

Review

Antipsychotics Promote Metabolic Disorders Disrupting Cellular Lipid Metabolism and Trafficking

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Antipsychotics frequently cause obesity and related metabolic disorders that current psychopharmacological/endocrinological theories do not explain consistently. An integrative/alternative theory implies metabolic alterations happening at the cellular level. Many observations *in vitro* and *in vivo*, and pivotal observations in humans, point towards chemical properties of antipsychotics, independent of receptor binding characteristics. Being amphiphilic weak bases, antipsychotics can disrupt lysosomal function, affecting cholesterol trafficking; moreover, by chemical mimicry, antipsychotics can inhibit cholesterol biosynthesis. These two molecular adverse effects may trigger a cascade of transcriptional and biochemical events, ultimately reducing available cholesterol while increasing cholesterol precursors and fatty acids. The macroscopic manifestation of these molecular alterations includes decreased high-density lipoprotein and increased very low-density lipoprotein and triglycerides that may translate into obesity and related metabolic disorders.

Antipsychotic-Induced Dysmetabolism: Clinical Significance

Excessive appetite and weight gain are evident in most patients treated with **antipsychotic drugs** (APs) (see [Glossary](#)), together with obesity and obesity-related metabolic disorders, such as dyslipidemia, insulin resistance, and hyperglycemia. The long-term administration of APs during psychiatric rehabilitation [1,2] or maintenance therapies [3] increases the risk of developing type 2 diabetes mellitus and/or a metabolic syndrome [4,5].

These adverse metabolic events occur in part due to psychopathologies but mostly due to adverse effects of APs, and more with atypical than typical APs (Tables 1 and 2). The differences of receptor binding affinities between atypical and typical APs have promoted many mechanistic studies focused on adverse effects on the central nervous system and the enteral neuroendocrine/cytokine system ('brain-gut' regulations). However, shortcomings and inconsistencies of such explanations, based on AP effects on neurotransmitters, hormones, and cytokines, are being increasingly reported ([Box 1](#)).

In parallel, the evidence of complementary mechanisms, yet to be characterized, is expanding. APs can reduce the endurance capability and explosive force output of psychiatric patients engaged in physical challenges, compared to AP-untreated psychiatric patients [23,24]. This suggests that APs may reduce the capability of spending energy for exercise, that is, a biochemical mechanism, rather than reduce the motivation to exercise, that is, a psychopharmacological mechanism. An insufficient energy allowance for consumption may, in turn,

Highlights

The severe adverse metabolic effects of antipsychotics including obesity, diabetes, and related metabolic disorders are limiting their use.

Models for antipsychotic-induced dysmetabolism based on psychopharmacological or endocrinological actions are not fully consistent and explanatory.

Interferences of antipsychotics with cholesterol inward and outward trafficking, cholesterol metabolism, and lipid metabolism have been recently found at the cellular level, especially in hepatocytes, myocytes, and adipocytes that are key regulators of the energetic metabolism.

We propose that these effects of antipsychotics at the cellular level can disrupt energetic homeostasis, possibly leading to compensatory behaviors and hormonal regulations. Cellular and whole-body alterations may together contribute to the development of clinically evident obesity and related metabolic disorders.

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Table 1. List of Most Frequently Used Typical or Atypical APs.

Typical APs ^a			Atypical APs ^b				
D2 antagonism	Low	Chlorpromazine	CHL	5-HT _{2a} antagonism/D2 antagonism ratio	↑ Higher (more atypical)	Aripiprazole	ARI
		Mesoridazine	MES			Clozapine	CLO
		Periciazine	PER			Asenapine	ASE
		Promazine	PRO			Zotepine	ZOT
		Thioridazine	THI			Sertindole	SER
	Medium	Loxapine	LOX		Olanzapine	OLA	
		Perphenazine	PER		Ziprasidone	ZIP	
					Paliperidone	PAL	
	High				Risperidone	RIS	
		Droperidol	DRO		Lurasidone	LUR	
		Fluphenazine	FLU		Iloperidone	ILO	
		Haloperidol	HAL		Quetiapine	QUE	
		Pimozide	PIM		Molindone	MOL	
		Trifluoperazine	TFP		Amisulpride	AMI	
	Zuclopenthixol	ZUC	Sulpiride	SUL			
			↓ Lower (less atypical)				

^aTypical APs are ordered in three groups following their D2 antagonism potential, and then in alphabetical order.

^bAtypical APs are ordered in a continuous list by their 5-HT_{2a}/D2 antagonism ratio, which determines the degree of being atypical.

generate biochemical/hormonal signals of energy shortage. These may reverberate on appetite, being etiopathogenic for overeating and weight gain.

The existence of 'weight-neutral' APs, such as ziprasidone and molindone (Table 1) corroborates the hypothesis that the metabolic effect of most APs may depend, at least partially, on a characteristic that is not related to their psychopharmacological effect. In fact, although having receptor binding profiles similar to those of other APs [6,9], ziprasidone and molindone promote no weight gain or metabolic deterioration in patients (Table 2) [25,26].

One hypothesis is that APs, by virtue of their chemical structure of amphiphilic weak bases ($pK_a \sim 8$), may interfere with intracellular lipid vesicles, altering the cellular trafficking and metabolism of cholesterol and lipids. Thus, the strong bases ziprasidone and molindone ($pK_a > 14$ and > 15 , respectively) are expected to be exempted from this biochemical 'class-effect'.

Herein, we review a considerable amount of literature supporting the hypothesis that APs induce cellular metabolic disruptions that may underlie the development of obesity and related metabolic disorders. Evidence currently comes mostly from *in vitro* and *in vivo* models but also from pivotal studies conducted in patients. We propose a new paradigm, to be further confirmed, that opens new directions for the prevention, diagnosis, and treatment of AP-induced obesity and obesity-related metabolic disorders.

The Way In: Effects of Antipsychotics on Cholesterol Uptake and Trafficking

The main route by which cholesterol is delivered to most cells *in vivo* is through uptake of exogenous lipoproteins. **Low-density lipoproteins** (LDLs) are the major cholesterol carriers in

Glossary

AMP-activated protein kinase:

fundamental regulator of energy management inside every cell. It keeps the levels of ATP, ADP, and AMP balanced by activating or deactivating anabolic and catabolic processes. AMPK is mainly activated by increases in the AMP/ATP ratio. It also has a role for whole-body metabolic regulations by integrating hormonal and psychopharmacological signals at the hypothalamic level.

Antipsychotic drugs: a class of drugs used to treat psychoses, schizophrenia, mania, and irritability. Antipsychotics must have antidopaminergic, serotonergic, or antihistaminergic properties. The combination of these properties determines whether an antipsychotic is typical or atypical and suggests its place in therapy. See also Tables Table 11 and Table 22.

Apolipoprotein: scaffold protein that allows the assembly of lipids and cholesterol into macro-complexes and facilitates the interaction with receptors and transporters. The main apolipoproteins are ApoA1, ApoB100, ApoC, and ApoE.

ATP-binding cassette transporter:

a family of transmembrane transporters that can mediate the efflux of various molecules by using ATP.

Endoplasmic reticulum:

intracellular compartment dedicated to the production of biological macromolecules, including proteins, cholesterol, and lipids.

Golgi apparatus: intracellular compartment dedicated to the post-translational modification of biological macromolecules and to vesicular trafficking.

High-density lipoprotein:

a complex composed predominantly of ApoA1 and cholesterol, mainly produced by hepatocytes to allow the circulation of cholesterol throughout the body. As HDLs deliver cholesterol to peripheral tissues and are enriched in lipids, they progressively become intermediate- or low-density lipoproteins that are destined to hepatic recycling.

Hydroxymethylglutaryl-CoA

reductase: the rate-limiting enzyme for cholesterol biosynthesis that is

Table 2. Summary of Evidence from Network Meta-Analyses on the Potential of APs to Cause Weight Gain in Patients^a.

weight gain ↑ Less ↓ More		Adult ^b	Pediatric ^c
			MOL ^d
			ZIP ^d
		Placebo	Placebo
		HAL	
		ZIP ^d	
		LUR	LUR
		ARI	ARI
		ASE	ASE
			HAL
		PAL	RIS
		RIS	PAL
		QUE	CLO
		CLO	QUE
	OLA	OLA	

^aSee Table 1 for full name of each AP abbreviation.

