduced glucose uptake without alterations in insulin sensitivity or in Akt/protein kinase B activation [79]. In the same line, Robinson et al. found that therapeutic concentrations of olanzapine (7–350 nM, see Table 2) failed to alter basal and insulin-induced glucose transport in 3T3-L1 cells [80]. These results suggest that the effects of SGAs on glucose transport are dose-dependent. Moreover, FGAs did not affect lipolysis in response to isoproterenol or glucose uptake in response to insulin, which might explain why conventional therapies are less associated with secondary metabolic side effects [37].

### 2.4. White adipose tissue and antipsychotic effects in animal models: dysregulation of lipid metabolism

Animal models provide a translational platform to decipher the molecular basis of the metabolic side effects associated with SGAs. Several studies have reported that clozapine and olanzapine are responsible for the higher weight gain associated with deficits in glucose and lipid metabolism in rodents [81–84]. For example, Yang et al. described that female Sprague-Dawley rats treated with olanzapine (2 mg/kg twice a day by oral gavage) for 2 weeks show increases in total body weight gain, which is mainly due to an increase in liver and WAT weight [85]. Moreover, the animals develop hyperlipidemia, hyperglycemia, hyperinsulinemia, insulin resistance and present elevated serum IL-6 levels together with tissue chromium depletion. Interestingly, daily supplementation of chromium during olanzapine treatment produces a milder phenotype and supplementation with AICAR, an 5′ adenosine monophosphate-activated protein kinase (AMPK) activator, ameliorates olanzapine-induced hyperglycemia and hyperlipidemia, suggesting that low chromium and AMPK activity are related to olanzapine-induced metabolic dysfunction [85]. Interestingly, female rats are reported to be more susceptible than male rats to the metabolic damaging effects of SGAs [81,86]. Albaugh et al. reported that an increasing dosing regimen of olanzapine mixed in cookie dough (4 mg/kg from day 0–6; 8 mg/kg from day 7–20 and 12 mg/kg from day 21–29) increases the body weight of female, but not male, Wistar and Sprague-Dawley rats [81]. Comparable results were previously reported by Pouzet and co-workers in female Wistar rats treated with 5 and 20 mg/kg of olanzapine *via* oral administration for 21 days [86]. Furthermore, Goudie et al. showed that female Wistar rats injected intraperitoneally with olanzapine (4 mg/kg) for 19 days increased their body weight [83]. Of note, it has been described that the weight gain is very fast at the beginning of the treatment and is reversible once treatment is ended [83,86]. In this respect, Albaugh et al. provided evidence for the association between body weight gain and hyperphagia in female rats receiving olanzapine self-administered *via* cookie dough, starting from the first 24 h of treatment [81]. In the same study, olanzapine consumption led to an increase in leptin levels and adiposity, and induced mild insulin resistance from day 12 of treatment, suggesting that olanzapine-induced weight gain could be a secondary effect of hyperphagia related to leptin resistance (hyperleptinemia). By contrast, in a study performed in female Sprague-Dawley rats injected intramuscularly with olanzapine (100 mg/kg) for 14 days in a 4-injection treatment, Horska et al. reported body weight gain together with a significant increase in the amount of visceral white fat, but without hyperphagia [87]. Furthermore, in addition to increases in both adiposity and adipocyte size, Tan et al. reported that olanzapine treatment (ranging from 0.003 mg/ml to 0.03 mg/ml) in the drinking water causes morphological alterations in subcutaneous WAT of female Wistar rats, an effect related to an increase in the number of undifferentiated adipose cells in this depot that was detected as early as the third day of treatment in a dose- and time-dependent manner, but independently of the body weight gain [87,88].

In contrast to what is found in female animals treated with SGAs, the body weight increase is more difficult to mimic in males. Minet-Ringuet et al. showed that male Sprague-Dawley rats fed with an SGAs-supplemented diet for 5 weeks (1 mg/kg haloperidol or olanzapine and 10 mg/kg ziprasidone) did not gain body weight but did show increased adiposity in subcutaneous and visceral adipose depots [86]. A deeper analysis of adipocytes isolated from these treated animals showed no alterations in basal lipolytic activity; however, olanzapine reduced the lipolytic effects triggered by the β-adrenergic agonist isoprenaline or the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX). Moreover, the anti-lipolytic actions of insulin, the adenosine analogue phenylisopropyladenosine, or the α2-adrenergic agonist UK14304, were unaltered in olanzapine-treated primary adipocytes. Overall, the results of this study point to a reduction of β-adrenergic receptor sensitivity by olanzapine, which might explain its negative impact on lipid metabolism. However, the fact that IBMX-induced lipolysis was also impaired suggests that the molecular mechanisms might be more complex than the blockade of β-adrenergic receptors by SGAs [89]. On the other hand, olanzapine, haloperidol and ziprasidone are moderate α1 and α2-adrenergic receptor antagonists [90], but since α1-adrenergic receptors are not involved in lipolysis in rats [91], it is unlikely that they are implicated in the mechanism of action of these drugs.

**Table 2**
Therapeutic reference ranges of different SGAs in accordance with the steady-state plasma concentrations in patients under treatment.

| Second-generation antipsychotics | Therapeutic reference range (ng/ml) | Therapeutic reference range (nM) |
|---|---|---|
| Clozapine | 350–500 | 1073–1533 |
| Olanzapine | 20–40 | 64–128 |
| Aripiprazole | 150–210 | 335–468 |
| Quetiapine | 50–500 | 130–1304 |
| Risperidone | 20–60 | 49–146 |
| Ziprasidone | 50–130 | 121–315 |

Moreover, in the study by Minet-Ringuet et al., the fact that SGAs treatment failed to counteract the inhibition of lipolysis induced by α2-adrenergic receptor agonists [89] discounts these receptors in the mechanism by which SGAs disrupt lipid mobilization.