^bThe order is taken from a network meta-analysis on adult patients [6].

^cThe order is taken from a network meta-analysis on pediatric patients [7].

^dKnown to be consistently weight-neutral in patients.

the plasma. After LDL uptake through receptor-mediated endocytosis, low-density **lipoprotein receptors** (LDLRs) are freed and return to the plasma membrane. Unbound LDLs are instead delivered to late endosomal compartments and are degraded in lysosomes to liberate free unesterified cholesterol that is then transported to other organelles, including the **endoplasmic reticulum** (ER) and plasma membrane. LDL-derived cholesterol can be delivered to the ER by different routes: (i) transport to the plasma membrane and from there to the ER, (ii) trafficking from late endosomes to the **Golgi apparatus** (Golgi) and then via retrograde transport to the ER, and (iii) direct transfer from late endosomes to the ER. In the ER, cholesterol can be esterified as a storage form, and its levels are sensed by key regulatory machinery that regulates cellular cholesterol content.

APs affect intracellular cholesterol trafficking by interfering with the cholesterol egress from the endosomal/lysosomal compartment and with its translocation to the ER [27–29]. Indeed, cholesterol accumulation in endosomes/lysosomes and enlargement of the endolysosomal compartment are reported in cells treated with APs, including haloperidol, clozapine, risperidone, and ziprasidone [27,29,30]. Several APs (haloperidol, pimozide, aripiprazole, clozapine, quetiapine, olanzapine, risperidone, and ziprasidone) impair the

controlled through inhibitory feedback. Given the substantial energy cost of producing cholesterol, this control step is essential to prevent excessive energy consumption and/or unnecessary cholesterol synthesis.

Intermediate-density lipoprotein and low-density lipoprotein:

complexes mainly composed of ApoB100 and variable amounts of lipoproteins, cholesterol, phospholipids, and triglycerides. Intermediate-density lipoproteins and low-density lipoproteins are the result of the progressive modification of HDLs or VLDLs.

Lipoprotein receptor: owing to the presence of apolipoproteins, specific lipoprotein receptors can bind to the VLDL-LDL-IDL-HDL complexes and allow interaction. This includes the extraction or addition of energetic substrates and apolipoproteins that change the nature of the complexes, or the internalization of whole complexes that can be destined to the endolysosomal compartment.

Liver X receptors: transcriptional activators that respond to a series of metabolic stimuli. See Box 2 for further information.

Sterol regulatory element:

transcriptional response elements that are activated by cholesterol levels through sterol regulatory-element binding proteins. Sterol regulatory elements are typically contained in all genes related to cholesterol metabolism and trafficking.

Sterol regulatory-element binding protein:

transcription factors that are able to bind cholesterol and target sterol regulatory elements with an activating or inhibiting effect.

Very low-density lipoprotein: a complex composed of ApoB100, ApoC, and ApoE that contains a much higher ratio of triglycerides and phospholipids than cholesterol. Very low-density lipoproteins are produced by hepatocytes and adipocytes to allow the circulation of triglycerides and phospholipids throughout the body. As VLDLs are depleted of lipids, they progressively become low- or intermediate-density lipoproteins that are destined to hepatic recycling.

Box 1. Criticism of Current Explanations for AP-Induced Metabolic Disorders

- Antagonizing central serotonin 5-HT_{2C}, histamine H₁, and acetylcholine M₁ receptors, most APs increase appetite [6,8,9] and caloric intake. AP administration to adults causes additional intakes of about 350–600 kcal per day, resulting in short-term (2- to 4-week) excess weight gains of 2.6–3.6 kg [10,11]. This weight cannot be gained exclusively by increased intake, that is, 600 kcal per day for 4 weeks cannot produce 3.6 kg overweight.
- Reported weight changes are not consistent: even unchanged caloric intakes were reported after AP treatment, although with increased waist size [12]. This suggests energy redistribution towards fat accumulation.
- To explain inconsistencies, APs should also reduce energy expenditures. Psychiatric patients requiring AP treatment are physically inactive both before and after treatment [11]. However, APs may further reduce behavioral drives for energy expenditure, that is, the will to move, through H₁- and M₁ antagonism-mediated sedation. Not all AP molecules/doses are sedative and this concept is not proven in humans, while murine models gave contradictory results.
- An effect of APs on resting energy expenditures is not confirmed in humans [13]. The only clear evidence is that olanzapine interferes with estrogens in female rodents, stimulating appetite and brown adipose tissue thermogenesis [14,15].
- APs can alter brain and gut neuroendocrine signaling, impairing senses of hunger and satiety and disrupting the regulation systems of insulin, leptin, incretins, oxytocin, and inflammatory cytokines that converge on AMPK (reviewed in [16]). Therefore, adverse metabolic effects of APs on lipid trafficking and metabolism may be independent or precursors to weight gain/overeating, questioning the etiopathogenic role of overeating and inactivity for the AP-induced metabolic syndrome.
- A review of therapies for AP-induced metabolic disorders, based on the above-mentioned brain–gut mechanisms, showed scant or no efficacy [17]. This supports the relevance of other non-brain–gut mechanisms.
- Healthy volunteers treated with low dose APs demonstrate impaired oral glucose tolerance, increased leptin and triglycerides, and decreased free fatty acids and HDL, without changes in appetite or body weight or composition [18–20]. This suggests that APs may affect glucose and lipid metabolism before causing overweight and/or overeating or that metabolic disruption and weight gain might be independent adverse effects of APs.
- Metformin treatment in AP users resulted in decreased BMI in previously AP-naïve patients, while long-time AP users experienced only a slowing of BMI gain [21]. A review of pharmacologic interventions (mostly metformin) for the adverse metabolic effects of APs concluded that treatments initiated before the onset of weight gain were more efficacious compared with those that started afterwards [22]. These observations suggest that metformin may act on mechanisms that precede and/or cause overweight and/or overeating and support that overweight and/or overeating may be secondary signs.

delivery of LDL-derived cholesterol from the endolysosomal compartment to the ER, reducing esterified cholesterol levels [28]. Moreover, AP exposure increases LDL receptor transcription, stimulating endocytosis and worsening the intracellular accumulation of LDL-derived lipids [30].

The Way Out: Effects of Antipsychotics on Cholesterol Recycling and Formation of Endogenous Cholesterol-Containing Particles

Cholesterol may be delivered [31] outside the cell through **high-density lipoproteins** (HDLs) or **very low-density lipoproteins** (VLDLs). HDLs are generated as complexes mainly constituted of **apolipoprotein AI** (ApoAI) that is secreted outside the cells and binds the **ATP-binding cassette transporter A1** (ABCA1) on the cell surface. The ApoAI complexes are then loaded with cholesterol and phospholipids through ABCA1, becoming a native HDL. ABCA1 transcription is activated by **liver X receptors** (LXRs) in the presence of high levels of cholesterol intermediates. HDLs contain a minimal load of triglycerides and, while circulating, they progressively draw lipids from tissues and from other circulating cholesterol-containing particles.

VLDLs are instead characterized by the predominance of ApoB100, ApoE, and ApoC and by the heaviest loads of phospholipids and triglycerides (regulators of VLDL assembly), and they already contain a low amount of both free and esterified cholesterol at the time of secretion. While circulating, VLDLs progressively get depleted of lipids by tissues and other circulating cholesterol-containing particles, thereby fulfilling their role as lipid suppliers during fasting.