Lipid homeostasis is the balance between lipogenesis and lipolysis. Interestingly, male Sprague-Dawley animals treated with olanzapine present an increased expression of FAS and a decreased expression of hormone-sensitive lipase (HSL) in adipocytes, thus favoring lipid synthesis over lipolysis, and providing a possible explanation for the increase in adipose depots in rats [89]. By contrast, Victoriano et al. reported that male Sprague-Dawley rats treated with olanzapine (2 mg/kg) or haloperidol (1 mg/kg) mixed in the food for 46 days showed no changes in the levels of lipogenic markers (FAS, and acetyl-CoA carboxylase (ACC)) and lipolytic enzymes (LPL and HSL) in WAT [92]. Clearly, more preclinical studies are needed to fully understand the molecular basis of the impact of SGAs in lipid metabolism, including gender intrinsic susceptibility, to be translated to humans.

### 2.4.1. Effect of second-generation antipsychotics on hormone/cytokine expression and secretion in WAT

As stated earlier, adipose tissue is now recognized as a main player in the development of systemic inflammation and, consequently, of insulin resistance and metabolic dysfunction [93,94]. While the mechanisms through which SGAs influence systemic and/or local inflammation remain unclear, it has been hypothesized that adipose tissue is responsible for the inflammatory response induced by SGAs, which in turn is the main cause of the metabolic dysfunctions associated with antipsychotic therapies [93]. By treating female Balb/c mice and Sprague-Dawley rats intraperitoneally with olanzapine (10 mg/kg) for 8 weeks, Li and co-workers found an increase in the levels of circulating pro-inflammatory cytokines TNF-α, IL-6, IL-8 and IL-1β, which correlated with their elevated mRNA expression in WAT [95]. Similarly, administration of olanzapine (10 mg/kg) *via* osmotic mini-pumps in male Sprague-Dawley rats increases the levels of IL-6 and F4/80 immunostaining in WAT samples, which positively correlated with the levels of translocator protein (TSPO) – a target for radiotracers putatively indicating microgliosis in clinical neuroimaging studies – whereas no changes were found in the abundance of IL-1β, IL-4, IL-5, IFN-γ or TNF-α [93]. Using oral administration of olanzapine (1 mg/kg olanzapine, 3 times daily) in female Sprague-Dawley rats, Zhang et al. reported an enhancement in monocyte infiltration in WAT in parallel with body weight gain and an increase in adipocytes size [96]. Moreover, the authors found a high correlation between adipocytes size and macrophage infiltration in WAT, and this was accompanied by an upregulation of TNF-α, IL-1β and IL-6. Interestingly, the inflammatory response in the WAT of SGAs-treated animals also might have a gender-dependent component. For example, Davey et al. tested the effects of olanzapine (2 or 4 mg/kg) administered intraperitoneally twice daily in male and female Sprague-Dawley rats, finding that female rats gained more body weight than males, an effect associated with hyperphagia [97]. Also, female, but not male, animals showed an up-regulation of IL-6 in WAT and elevated plasma levels of IL-8 and IL-1β. However, adiposity and macrophage infiltration was increased by olanzapine treatment in both genders, together with alterations in the gut microbiota [97]. The aforementioned study of Victoriano et al. performed in male Sprague-Dawley rats treated with olanzapine or haloperidol mixed in the food also described WAT inflammation that manifested as elevated TNF-α mRNA levels and infiltration of CD68-positive cells [92]. Since SGAs treatment leads to NF-κB overexpression in human ASCs [61], and the relationship between inflammation and insulin resistance is well documented [98], a shift to a more pro-inflammatory profile might explain some of the metabolic dysfunctions described in adipose tissue after SGAs treatment. Accordingly, NF-κB could be a potential target to prevent and/or mitigate olanzapine-induced insulin resistance due to WAT inflammation [95].

It is important to mention that the action of SGAs in the CNS might also play a relevant role in appetite and food intake patterns, since olanzapine might activate the melanin-concentrating hormone system (feeding-initiation system) in the lateral hypothalamus that has projections into the nucleus accumbens [42] enhancing food intake. Regarding the CNS-mediated effects of SGAs that result in a pro-inflammatory profile, Guesdon et al. described an effect of olanzapine (1 mg/kg) administration in male Wistar rats for 13 days *via* implanted mini-pumps by moderately increasing the mRNA levels of melanin-concentrating hormone receptor in the nucleus accumbens shell [41]. Over time, this could lead to increased food intake and, consequently, in adipose tissue deposition that can *per se* recruit immune cells to the fat depots, thereby triggering inflammatory processes. However, as mentioned above, the link between peripheral and central effects following SGAs treatment remains poorly understood and needs to be investigated in future studies.