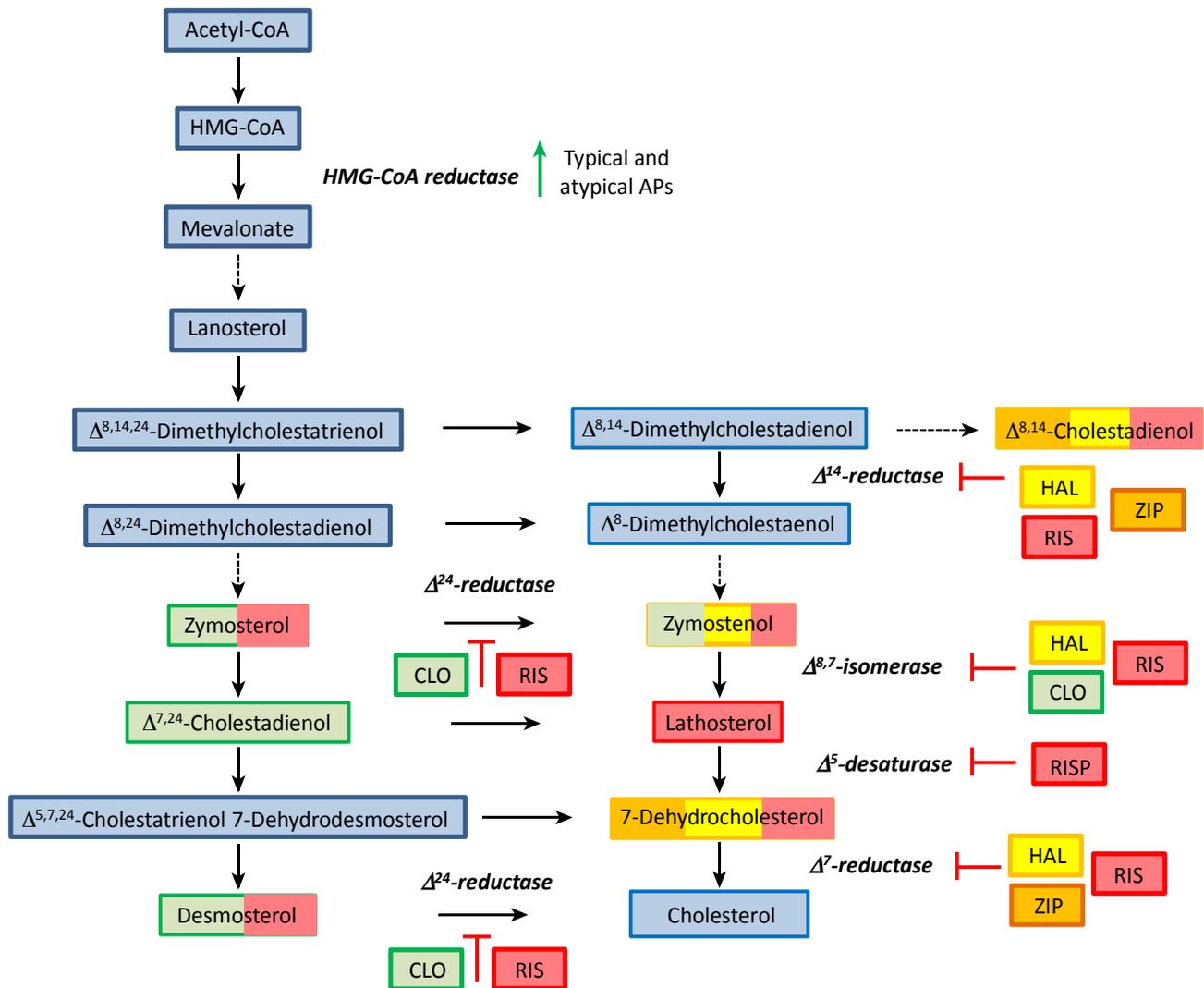
APs can affect cholesterol-containing particles. Clozapine, olanzapine, and haloperidol induce an increase in ABCA1 levels [32,33], while a rapid and temporary reduction in ABCA1 expression was reported with clozapine, risperidone, ziprasidone, and haloperidol in association with reduced cholesterol levels [29]. Several studies on patients treated long-term with atypical APs consistently reported decreased plasma HDLs [18,34,35]. Whether this reduction is related to changes in ABCA1 expression is not yet known. In patients treated with clozapine, haloperidol, ziprasidone, risperidone, and olanzapine, increased ApoB100 secretion has been determined, together with increased VLDL production [34].

Effects of Antipsychotics on Cholesterol Biosynthesis Enzymes

Cholesterol can also be synthesized in the ER starting from acetyl-CoA, through the mevalonate pathway, and involves several enzymes (Figure 1). APs (haloperidol, pimozide, aripiprazole, clozapine, quetiapine, olanzapine, risperidone, and ziprasidone) inhibit enzymes in the final steps of cholesterol biosynthesis *in vitro*, reducing cellular cholesterol content and inducing the accumulation of sterol intermediates [27–29,36]. Aripiprazole and ziprasidone have the most marked effects, and they have also been reported to inhibit fatty acid synthesis [28,29]. In brief, haloperidol, risperidone, ziprasidone, and to a minor extent clozapine affect $\Delta^{8,7}$ -isomerase, inducing zymosterol accumulation (Figure 1) [29]. Haloperidol and ziprasidone also inhibit Δ^7 -reductase and Δ^{14} -reductase, inducing the accumulation of 7-dehydrocholesterol and $\Delta^{8,14}$ -cholestadienol [27]. Moreover, only clozapine highly affects Δ^{24} -reductase, increasing C^{24} -unsaturated sterols [29]. Thus, different APs seem to target the same enzymes in the same way (Δ^7 -reductase > $\Delta^{8,7}$ -isomerase > Δ^{14} -reductase), although with different efficacy: ziprasidone > haloperidol > risperidone. Consistently, cholesterol is mostly depleted during exposure to ziprasidone, whereas clozapine has notably limited effects [28,29]. AP effects are limitedly variable among cell lines (HepG2, HL-60, and SH-SY5Y), depending on the relative activity of each enzyme.

Effects of Antipsychotics on the SREBP Pathway

The adverse metabolic effects induced by APs seem to be related to their actions on lipid and cholesterol metabolism genes. Cells maintain a steady-state concentration of cholesterol by controlling its biosynthesis and uptake through classic end product feedback inhibition of the **hydroxymethylglutaryl-CoA reductase** (HMGCR) and via LDLR-mediated endocytosis of LDL-cholesterol (Figure 2, Key Figure). Cholesterol metabolism genes contain a **sterol regulatory element** (SRE) in their promoter, and their expression is mainly regulated by **sterol regulatory-element binding proteins** (SREBPs), helix-loop-helix leucine zipper transcription factors that bind as dimers to the SREs, inducing gene transcription in response to lowered sterol levels [37]. SREBPs are conserved from yeast to humans and are key actors in cellular lipid metabolism and homeostasis. SREBPs participate in many physiological and pathophysiological processes, such as anabolism and growth, ER stress, inflammation, autophagy, and apoptosis and contribute to many metabolic diseases [38]. SREBPs are encoded by the SREBF1 and SREBF2 genes. The three major SREBP isoforms have distinct physiological roles, although their functions overlap to some extent: SREBP1a is involved in overall lipid synthesis, SREBP1c regulates fatty acid synthesis and energy storage, and SREBP2 is specific for cholesterol metabolism regulation. The biological role of the two SREBP1 isoforms is dependent on their pattern of expression across different tissues: SREBP1a is predominant in spleen and intestine cells, where it drives cell growth, and is also highly expressed in cultured cell lines; SREBP1c is predominant in metabolically active tissues, such as liver, fat, and skeletal muscle, where it regulates energy homeostasis [39].



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Figure 1. Effects of Antipsychotics on the Cholesterol Biosynthesis Pathway. Inhibitory effects of antipsychotics on enzyme activities are indicated by red squared arrows. Accumulated intermediates are shown in the same color as the antipsychotic inducing the accumulation. All antipsychotics induce an increase in HMG-CoA reductase levels (green arrow). Abbreviations: AP, antipsychotic drugs; CLO, clozapine; HAL, haloperidol; RIS, risperidone; ZIP, ziprasidone.

The activity of SREBPs is controlled at the transcriptional and post-transcriptional levels. SREBP1a and SREBP2 are transcribed in the presence of low intracellular cholesterol levels, while SREBP1c expression is induced by insulin and LXR, which is activated by oxysterol and by cholesterol synthesis intermediates, and inhibited by polyunsaturated fatty acid (Box 2) [40]. In the liver, SREBP1c is the major lipogenic transcription factor, whereas adipocyte fatty acid biosynthesis (*de novo* lipogenesis) is mainly regulated by carbohydrate response element-binding proteins (ChREBPs), which respond to glucose excess by triggering lipogenesis [46].