### 2.4.2. In vivo insulin-related disturbances in WAT by second-generation antipsychotics

Several animal studies have reported a direct influence of SGAs in the response of WAT to insulin. In this line, Cui et al. reported that the increased body weight in female C57Bl/6 female mice treated with olanzapine (3 mg/kg/day) mixed in the food for 2, 4 or 8 weeks associates with hyperinsulinemia and insulin resistance [99]. These findings were corroborated by Coccurello et al. in the same animal model [100], and also by Hou et al. in female C57Bl/6 mice receiving olanzapine (6 mg/kg) *via* oral gavage for 7 weeks [101]. Calevro et al. hypothesize that the insulin resistance in treated animals is closely related to inflammation since olanzapine increases macrophage infiltration and pro-inflammatory cytokine expression in WAT, particularly IL-6 [93]. Also, the effect of SGAs on the Wnt signaling pathway seems to be relevant for the alterations of glucose metabolism and insulin sensitivity in adipose tissue. In this regard, Li and coworkers found that, in addition to an increase in insulin levels during fasting, the expression of TFC7L2, a key effector of the Wnt pathway, was increased in the WAT, liver and skeletal muscle of male C57Bl/6 male treated with olanzapine (4 mg/kg/day) *via* oral gavage for 8 weeks. The addition of metformin, an anti-hyperglycemic drug, effectively blocked the changes in insulin plasma levels and TFC7L2 expression, suggesting a potential correlation between olanzapine-induced insulin dysfunctions and TCF7L2 [102]. Also, at the molecular level, the final stage of the insulin-signaling cascade in adipose tissue is the translocation of glucose transporter-4 (GLUT4) to the plasma membrane. However, hyperinsulinemic-euglycemic clamp studies performed in male Sprague-Dawley rats treated with olanzapine mixed in cookie dough with an increasing dose regimen (day 0–6: 4 mg/kg/day; day 7–13: 8 mg/kg/day and day 14–29: 12 mg/kg/day) failed to find alterations in adipose tissue glucose uptake although glucose uptake was impaired in skeletal muscle [103].

Besides the general direct relationship of insulin resistance and SGAs, a study in obese male C57Bl/6 mice found that olanzapine treatment (2 mg/kg/day) *via* oral gavage, once daily for 4 weeks, increased insulin sensitivity by lowering glucose and insulin plasma levels, an effect related to autophagy by potentiation of lysosomal function in adipocytes [104]. Thus, further research will provide new insights to fully understand the effect of these therapies *in vivo* in order to unravel their clinical relevance.

## 2.5. Direct and indirect effects of second-generation antipsychotics in human WAT

Human studies are indispensable to translate data on the mechanisms of action of SGAs identified in cellular or animal models, since the latter do not fully recapitulate the disease in patients. Regarding the effect of SGAs in adipokine expression and insulin resistance, the levels of the pro-inflammatory adipokine resistin are known to be elevated in patients with schizophrenia under stable therapy with clozapine [105,106], and correlate with circulating IL-1Ra, TNF-α and C-reactive protein [107]. In line with these results, Sapra and co-workers compared body weight and several inflammatory indicators between a group of 8 non-diabetic men with schizophrenia under treatment for at least 6 months with SGAs (independently of the chosen agent) and age- and body mass-index (BMI)-matched healthy men, finding that adiponectin plasma levels were lower and C-reactive protein levels were higher in the former after an overnight fast, which associated with increased insulin resistance [108]. Likewise, in an open-label prospective single-center study with 113 patients treated either with risperidone (54 patients with an average dosage of 4.4 mg/day) or olanzapine (59 patients with an average dose of 17.4 mg/day), body weight gain and the prevalence of metabolic syndrome were significantly greater in the olanzapine group than in the risperidone group. Also, whereas adiponectin levels significantly increased in the risperidone group over time, they significantly decreased in olanzapine-treated patients. By contrast, no significant differences were found between the groups for fasting glucose, insulin levels and insulin resistance, suggesting that the effect of olanzapine on adiponectin levels precedes dysfunctions in whole-body glucose metabolism [109]. In a similar type of study, Richards et al. [110] examined the effects of olanzapine and other SGAs on the levels of adiponectin in patients with schizophrenia *versus* matched healthy controls, finding olanzapine-associated hypoadiponectinemia with a specific decrease of the high molecular weight forms of the protein. However, the study failed to find alterations in adiponectin expression or in multimer composition in primary adipocytes isolated from subcutaneous WAT and treated *ex vivo* with olanzapine (10 ng/ml) for up to 7 days. These results point to the notion that alterations in adiponectin expression and secretion might occur progressively.

Beyond the specific effects of SGAs on metabolic parameters, disease-specific changes should also be considered. In a study performed with 9 medication-free non-diabetic patients and matched controls, Cohn et al. provided evidence for schizophrenia-related insulin resistance with an inadequate compensation in insulin secretion [111]. However, this was not associated with a significant loss of adiponectin levels, as reported in another study of medication-free patients with schizophrenia [112]. It is known that adiponectin levels can be influenced by gender, as testosterone modulates adiponectin expression and the secretion of multimers *in vitro* [113]. Therefore, indirect and direct drug effects, inflammatory phenomena and/or hypoadiponectinemia can also be considered as potential mechanisms for the metabolic disturbances induced by SGAs [108]. Likewise, plasma levels of calcium binding-protein B (S100B) are increased in female, but not male, patients treated with clozapine (125–900 mg/day) and positively correlate with BMI, pointing to a possible link between this protein and increased adiposity [114]. It should be mentioned that despite the belief for astrocytes as the only cells that express and secrete S100B, adipocytes have also been shown to secrete S100B in a process that is negatively controlled by insulin [115]. S100B inhibits adenylate cyclase by activating D2 receptors and enhancing the extracellular signal-regulated kinases (ERK) in astrocytes [116] and, accordingly, the S100B/D2-receptor complex is a potential molecular target of the SGAs [114].