Post-transcriptional regulation of SREBP1 and SREBP2 activity is mediated by sterols that inhibit their proteolytic activation [47]. SREBPs are indeed synthesized as inactive 120-kDa

Key Figure

Effects of Antipsychotics on Cholesterol and Lipid Metabolism and Trafficking.

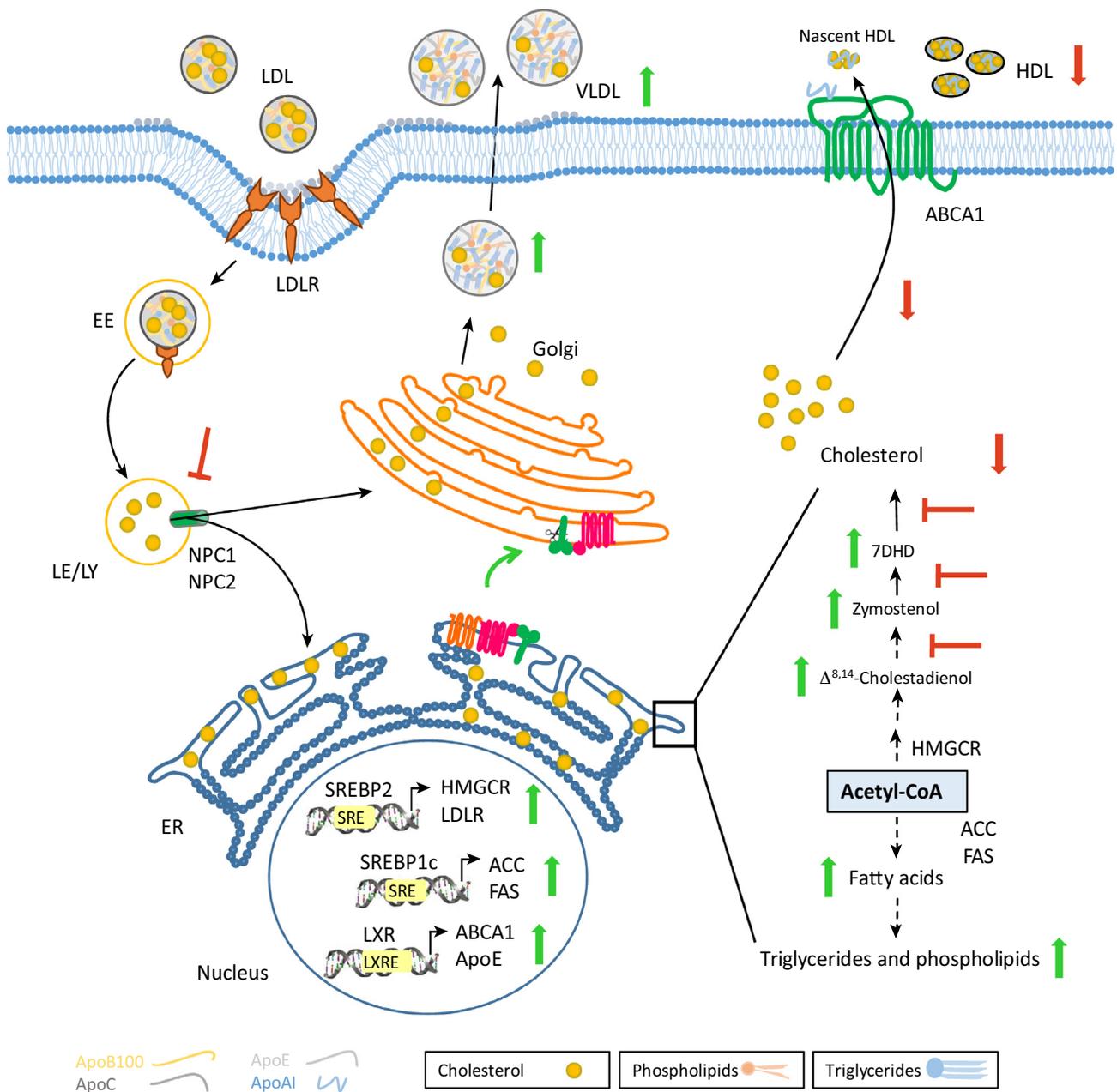


Figure 2. Green arrows indicate increases due to antipsychotics; red arrows indicate decreases due to antipsychotics; inhibitory effects of APs are indicated by red squared arrows. LDLs bind LDLRs on the cell surface and are internalized through the endolysosomal pathway. APs act at the level of lysosomes by dissipating the acidic pH, inhibiting lysosomal enzymes, and hindering the exit of free cholesterol towards the cytosol. Low (oxy-)cholesterol levels are sensed within the ER, leading to INSIG degradation. In this condition, the SREBP-SCAP complex can be translocated and processed in the Golgi to generate the active SREBP homodimer that is (Figure legend continued on the bottom of the next page.)

Box 2. SREBP Activity Regulation by Liver X Receptors

In the liver, a major site of cholesterol and polyunsaturated fatty acids synthesis, the SREBP1c pathway is subject to transcriptional regulation mediated by liver X receptor (LXR) α and LXR β that heterodimerize with retinoid X receptors (RXRs) and bind to liver X-responsive elements (LXREs) at target promoter regions.

LXRs are activated mainly by oxysterols, well-known inhibitors of SREBP processing, but also by a variety of sterols including intermediates in the cholesterol biosynthetic pathway, such as desmosterol.

While LXR target genes, such as the ATP-binding cassette subfamily (ABCA1, ABCG5, and ABCG8), CETP, ApoE, and CYP7A1 have one LXRE, SREBP1c contains two LXREs, suggesting a stronger transcriptional response. LXREs are also found in the promoter of several SREBP1c-induced lipogenic genes, such as FASN, ACC, and SCD-1; therefore, LXRs may activate lipogenic gene transcription directly as well as indirectly through the induction of SREBP1c [41].

In the presence of sterol overload or high levels of cholesterol intermediates LXR (or RXR) markedly increases the transcription of its target genes and of SREBP1c, with the consequent induction of fatty acid synthesis. On the contrary, polyunsaturated fatty acids antagonize the action of LXR and inhibit the expression of SREBP1c, decreasing lipogenesis [42].

It is generally believed that LXRs function also in insulin-mediated lipogenesis: glucose and glucose 6-phosphate can bind directly to LXRs, stimulating the transcription of their target genes [43]. Moreover, insulin can significantly upregulate LXR expression [44].

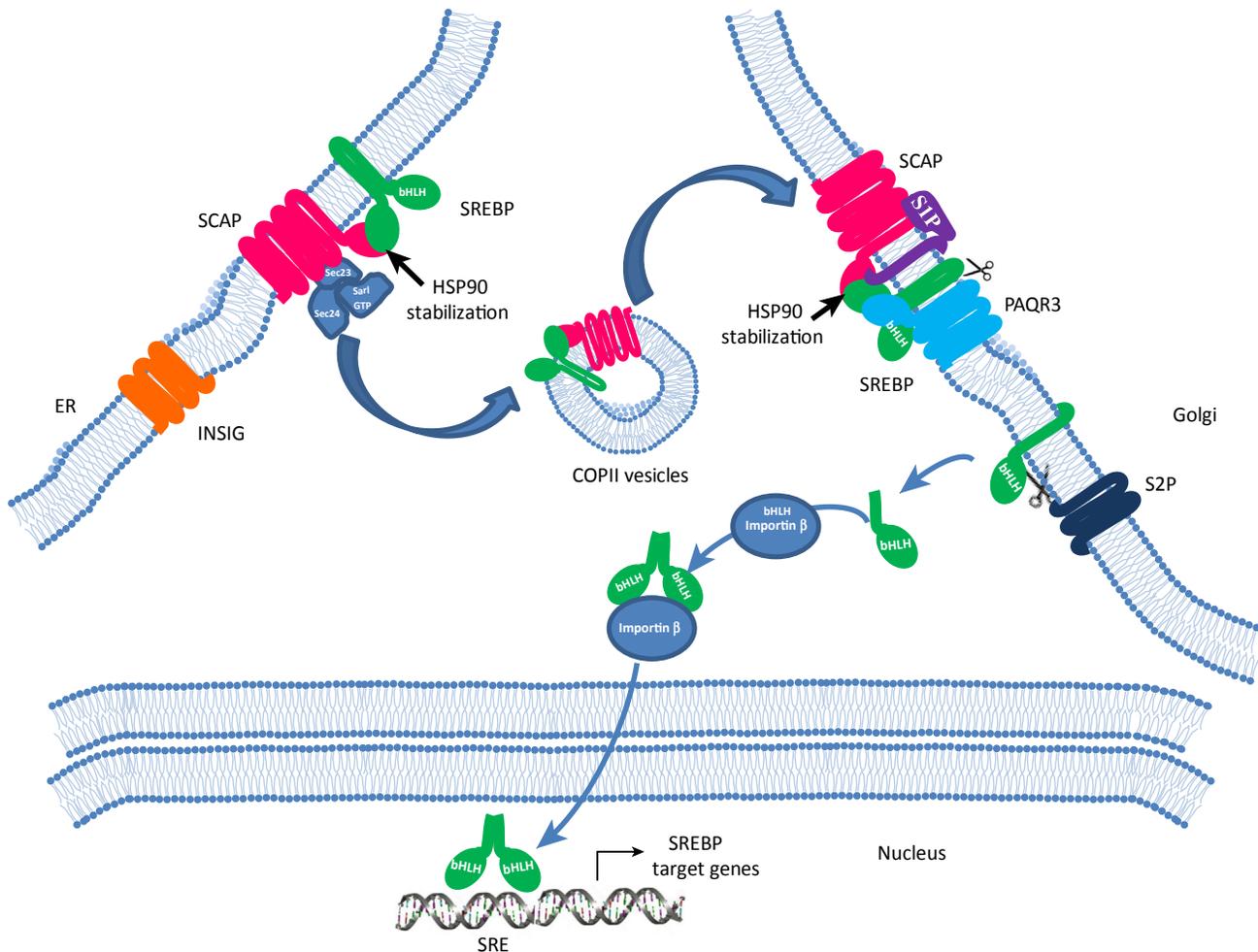
LXRs can also elevate INSIG2 mRNA and protein, with subsequent retention of SREBP1c in the ER. In the presence of insulin, which downregulates INSIG2, LXR agonists strongly induce the transport of the SREBP-SCAP complex to the Golgi, enhancing lipogenesis. This crosstalk between LXR and insulin prevents lipid overabundance [42].

A transcriptional activation of the LXR target genes involved in cholesterol efflux, such as ApoE and ABCA1, is reported for clozapine, olanzapine, and haloperidol in several cell lines after the activation of SREBP pathways [32,33], and for clozapine in rats [45]. Instead, a direct effect of APs on LXR transcription factor has been excluded, suggesting that LXR could be activated by the accumulation of intermediates/products of the cholesterol synthesis pathway [28].

membrane-bound precursors in the ER. Only in the presence of low cholesterol levels are they cleaved in the Golgi to generate a transcriptionally active 60- to 70-kDa fragment that is translocated to the nucleus, where it induces the expression of cholesterol and fatty acid biosynthesis genes.

Several proteins are involved in the crucial trafficking steps of SREBPs between ER, Golgi, and the nucleus (Figures 2 and 3). They include SREBP cleavage-activating protein (SCAP) and insulin-induced genes (INSIGs), two ER-resident protein types that contain a sterol-sensing domain and regulate SREBP stability and function in response to cellular sterol levels. SCAP forms a heterodimeric complex with SREBPs and, in the presence of high cholesterol levels, prevents the SREBP-SCAP complex from exiting the ER. INSIGs present with two isoforms: INSIG1 and INSIG2. INSIG1 is a SREBP target gene, induced by increased SREBPs activation.

transported to the nucleus. In the nucleus, SREBP target genes are activated, increasing the production of several transcripts connected with cholesterol and lipid metabolism. LXR target genes are also induced. The cholesterol and lipid biosynthesis pathways are both activated by SREBPs, however APs inhibit cholesterol biosynthesis at several steps (red squared arrows). This leads to cholesterol shortage with the accumulation of cholesterol precursors. Lipid biosynthesis is not inhibited and results in increased levels of fatty acids, triglycerides and phospholipids. The imbalance between cholesterol and lipid levels leads to a decreased production of HDLs and an increased production of VLDLs. Abbreviations: 7DHD, 7-dehydrocholesterol; ABCA1, ATP-binding cassette transporter A1; ACC, acetyl-CoA carboxylase; AP, antipsychotic drug; EE, early endosome; ER, endoplasmic reticulum; FAS, fatty acid synthase; HDL, high-density lipoprotein; HMGCR, hydroxymethylglutaryl-CoA reductase; INSIG, insulin-induced gene; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LE, late endosome; LXR, liver X receptor; LXRE, liver X-responsive element; LY, lysosome; NPC, Niemann-Pick; SCAP, SREBP cleavage-activating protein; SREBP, sterol regulatory element-binding protein; VLDL, very low-density lipoprotein.



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Figure 3. SREBP Interactors and Activators. The PAQR3 protein anchors the SREBP-SCAP complex to the Golgi membrane, depending on cholesterol levels. HSP90 stabilizes the SREBP-SCAP complex both in the ER and the Golgi. SCAP escorts SREBPs from the ER to the Golgi with the assistance of the COPII vesicle-binding proteins Sar1 and Sec23/24. The small GTP-binding protein Sar1 attaches to the ER membranes, Sec24 binds to SCAP, and Sec23 attracts other proteins to form the coat of the budding vesicle. In the Golgi, SREBPs are proteolytically activated in two steps: the ER luminal loop of SREBPs is cleaved by site-1 protease (S1P) generating a halved SREBP; next, the N-terminal region is eliminated by site-2 protease (S2P). Importin β then allows active SREBP homodimers to enter the nucleus [38]. Abbreviations: bHLH, basic-helix-loop-helix; ER, endoplasmic reticulum; HSP90, heat shock protein 90; INSIG, insulin-induced gene; PAQR3, progesterin and adipoQ receptor family member 3; SCAP, SREBP cleavage-activating protein; SRE, sterol regulatory element; SREBP, sterol regulatory element-binding protein.

INSIG2 is a constitutively expressed gene that is downregulated by insulin, and its activity is modulated by sterol levels [33,48]. The interaction of INSIG2 with the SREBP-SCAP complex regulates its transport to the Golgi [47]. High oxysterol levels in the ER stabilize INSIG2 and can, in turn, inhibit the SREBP-SCAP complex from translocating to the Golgi. Concomitantly, INSIG2 accelerates the degradation of HMGCR, reducing cholesterol synthesis. When intracellular cholesterol levels decrease, oxysterol levels in the ER drop, and INSIG2 is released from the SREBP-SCAP complex and rapidly degraded by proteasome; thus, the SREBP-SCAP complex can translocate to the Golgi, where SREBPs are proteolytically activated [49]. Active SREBPs enter the nucleus and bind to SRE elements in the promoter of sterol-responsive genes, inducing their expression (Table 3).