Healthy volunteers are used in clinical trial studies because they are normally naive to the tested pharmacological treatments and can provide information on the of the treatment *per se*, independently of pathology. In healthy volunteers, a single dose of olanzapine (10 mg) elevates fasting glucose levels in the first 4.25 h after administration, without altering body weight, and also decreases serum cortisol and FFA levels [117]. Since cortisol activates hepatic-sensitive LPL and ATGL in adipocytes [118], its decrease could explain the reduction in FFA levels. A decrease in fasting and postprandial FFA concentrations was also found in healthy volunteers that received olanzapine (10 mg/day) over 8 days, which was associated with increased nocturnal adiponectin levels independently of BMI alterations [119]. Moreover, 15 days treatment of olanzapine (10 mg/day) in healthy men elevates the levels of adiponectin, leptin and TNF-α, and decreases those of ghrelin, with no correlation to changes in adiposity [120]. Overall, these studies illustrate the direct effect of SGAs on the organism independently of alterations in body weight or adiposity [117].

Because schizophrenia can manifest in early adulthood, some studies have focused on younger cohorts of patients. A study on SGAs-naive 6–18-year-old patients diagnosed with disruptive behavior disorders and treated randomly with aripiprazole, olanzapine or risperidone (commonly used in children) for 12 weeks showed that olanzapine leads to a higher weight gain (4.12%) when compared with aripiprazole (1.66%) or risperidone (1.18%), in association with an increase in subcutaneous fat [119]. Also, patients treated with olanzapine or aripiprazole show a reduction in insulin-stimulated glucose uptake (29.34% and 30.26%, respectively), indicating insulin resistance. The increase in body weight and reduced insulin sensitivity in the first 12 weeks of treatment might be responsible for the future cardiometabolic morbidity and mortality associated with SGAs therapy in the young population [121].

The recent introduction of co-therapies has ameliorated some of the SGAs-induced metabolic side-effects. For instance, in a cohort of 30 patients with schizophrenia under stable treatment with olanzapine, Taveira and co-workers tested the effect of co-administration of naproxen, an opioid receptor antagonist. This combinatorial therapy decreased body fat mass and increased free-fat mass, conferring protection against weight gain. This study does not prove that olanzapine-associated weight gain is induced by activation of the opioid system since by itself this system has the ability to improve body weight control, but suggests that blockade of opioid receptors could counteract the dysregulation of lipid metabolism associated with SGAs treatment [122].

The molecular impact of SGAs therapy for human adipose tissue is still poorly understood and many contradictory reports have appeared in the literature. A possible reason for these discrepancies is the mismatch of the control groups used in clinical studies (age, BMI, sex and smoking status, among other parameters). Moreover, the effect of SGAs is clearly different between healthy volunteers and patients with schizophrenia or other psychotic disturbances, and so it is possible that disease-specific alterations are overlooked when using healthy subjects. Of interest, alterations in adiposity and increased BMI observed in individuals undergoing SGAs treatments are closely related to cardiometabolic comorbidity and mortality. Accordingly, further studies are required to understand the direct (or indirect) impact of these agents in human adipose tissue with regard to differentiation, gene expression, metabolic routes and secretory patterns.

## 3. Effect of second generation antipsychotics in brown adipose tissue

BAT is the main player in adaptive thermogenesis, which functions to maintain the core body temperature in cold environments. In contrast to WAT, BAT is defined by the expression of the UCP-1, which burns metabolic substrates and dissipates energy in the form of heat [21–23]. Despite their different functions, however, the differentiation of white and brown adipocytes is controlled by similar transcription cascades [123], although brown adipogenesis requires the expression of additional transcription factors such as the zinc protein PRDM16, which forms a complex with C/EBP-β and activates PPAR-γ2, resulting in the expression of BAT-specific markers such as UCP-1, peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) and Cidea [124].

### 3.1. *The impact of second-generation antipsychotics on BAT thermogenesis*

BAT was recognized for many years to be functionally important for cold acclimatization in small mammals, such as mice and rats, and in newborn humans. Recent studies using $^{18}$F-fluorodeoxyglucose positron emission tomography combined with computed tomography have proved the existence of discrete areas of metabolically-active BAT in adult humans [24,125], which is functionally controlled by both catecholamines and insulin [126]. Whereas healthy brown adipocyte differentiation increases energy expenditure and contributes to the reduction of weight gain [127], diminished brown adipogenesis is related to obesity and insulin resistance [128].

In an attempt to unravel the involvement of brown adipocytes in SGAs-induced weight gain, Oh et al. differentiated a murine brown adipocyte cell line in the presence of clozapine (40 μM), quetiapine (30 μM) or ziprasidone (10 μM). At day 8 of differentiation, clozapine inhibited almost completely the expression of PRDM16, UCP-1 and Cidea. Quetiapine also reduced the expression of these genes, but its effect was less robust. Moreover, ziprasidone treatment inhibited PPAR-γ expression at the initiation, but not at the end, of the differentiation process. These findings correlated with the Oil Red-O staining of lipids, which showed an almost complete inhibition of brown adipogenesis by clozapine, moderate inhibition by quetiapine, and no inhibition by ziprasidone [129]. In parallel to the thermogenic program, it is well known in rodents that brown adipocytes differentiate at the end of fetal life *via* an adipogenic program related to lipid synthesis and the expression of lipogenic enzymes, resulting in a multilocular fat droplets phenotype [130]. In the aforementioned study, clozapine reduced the expression levels of genes encoding ACC, SCD1, GLUT4, adipocyte protein 2 (AP2) and CD36, but not FAS. In contrast to the response of clozapine, quetiapine increased ACC and FAS levels, whereas ziprasidone treatment did not modulate lipogenic gene expression [129] (Fig. 2, panel A). Of interest, the effects of SGAs in brown adipocytes differentiation correlate with their ability to induce weight gain in patients (clozapine > quetiapine > ziprasidone). Overall, these results support the hypothesis that inhibition of brown adipogenesis may be a mechanism by which SGAs induce weight gain as a side-effect [129]. Contrasting with these findings, Ota et al. reported that male Sprague-Dawley rats treated subcutaneously with risperidone (0.1 mg/kg/day) for 21 days present hypothermia without changes in adipogenic, lipogenic or thermogenic gene expression programs in BAT [131].

As mentioned above, hyperphagia is believed to be the main cause of weight gain induced by short-term SGAs treatments. However, in long-term treatment, when food intake is normalized, a reduction in energy expenditure due to diminished thermogenesis and locomotor activity is likely responsible for body weight increase. Zhang and coworkers found in female Sprague-Dawley rats that a 34-day treatment with olanzapine (1 mg/kg, 3 times daily) mixed in cookie dough significantly reduces BAT temperature detected at 45–150 min post-treatment. This decrease was associated with reductions in UCP-1 and PGC-1α levels and a diminished abundance of brown adipocytes, suggesting that olanzapine induces both BAT morphological alterations and deficiency in its thermogenic function [132]. In addition to these studies, it should be noted that a direct link between hyperphagia and BAT thermogenesis has been recently identified. After a meal, an increase in the gut hormone secretin in circulation activates BAT thermogenesis by binding to its receptor in brown adipocytes thereby stimulating lipolysis, which is sensed in the brain and promotes satiation [133]. Whether this regulatory mechanism is affected by SGA treatments remains to be elucidated.

The demonstration of active BAT metabolism in adult humans [24–26,125,134] has led to the hypothesis that heat production in BAT could contribute to emotional hyperthermia. Indeed, mild psychological stress has recently been shown to activate BAT thermogenesis in adult humans [135]. Related to this, Blessing et al. conducted a study in male Sprague-Dawley rats treated subcutaneously or intraperitoneally (catheter) with the FGA chlorpromazine (0.1–5 mg/kg) or with the SGA clozapine (30 μg–2 mg/kg) or risperidone (6.25 μg–1 mg/kg) and exposed to an intruder rat. The objective of the study was to understand the effect of the antipsychotic agents on emotional hyperthermia, which activates BAT thermogenesis and tail artery constriction. All of the antipsychotics tested strongly reduced the intruder-elicited BAT thermogenesis and artery tail vasocontriction, diminishing the emotional hyperthermia in a dose-dependent manner [136]. Moreover, all of the doses required to elicit an effect on the thermogenic capacity of BAT were lower than those that impact cardiovascular parameters induced by open field stress [137], acoustic startle response [138] or pre-pulse inhibition [139–141], indicating a higher sensitivity of BAT to these drugs. Even though the D2 antagonist chlorpromazine could inhibit the thermoregulatory actions in a manner similar to clozapine or risperidone, this does not necessarily mean that the mechanism of action is dependent on D2 receptor blockade, as a previous study with the FGA haloperidol, another potent D2 antagonist, failed to show an acute effect on resting body temperature when administered subcutaneously to male Sprague-Dawley rats in one single dose (up to 3 mg/kg) [142]. Blessing et al. also tested the selective and potent D2 antagonist raclopride, finding that it did not reduce intruder-elicited BAT thermogenesis [136]. By contrast, the hypothermic action of the D2 agonist apomorphine was counteracted by haloperidol in a dose-dependent manner [142]. Similarly, a low dose of the D2 antagonist spiperone ablated apomorphine-induced hypothermia as well as quinpirole (D2 agonist)-mediated inhibition of BAT thermogenesis. Moreover, spiperone diminished the tail artery vasoconstriction induced by clozapine [143]. All of these findings suggest that perhaps the stimulation, and not the blockade, of dopamine D2 receptors in the CNS reduces body

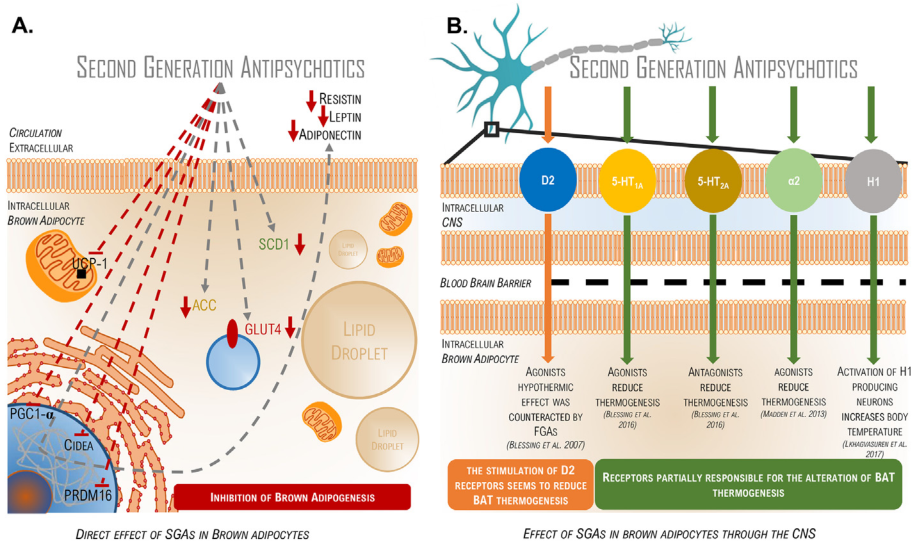

**Fig. 2.** Impact of SGAs in brown adipogenesis and BAT thermogenic function. A. Scheme of the molecular cell autonomous effects of SGAs in brown adipocytes, focusing on their effects in lipid-related and thermogenic proteins, as well as the inhibition of brown adipogenesis. B. List of the receptors in the CNS through which SGAs.