Table 3. SREBP2 and SREBP1c-Induced Genes

SREBP2 targets: cholesterol biosynthesis and metabolism		SREBP1c targets: lipid biosynthesis and metabolism	
Sterol regulatory element-binding protein 2	SREBP2	Sterol regulatory element-binding protein 1c	SREBP1c
ATP citrate lyase	ACLY	Acetyl-CoA synthase	ACS
Acetoacetyl CoA thiolase	ACAT2	Acetyl-CoA carboxylase	ACC/ACACA
HMG-CoA synthase	HMGCS1	Fatty acid synthase	FAS/FASN
HMG-CoA reductase	HMGCR/RED	Stearoyl CoA desaturase 1	SCD-1
Mevalonate kinase	MVK	Stearoyl CoA desaturase 2	SCD-2
Phosphomevalonate kinase	PMVK	AcylCoA binding protein	ACBP
Diphosphomevalonate decarboxylase/mevalonate pyrophosphate decarboxylase	MVD	Glycerol-3-phosphate acyltransferase	GPAT
Geranylgeranyl pyrophosphate synthase	GGPS1	HDL receptor	HDLR
Isopentenyl-diphosphate delta isomerase/isopentenyl pyrophosphate isomerase	IDI1	Malic enzyme	ME
Farnesyl pyrophosphate synthase/farnesyl diphosphate synthase	FPPS/FDPS	Glucose-6-phosphate dehydrogenase	G6PD
Squalene synthase/farnesyl-diphosphate farnesyltransferase-1	SQS/FDFT1	Phosphoglycerate dehydrogenase	PGDH
Squalene epoxidase	SQLE	Glucokinase	GK
Lanosterol synthase	LSS	Liver-type pyruvate kinase	L-PK
Lanosterol 14a-demethylase	CYP51	Lipoprotein lipase	LPL
Lathosterol oxidase/sterol-C5-desaturase-like	SC5DL	Peroxisome proliferator-activated receptor gamma	PPAR γ
7-Dehydrocholesterol reductase/ Δ -7-sterol reductase	7DHCR/DHCR	Spot 14	S14
LDL receptor	LDLR	HDL receptor/scavenger receptor class B type 1	HDLR/SRB1
		Elongation of very long chain fatty acids protein 5	ELOVL5
		Elongation of very long chain fatty acids protein 6	ELOVL6
		Ω 5 fatty acid desaturase-1	FADS1
		Ω 6 fatty acid desaturase-2	FADS2
		Microsomal triglyceride transfer protein	MTTP
		Phosphoenolpyruvate carboxykinase	PEPCK

Along with SREBPs, the small noncoding RNAs miR-33a and miR-33b, encoded in the introns of the SREBP2 and SREBP1 genes, act synergistically to regulate intracellular cholesterol and fatty acid levels, through the downregulation of their target genes (Box 3) [50].

The effects of APs on the expression of SREBP target genes have been analyzed *in vitro* in several cell lines derived from brain, liver, and fat [28,29,59–65] and *in vivo* [33,45,66–72]. *In vitro*, APs induce SREBP1c and SREBP2 activation and the expression of cholesterol and fatty acid biosynthesis genes, in particular the cholesterol biosynthesis rate-limiting enzyme HMGCR, LDLRs, and the fatty acid synthesis genes fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC) in all cell lines (Table 4). Among the APs, clozapine and chlorpromazine are the strongest SREBP stimulators [52,53] and clozapine is also the best INSIG2 inhibitor [65]. However, *in vitro* analyses of the effect of ziprasidone evidenced inconsistencies, reporting either a ziprasidone-induced activation of SREBP pathways [28,29,64] or no effect [60,61].

Box 3. Regulation of Cholesterol Metabolism through miRNAs

miRNAs have been implicated in cholesterol biosynthesis (miR-223, miR-195, miR-21, and miR-29), uptake (miR-27, miR-185, and miR-199a), efflux (miR-33), intracellular transport (miR-33), and in HDL metabolism (miR-33).

miR-33a and miR-33b, collectively named miR-33, are encoded within the introns of SREBP2 and SREBP1 genes and exert a key regulatory role [50].

Targets of miR-33 are ABCA1, Niemann-Pick 1 (NPC1), carnitine *O*-octanoyltransferase (CROT), hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit β (HADHB), carnitine palmitoyltransferase IA (CPT1A), and AMP-activated protein kinase α 1 (AMPK α 1). ABCA1 and NPC1 are associated with cholesterol metabolism; the others are involved in fatty acid oxidation [51].

In the presence of low available cholesterol levels, miR-33a is cotranscribed along with SREBP2. miR-33a prevents cholesterol efflux and HDL formation by downregulating cholesterol transport protein ABCA1 [52,53]. Silencing miR-33a promotes ABCA1 activity and cholesterol externalization, resulting in increased HDL circulation [54].

miR-33b is transcribed along with SREBP1c and works in concert with SREBP1-induced fatty acid synthesis, downregulating genes involved in fatty acid oxidation, such as CPT1A, CROT, and HADHB [50].

miR-33 also regulate intracellular cholesterol trafficking, by downregulating the cholesterol transport protein NPC1 that, together with NPC2, controls the exit of cholesterol from lysosomes [55].

Increased hepatic expression of miR-33 and SREBP2 has been reported following the activation of farnesoid X receptor (FXR), a nuclear receptor highly expressed in tissues involved in cholesterol metabolism, such as liver and intestine [56]. In liver, FXR directly downregulates the expression of ApoA1, a major HDL constituent [57]. FXR activation leads to the transcription of both SREBP2 and miR-33; however, the effects of miR-33 are immediate, while those of SREBP2 are still under the control of cholesterol levels [58].

Further investigations are needed to define the role of miR-33 in translational repression, the action on NPC1 or NPC2, and the contribution to FXR-dependent regulation of ABCA1. Dedicated *in vitro* experiments, as well as measurements taken in patients with and without AP therapy, could clarify the contribution of miR-33 and other miRNAs for the instauration of a pathological metabolic phenotype.

As for *in vitro* models, *in vivo* studies highlighted that AP effects are subject to discrepancies depending on several factors. Sex-specific metabolic effects of APs have been demonstrated in rodents. Female mice [73] and rats [74] exhibit remarkable concordance with the clinical effects of APs, showing increased appetite and weight gain, while less or no concordance with clinical effects was observed in male rats [66,75]. Moreover, it has been clearly shown that in female rats, APs upregulate lipogenic SREBP target genes both in liver and adipose tissue, increasing circulating triglycerides [45,68,71–73,76], while only one study on male rats has shown an effect of APs on SREBP induction [66]. The food allowance of animals was also shown to be determinant for experimental results, since restricted feeding prevented AP-induced weight gain. Another interesting source of heterogeneity in animal models is the fluctuation of AP plasma levels following bolus or oral administration of APs, which has led to the use of long-acting AP formulations *in vivo* [70,77]. Finally, drug dose affects the occurrence and magnitude of AP effects [71].

Antipsychotics-Induced Lipid Accumulation

APs induce the trapping of LDL-derived cholesterol in the endolysosomal compartment, impairing cholesterol trafficking to the ER [27–29] (Figure 2). Reduced cholesterol in the ER is sensed by SCAP, resulting in SREBP1c- and SREBP2-mediated transcriptional responses [69,62].

SREBP1c activation determines the expression of fatty acid biosynthesis genes and elevates the levels of free fatty acids, triglycerides, and phospholipids in AP-treated cells [28,29]. APs

Table 4. Effects of APs on SREBPs and on SREBP-Target Genes *In Vitro*

Cell line	Anti-psychotic ^a	SREBP1 ^{cb}		SREBP1 targets							SREBP2 targets							Other effects	Refs
		SREBP1 ^{cb}	SREBP2 ^b	ACC	FASN	SCD-1	FADS1	FADS2	ACAT2	HMGCS1	HMGCR	FDPS	SCSCL	DHCR	LDLR				
GaMg ^c	CLO, HAL				+	+	+	+	+	+	+	+	+	+			[59]		
	CLO, HAL > OLA, CHL	ex+	ac+		+	+	+	+		+	+	+	+	+			[61]		
GaMg ^c	RIS, ZIP														/				
	CLO, HAL > OLA		ac+							+		+					[60]		
3T3-L1 ¹	ZIP														/				
	OLA	ex+	ac+		+												[62]		
ARPE-19 ⁹	ARI, CLO, OLA, HAL, CHL, QUE				+	+	+	+	+										
	RIS, ZIP, TRIL, PER, LOX, ILO, FLU, MES, MOL, THI				+	+	+	+	+				+				[63]		
GaMg ^c , CCF, STTG1 ¹⁰ , SH-SY5Y ¹¹ , HepG2 ²	CLO, OLA, HAL																		
	CLO									+							[62]		
GaMg ^c , HepG2 ²	CLO																		
	HAL																[62]		
3T3-L1 ¹	CLO, RIS	ex+																	
	PIM, OLA																[137]		
IMR-32 ¹ , HT29 ⁹	HAL, PIM																		
	HAL, PIM, ARI, CLO, QUE, OLA, RIS, ZIP			+	+														
CHO ⁸	ARI, ZIP > HAL, PIM, CLO, QUE, OLA, RIS																		
	ARI, ZIP																[28]		
Primary rat hepatocytes	CLO, OLA, HAL	ex+		+	+														
	CLO, OLA		ac+														[33]		