temperature [136,143]. Additionally, pharmacological studies demonstrated that 5-$HT_{1A}$ agonists and 5-$HT_{2A}$ antagonists reduce BAT thermogenesis and cutaneous vasoconstriction thereby both contributing to hypothermia [144,145], and also agonists of α2-adrenergic signaling reduce BAT thermogenesis [146]. Conversely, activation of $H_1$ receptors in histamine-producing neurons increases body temperature in association with stimulation of the ascending arousal system [147]. Due to their shotgun binding profile, clozapine and risperidone impact all of the aforementioned families of receptors, motivating the hypothesis that perhaps the influence of these SGAs on BAT thermogenic function is a consequence of synergistic interactions among them [136] (Fig. 2, panel B). These observations are still preliminary and further investigation is needed to understand how antipsychotics impact on BAT thermogenesis.

BAT activation may be critical for the gender differences found in weight gain upon SGAs treatment. This hypothesis was tested by Ferno et al. in male Sprague-Dawley rats treated with two intramuscular injections of commercially available olanzapine, pamoate depot formulation (ZypAdheras®, 100 mg/kg) at days 0 and 9, and sacrificed at day 17 after the first injection [148]. The dose was previously used by the authors to induce hyperphagia in female Sprague-Dawley rats [149]. A second group of animals fed a high-fat diet was also treated. The authors found that olanzapine transiently increases food intake in the days following its administration, with a prolonged effect in the group fed a standard diet. However, despite the induction of temporary hyperphagia, the olanzapine-treated groups (independently of diet) presented a decrease in body weight, suggesting reduced feed efficiency. Yet, the treatment failed to modify the mRNA levels of UCP-1 and PGC-1α in the BAT of the experimental groups, likely excluding an effect mediated by increased BAT activity. The authors also treated another cohort of animals with a single higher dose of the same formulation of olanzapine (150, 200 or 250 mg/kg) and sacrificed them at day 13 post-injection. This acute treatment also led to a transient increase in food intake accompanied by a decrease in cumulative weight gain which, contrary to the previous treatment, could be explained by an up-regulation of UCP-1 and PGC-1α in the BAT of olanzapine-treated animals indicating increased energy expenditure [148]. This is contrary to what has been reported in female animals [149,150]. For example, Skrede et al. described that treatment of female Sprague-Dawley rats with olanzapine (6 mg/kg) twice daily by oral gavage for 13 days decreases both UCP-1 and PGC-1α in BAT and increases weight gain. Of note, when the animals were treated with aripiprazole in the same experimental set-up, only PGC-1α expression decreased [151]. In another study [150], female Sprague-Dawley rats treated orally with olanzapine (6 mg/kg/day) for 24 days displayed a transient increase in food intake together with increased body weight gain along the treatment, as compared with non-treated controls. Likewise, in pair-fed rats receiving olanzapine, body weight increased along the treatment as compared with vehicle-treated rats, in association with decreased energy expenditure, a reduction in BAT temperature and a decreased expression of UCP-1 protein. Interestingly, an increase in FOS protein was found in spinal cord neurons projecting to discrete sites in the brainstem and hypothalamus, suggesting their excitatory activation, and some of these neurons, specifically those located at the perifornical region of the lateral hypothalamus, were positive for orexin A [150], a key neuropeptide in BAT-directed neurons in the lateral hypothalamus [152]. In a previous study, the activation of these neurons upon olanzapine treatment was associated with hyperphagia, likely due to their projections to the areas associated with hunger in the cerebral cortex [153]. Additionally, the same perifornical orexin A-positive neurons in the anterior cingulated cortex project axon collaterals to sites that are related to food intake and thermogenesis [154], corroborating that the activation of these neurons by olanzapine provides a possible explanation for SGA-mediated changes in both feeding and energy expenditure, thereby leading to weight gain. Another possible factor related to the decrease in thermogenesis by SGAs is the stimulation of different sub-regions of the lateral hypothalamus that induce inhibitory responses, constraining sympathetic nerve activity [155], although it has yet to be tested in BAT. In this scenario, the response to olanzapine would mediate inhibitory actions at the spinal-cord level, but more work is needed to fully understand the involvement of hypothalamus-BAT axis in the side effects induced by SGAs [150]. Moreover, S100B and its chaperone calsyntenin 3β have been shown to positively influence sympathetic innervation of BAT, while their deficiency predisposes mice to diet-induced obesity [156]. As aforementioned, S100B is increased in plasma of female patients under clozapine treatment [114]; however, it should be mentioned that an elevation in circulating S100B does not necessarily mean a positive effect in the functional innervation of BAT, since this adipokine can activate innate immune cells by interaction with receptors for advanced glycation end-products [157], which might result in defective insulin sensitivity and thermogenic function in BAT [158]. Further studies are required to address the impact of the higher plasma levels of S100B upon SGA treatment.

Nevertheless, other reports describe different effects of SGAs on BAT gene expression. Treatment of female C57Bl/6 J mice with risperidone for 3 weeks (4 mg/kg/day, intraperitoneal) increases food intake and body weight, and is associated with reduced locomotor activity in the first 2 days after the first injection, and without alterations in the core body temperature at this time period [159]. Yet, at the end of the third week, the body temperature of animals receiving risperidone increased, and this was associated with lower locomotor activity during the dark phase. Also, expression of UCP-1 in BAT and UCP-3 in gastrocnemius was elevated by risperidone, whereas the mRNA encoding orexin A was decreased in the hypothalamus. These results suggest that risperidone-induced weight gain in mice is a consequence of increased energy intake and reduced activity, whereas the higher body temperature may be a result of diet-induced thermogenesis and elevated UCP-1 and UCP-3 together with reduced hypothalamic orexin A expression [159]. By contrast, using male Sprague-Dawley rats treated with olanzapine (1 mg/kg/day) in food for 6 weeks, Minet-Ringuet and co-workers reported no alterations in mitochondrial thermogenesis in the BAT of olanzapine-treated animals. Even in the presence of guanosine diphosphate, which inhibits UCP-1, the respiratory rates at different membrane potentials showed no alterations in proton conductance after the treatment [160]. Considering the apparent contradictory results in some of these studies, we cannot discard that these outcomes might be related, at least in part, to the different binding affinities, doses and/or administration routes of the SGAs tested.

Co-therapies have also been tested to counteract the side effects of SGAs in BAT function. Specifically, and since the antagonism of these drugs to histaminergic receptors was identified as a main contributor to body weight alterations, the $H_1$ receptor agonist and $H_3$ receptor antagonist betahistine was tested in female Sprague-Dawley rats as a co-therapy together with olanzapine (3 mg/kg/day in cookie dough) for 3 weeks after 23 days of olanzapine treatment and then followed by 19 days of washout [161]. Betahistine co-therapy reduced (45%) the body weight gain induced by olanzapine and counteracted the olanzapine-induced increases in $H_1$ receptor protein levels and AMPKα phosphorylation in the hypothalamus, as well as the decrease in UCP-1 and PGC-1α in BAT. Importantly, this work led to the hypothesis that olanzapine-induced AMPK activation in the hypothalamus mediates weight imbalances by diminishing BAT thermogenesis through the hypothalamic $H_1$ receptor-AMPK pathway [161]. These results align with the effects of specific genetic activation of hypothalamic AMPK in the ventromedial nucleus of the hypothalamus, which counteracts the central effect of the thyroid hormone triiodothyronine in BAT activation [162]. Another tested co-therapy is the nonselective β-adrenergic-blocker propranolol, which can mitigate risperidone (0.75 mg/kg/day, oral gavage for 4 weeks)-induced alterations in BAT in C57BL/6 J females by increasing UCP-1 and PGC-1α levels [163].

### *3.2. The impact of second-generation antipsychotics on BAT cytokine expression*

Beyond the impact of SGAs on BAT adipogenesis, lipogenesis and function, the aforementioned study by Oh et al. [129] showed that differentiation of murine brown pre-adipocytes in the presence of clozapine also modulates the expression of adipokines by reducing resistin, leptin and adiponectin. The same outcome was observed for resistin and leptin levels in quetiapine-treated cells, whereas ziprasidone was unable to affect adipokine expression. Again, the effect of these SGAs in leptin expression reflects their ability to induce weight gain [129]. Of interest, leptin synthesis and secretion is modulated by insulin [164] and it has been reported that SGAs treatment interferes with insulin signaling [165], although it has not yet been reported in BAT. In such case, the impact of SGAs on the insulin response in BAT might potentiate the reduction of leptin levels [129]. Additionally, the role of UCP-1 in augmenting the anorexigenic effect of leptin should also be considered [166], suggesting that in synergy with the direct suppression of leptin expression, clozapine also reduces leptin actions through inhibition of UCP-1 expression [129]. Independently of these studies, some reports suggest that clozapine increases the serum levels of this adipokine in patients with schizophrenia [167]. Of interest, the work of Zhang et al. discussed above [130] in rats treated with olanzapine showed increased infiltration of macrophages in BAT, which was accompanied by up-regulation of the inflammatory cytokines TNF-α, IL-1β and IL-6, suggesting that olanzapine can trigger peripheral inflammation. Moreover, these inflammatory cytokines are also up-regulated in the hypothalamus [96], an effect possibly related to the undermining of thermogenesis, as previously described outwith the context of SGAs treatment [168].