Table 4. (continued)

Cell line	Anti-psychotic ^a	SREBP1cb ^b	SREBP2 ^b	SREBP1 targets					SREBP2 targets						Other effects	Refs	
				ACC	FASN	SCD-1	FADS1	FADS2	ACAT2	HMGCS1	HMGCR	FDPS	SC5DL	DHCR			LDLR
	QUE, ARI			/	/	/					/				/	No effect on ABCA1 expression	
HepG2 ^d	HAL, CLO, RIS, ZIP	ex+	ex+		+						+				+	Inhibition of cholesterol synthesis; downregulation of LXR target ABCA1	[29]
Rat adipose-derived stem cell culture	CLO, OLA, RIS	ex+		+		+										Induced adipogenic differentiation; enhanced lipid droplets formation; reduced INSIG2 expression; increased expression of aP2 ^u	[65]

^aSee Table 1 for full name of each AP abbreviation.

^bex, expression; ac, activity; +, increased; /, unchanged; blank, not investigated.

^cHuman glioma.

^dHuman liver.

^eHuman hepatocellular carcinoma.

^fMurine adipocytes.

^gHuman retinal pigment epithelium.

^hSqualene synthase.

ⁱLanosterol synthase.

^jLanosterol 14a-demethylase.

^kPhosphate cytidyltransferase 2.

^lRAS oncogene family member 26.

^mHuman astrocytoma.

ⁿHuman neuroblastoma.

^oNiemann-Pick 1.

^pNiemann-Pick 2.

^qHuman neuroblastoma.

^rHuman lung carcinoma.

^sChinese hamster ovary.

^tACC I isoform.

^uAdipocyte protein 2.

also induce the expression of VLDL proteins ApoB100 and ApoE and VLDL assembly, as expected in the presence of increased triglyceride levels [29].

On the contrary, SREBP2 activation and the expression of cholesterol biosynthesis enzymes do not result in increased intracellular cholesterol levels but rather the accumulation of sterol intermediates [27–29]. This is due to the inhibitory effect of APs on the enzymes of the final steps of the cholesterol biosynthesis pathway [27,29].

The inhibition of cholesterol synthesis and the increase in sterol intermediates, induced by APs, may lead to further activation of lipogenic pathways. Presumably as a consequence of increased sterol intermediates levels, APs induce LXR activation (Box 2), increasing the expression of the fatty acid synthesis genes FAS and ACC and of SREBP1c, again boosting fatty acid synthesis [28,32]. The concurrent inhibition of INSIG2 expression by APs promotes SREBP1c activation, inducing the lipogenic pathways [65].

Therefore, the AP-induced cholesterol trapping and inhibition of cholesterol biosynthesis enzymes may cause an overproduction of both cholesterol precursors and lipids and a lack of cholesterol.

This mechanism of SREBP1c-induced lipogenic activation through inhibition of cholesterol synthesis [78,79], which was investigated in cell lines where SREBP1a is the predominant isoform, could be even more relevant for the development of AP-induced dyslipidemia *in vivo* and in humans, due to the neat prevalence of the SREBP1c isoform in metabolically active tissues [80].

Effects of Antipsychotics on AMPK

Recent findings suggest that APs may alter energetic metabolism by regulating the activity of **AMP-activated protein kinase** (AMPK) in both peripheral tissues and the hypothalamus. In the liver, activated AMPK suppresses lipogenesis by inhibiting the activity of SREBPs and ChREBPs [81] and inhibiting ACC and HMGCR, the rate limiting enzymes of cholesterol and fatty acid biosynthesis, respectively [82]. Moreover, AMPK stimulates fatty acid oxidation, enhancing the activity of peroxisome proliferator-activated receptor α (PPAR α) [83]. AMPK also regulates glucose trafficking in muscle and fat [84] and the mobilization of fat lipid storages during fasting or energy demand, via hormone-sensitive lipase [85] and adipocyte triglyceride lipase [86].

AP effects on SREBPs can also be an indirect consequence of AMPK activation in peripheral tissues, as reported by studies conducted *in vivo* and *in vitro*. Indeed, olanzapine and clozapine can reduce AMPK phosphorylation and activation in hepatocytes and 3T3-L1 cells, with a concomitant increase in SREBP1 and SREBP2 protein levels and in SREBP-dependent lipid synthesis [83,87]. Furthermore, olanzapine and aripiprazole were shown to inhibit AMPK in human mononuclear blood cells [88].

An effect of APs on hypothalamic AMPK was also reported. Hypothalamic AMPK serves as the integrator of psychopharmacological and hormonal signals of appetite and satiety and can determine whole-body energetic responses by modifying appetite and regulating the autonomous nervous system. AMPK is inhibited when the hypothalamus receives typical satiety signals such as leptin, insulin, and GLP1 but also CNTF, T3, and estradiol; AMPK is activated when the hypothalamus receives typical signals of energy shortage, such as Ghrelin, AgRP, adiponectin, and glucocorticoids but also cannabinoids and antipsychotics [89]. Therefore, at

the hypothalamic level, hormonal and psychopharmacological actions may interfere with responses that are usually biochemically determined by the whole-body energy availability. Indeed, experimental AMPK activation increases appetite [90] and the dietary preference for carbohydrates [91], while its deactivation reduces appetite [90] and increases brown fat thermogenesis, adipose tissue browning, and hepatic lipogenesis [92]. With specific regard to APs, olanzapine has been shown to stimulate hypothalamic AMPK in female rats by blocking the histamine H1 receptor [77,93,94], evidencing an opposite effect of APs on peripheral and hypothalamic AMPK activity, as reported for other molecules [83]. Further studies are necessary to clarify to which extent AMPK and SREBPs are involved, either jointly or separately, in AP-mediated metabolic alterations.

Evidence of Altered Lipid Metabolism in AP-Treated Human Subjects

AP-treated patients commonly manifest dyslipidemia, characterized by decreased HDL and increased triglycerides [95]. In particular, clozapine is associated with hypertriglyceridemia [96]. Olanzapine induces *de novo* biosynthesis of phosphatidylethanolamine and phosphatidylcholine, and hypertriglyceridemia, leading to a characteristic plasma lipid profile [97], possibly through the accumulation of oxysterols that activate the phospholipid biosynthetic enzyme CTP:phosphocholine cytidyltransferase α [98]. Olanzapine also increases the expression of the lipid biosynthesis genes stearoyl-CoA desaturase (SCD) and FASN in peripheral blood cells [99]. Risperidone induces a similar increase and also increases lysophosphatidylcholine [97]. AP inhibitory effects on cholesterol biosynthesis observed *in vitro* are variably observed in schizophrenic adults. APs have been associated with increased plasma levels of total and LDL-cholesterol as well as decreased plasma HDL-cholesterol [100]. This may be both a direct effect of the reduction in cholesterol levels and an indirect effect of the increase in triglycerides production, known to decrease HDL production in favor of VLDLs. Specifically, olanzapine increased total and LDL-cholesterol more than aripiprazole, risperidone, and ziprasidone, while all decreased HDL-cholesterol levels [101–104]. During fasting, total cholesterol levels were found to be increased by clozapine, but not haloperidol or risperidone [101]. Haloperidol was also reported to increase the plasma levels of the cholesterol synthesis intermediate 7-dehydrocholesterol [105]. Finally, the upregulation of genes involved in the ER-Golgi transport and in cholesterol and lipid biosynthesis genes were reported in postmortem liver samples of AP-treated schizophrenic patients [106].