## 4. Effect of second generation antipsychotics on beige adipose tissue

Beige adipose tissue is found within the white depots, most notably in subcutaneous depots. Like BAT, beige adipocytes are characterized by high amounts of mitochondria and by their ability to dissipate energy by thermogenesis due to the considerable levels of UCP-1 [16]. *Kristof* et al. showed that *ex vivo* treatment of human ASCs isolated from 20 to 65-year-old healthy volunteers (BMI < 29.9) with clozapine (100 ng/ml) during differentiation resulted in a 10-fold increase of their expression levels of UCP-1, pointing to a beiging/browning process of these precursors [169]. The up-regulation of UCP-1 mRNA levels was associated with increased expression of other browning marker genes, including TBX1. Indeed, the addition of clozapine to differentiating ASCs resulted in a 1.5-fold increase in the number of beige cells. This pattern of browning-related gene expression was up-regulated even when the cells were treated only in the last 2 or 4 days of the differentiation process, suggesting that clozapine can promote a white-to-beige shift in adipocyte cell fate in the latter stages of differentiation. However, in spite of the increase in browning, the cells were unable to activate basal thermogenesis unless they were stimulated with β-adrenergic agonists. Independently of this, clozapine added for 12 h to terminally-differentiated adipocytes from ASCs failed to alter their expression profile of beiging-related genes. This suggests that there is a critical stage of the differentiation process at which clozapine commits mesenchymal adipocyte progenitors to beige adipocytes. In addition, the lipid droplets found in the differentiated-clozapine treated cells were smaller and presented a multiocular distribution, which was associated with increased levels of mitochondrial DNA, but with diminished sensitivity to cAMP stimulation [169]. In this regard, defective cAMP signaling has been found in the brain of humans [170] and mice [171] under SGAs treatment. By contrast, and as mentioned above, treatment of white adipocytes with olanzapine during differentiation augments lipid accumulation. Moreover, differentiation of human ASCs in the presence of olanzapine, ziprasidone, risperidone, quetiapine, haloperidol (all used at 50 ng/ml) or aripiprazole (100 ng/ml) failed to alter UCP-1 expression [169]. Of interest, elevations of peripheral serotonin levels in obese mice have been described to inhibit beiging, as well as the sensitivity of both beige and brown cells to thermogenic stimulus, in a cell autonomous manner [172,173]. Also, increased levels of peripheral serotonin [174] and polymorphisms in the gene encoding tryptophan hydroxylase 1, which catalyzes the rate-limiting step of serotonin production outside the CNS, are associated with obesity [175]. The importance of serotonin signaling was demonstrated by Kristof and co-workers, who co-treated adipocytes [55] with exogenous serotonin and clozapine during differentiation, and found a partial reduction of browning caused by the lowered expression of brown-related genes. Of interest, the serotonergic receptor $HTR_{2A}$ was found up-regulated in clozapine-treated adipocytes [169], and it has recently been described that $HTR_{2A}$ activation results in Gq-mediated signaling capable of abolishing browning in mice and humans [176]. In fact, Gq protein expression negatively correlates with UCP-1 levels in the WAT in mice. Given this, and considering that clozapine antagonizes several serotonergic receptors with specific high affinity for $HTR_{2A}$ [177], the alteration in Gq signaling induced by serotonin can, at least in part, explain the unexpected results of Kristof and co-workers. Of interest, clozapine is a well-known agonist of H4 receptors [178,179], which are highly expressed in adipocytes. In a recent study where these receptors were knocked-down in subcutaneous WAT, cold-induced browning and lipolysis were abolished. By contrast, when 4-methylhistamine, a selective H4 receptor agonist, was adjacently injected in subcutaneous WAT, browning was increased through activation of p38/MAPK and ERK1/2/MAPK signaling pathways, together with an acceleration in metabolic rate and tolerance to hypothermia [180]. Considering these results, the increased browning in ASCs treated with clozapine described by Kristof et al. could be a consequence, at least in part, of H4 receptor activation. Nevertheless, more studies are required to understand the precise impact of SGAs in beige adipocytes and the browning process. In this sense, the study by Kristof et al. [169] is an excellent start. Moreover, it highlights specific signaling pathways that might affect the differentiation of the adipocytes by clozapine, with special importance to serotonergic signaling, pointing to a possible co-therapeutic approach to ameliorate the adverse effects of these drugs in the pool of beige adipocytes.

## 5. Concluding remarks

The study of adipose tissue depots as both metabolically-active tissues and endocrine organs that orchestrate peripheral insulin action, inflammation and energy expenditure is a relatively new field. Much effort is being directed to understand the relevance of adipose tissues as triggers of metabolic dysfunctions and/or possible therapeutic targets for metabolic pathologies including those associated with SGAs treatment. In this review, we have attempted to illustrate the modulation of adipocyte fate and their metabolic and endocrine functions by SGAs, both in cell-based systems and in pre-clinical and clinical studies. These drugs can modulate white adipocyte differentiation, resulting in increased lipid accumulation and adiposity that is related to the clinical manifestations of patients under SGAs therapy in an apparent gender-dependent manner. Also, SGAs exacerbate the chronic low-grade inflammatory processes of WAT by augmenting the expression and secretion of pro-inflammatory cytokines and the recruitment of immune cells, which is associated with altered secretory patterns of adipokines correlating with obesity, insulin resistance and hyperphagia. These processes are clearly main contributors to the evident metabolic dysfunctions under SGAs therapy. In addition, SGAs treatment impacts brown adipogenesis and BAT homeostasis through their ability to modulate the thermogenic differentiation program by controlling the expression of UCP-1, also in a gender-dependent manner. Similarly, browning of white adipocytes is altered by SGAs, particularly clozapine. Only one study so far has examined this particular adipose depot, but it could be a crucial factor in the secondary side-effects during SGAs treatments. Another important but less studied theme is how the CNS-adipose axis is affected by SGAs. The central outputs of this axis are major coordinators of peripheral adipose tissue function and homeostasis by supplying neurotransmitters and, in this regard, alterations in the neuronal circuits that control energy expenditure or food intake boost the peripheral disturbances in synergy with specific cell autonomous effects of the SGAs. Finally, and not covered in this work, metabolic deficits in patients under SGAs treatment might be due to the defective cross-talk between fat and liver/skeletal muscle, which are key tissues for *de novo* lipogenesis or glucose uptake, respectively.

In conclusion, despite the great strides made in our understanding of the metabolic consequences of the treatment with SGAs, further investigations are required to fully address the impact of SGAs in the different adipose tissue depots of the organism and the interactome between them either by inflammatory molecules, adipokines and/or other activator or inhibitory compounds, as well as the connections between the CNS and fat.

## Transparency document

The Transparency document associated this article can be found, in online version.

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## Declaration of competing interest

The authors declare they have no conflicts of interest to be declared.

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