Chemical Properties of Antipsychotics and Affinity to Membranes

Alterations of cholesterol metabolism and trafficking have been related to AP chemical structures. APs are cationic amphiphiles, that is, molecules with an amine group that can be protonated and that display both hydrophilic and hydrophobic properties. Because of this chemical feature, APs can indeed accumulate in the brain and in cell membranes, interfering with lipid organization, trafficking, and metabolism, independently from their receptor activity [27,30,107–110].

Physical interaction with plasma membrane lipids has been demonstrated for haloperidol and chlorpromazine [27,107]. AP preference for sphingomyelin and cholesterol-enriched membrane regions, such as raft-like structures, may change membrane compartmentalization, differentially modulating the signaling cascades of G protein-coupled receptors and impairing intracellular pathways. Many key signaling molecules, including the insulin receptor, localize to lipid rafts, and lipid raft alteration by haloperidol impairs insulin signaling in neuronal cell lines [27,107].

Similar to other weak base amphiphiles (e.g., U18666A, chloroquine, imipramine, and tamoxifen), APs can penetrate membranes and accumulate in lysosomes, being trapped after

intraluminal protonation [111]. In the unionized form, amines are weak basic and membrane permeable, but after permeating the lysosomes they are protonated and lose permeation capacity. The lysosomal trapping of APs affects lysosomal/endosomal functionality, morphology, and lipid metabolism. Indeed, haloperidol impairs the egress of LDL-derived cholesterol also by increasing lysosomal pH and reducing the activity of protease and β -galactosidase enzymes [30]. Chlorpromazine, thioridazine, and other APs alter lysosomal acidification; increase lipid biogenesis; and activate the nuclear translocation of transcription factor E-box (TFEB) [112]. TFEB controls lysosomal gene transcription in response to a variety of cellular and environmental stresses and regulates autophagy [113].

Lysosomotropic compounds may indeed cause toxicity and metabolic dysfunction via inhibition of autophagy. The finding that olanzapine activates autophagy to protect from mitochondrial damage supports a role of APs in lysosome-autophagy modulation [111]. Lysosomotropic compounds could inhibit the degradation of autophagolysosomes by increasing lysosomal pH and impairing acid hydrolases.

The propensity of amines to be trapped into lysosomes is highest for molecules with pK_a values near or slightly above neutrality, such as most APs except ziprasidone and molindone. Ziprasidone is a strong base amphiphile ($pK_a > 14$); nevertheless, it has been reported to alter cholesterol trafficking and to induce the expression of SREBP2 target genes, similar to weakly basic APs [28,29]. This inconsistency may be the result of the lower propensity of strong bases to be trapped into lysosomes, compared with weak bases: ziprasidone may be more slowly protonated; therefore, it may better escape lysosomes. The variable results concerning ziprasidone might also be read as the result of different cell culture and drug exposure conditions.

Cholesterol biosynthesis enzyme inhibition by APs could also be related to their chemical structure. Indeed, the cationic amphiphiles AY9944, U18666A, and tamoxifen also inhibit the last enzymes of cholesterol biosynthesis, by mimicry of their carbocationic high-energy substrates [114]. In particular, AY9944 has the same inhibition effect of haloperidol, ziprasidone, and risperidone on Δ^7 -reductase, $\Delta^{8,7}$ -isomerase, and Δ^{14} -reductase [115].

Can the Effects of Antipsychotics on Lipid Metabolism Explain Weight Gain?

SREBP activation and reduced INSIG2 expression may be crucial for AP-induced dyslipidemia, weight gain, and consequent cardiovascular diseases [116–118]. Several clinical observations and *in vivo* studies consistently supported that alterations of the SREBP pathways in metabolically active peripheral tissues happen in a time frame of tens of hours after AP treatment, which is insufficient to allow a meaningful weight gain.

In detail, clozapine or olanzapine in rats induced the transcription of SREBP target genes and affected carbohydrate and lipid metabolism 12–24 h after administration [72]. Clinical studies showed that lipid alterations are evident in AP-treated patients even independently of weight gain [20]. There are even clinical trials of acute doses of APs that demonstrated immediate metabolic effects (reduced glucose load tolerance, reduced fasting triglyceridemia) in the absence of weight or appetite modifications [18,19].

Moreover, in the last decade several INSIG2/SCAP/SREBP polymorphisms were associated to obesity and implicated with glucose metabolism, further supporting the involvement of this pathway in weight gain and metabolic dysfunction. Two studies on Han Chinese patients reported results yet to be verified in other ethnic groups: the T-allele of rs11654081 in SREBF1

was associated with an increased risk of AP-induced metabolic syndrome [119], and the A-allele of rs2267443 or rs1052717 in SREBF2 was associated with an increased risk of metabolic syndrome due to clozapine [120]. Genetic variants in SREBF1 and SREBF2 have been recently reported to influence the response to statin medications in AP-treated patients with schizophrenia [121]. Furthermore, several genetic variants in INSIG2 were associated with central obesity and regulation of body mass index (BMI) and adipocyte and cholesterol metabolism, suggesting that INSIG2 may play a role in metabolic dysregulation [122–124]. Specific INSIG2 polymorphisms were studied for their association with AP-related metabolic dysregulation in schizophrenic patients [116,117,125]. The combination of three INSIG2 variants (rs17587100, rs10490624, and rs17047764) significantly increased the risk of weight gain [117]. A significant gene–gene interaction between INSIG1 and INSIG2 was also found to increase the risk of developing a metabolic syndrome in AP-treated patients (rs11123469, rs10185316, and rs1559509) [116]. Moreover, AP-induced adipogenic differentiation of adipose-derived stem cells can be blocked by INSIG2 overexpression, suggesting that APs may promote adipogenesis through SREBP activation [65]. This picture suggests the INSIG2-SCAP-SREBP pathway as a therapeutic target for AP-induced obesity and obesity-related metabolic disorders.

However, evidence from *in vivo* studies suggests that lipid metabolism alterations are not sufficient to produce weight gain. Specifically, experiments conducted on pair-fed rats (whose food allowance is equal to the amount of food eaten by a control rat) showed that AP-induced SREBP activation is not associated with weight gain by itself; rather, these experiments showed that increased eating is also required [71,74]. In this context, other mechanisms investigated in animal studies could be important modulators of AP-induced SREBP alterations and overall metabolic dysfunctions, such as the psychopharmacological mechanisms of hyperphagia, the role of estradiol, and their relationships with peripheral and hypothalamic AMPK activity, which is also a prominent target of AP adverse metabolic effects and for innovative therapies for metabolic disorders [126].

In particular, *in vivo* studies conducted on female rats revealed an important contribution of estradiol in determining the outcome of AP-induced lipid metabolism alterations and weight gain [14]. Indeed, in sterilized female rats lacking estrogen, olanzapine failed to induce transcriptional changes of lipogenic target genes and induced less pronounced weight gain, compared to intact females. Consistently, peripheral estradiol administration partially restored the appetite-increasing action of olanzapine in sterilized female rats [14]. The supposed mechanism of action of estradiol may be ascribed to its influence on hypothalamic AMPK and consequently also on brown fat thermogenesis. Preliminary data from human subjects also demonstrate that estrogens can promote resting energy expenditure and physical activity [127,128]; therefore, their action, likely mediated by AMPK, may be potentially relevant for metabolic disorders and their treatment also in humans.

Moreover, the metabolic adverse effects of clozapine and olanzapine in schizophrenic patients have been shown to vary in occurrence and magnitude upon the presence of specific AMPK polymorphisms [129].

Concluding Remarks and Future Perspectives

Improving the knowledge of the mechanisms underlying AP-induced obesity and related metabolic disorders, and devising appropriate rescuing measures, are currently unmet needs for psychopharmacological therapy. Those adverse effects of APs especially concern patients in the developmental age and all patients undergoing long-term rehabilitation treatments, and

Outstanding Questions

Can the development of AP-induced overeating, weight gain, and metabolic syndrome be explained by brain–gut theories only, or is lipid trafficking and metabolism in peripheral tissues a relevant aspect? What is the quantitative relevance of AP-induced trafficking and metabolic alterations in determining the etiopathogenesis of an AP-mediated metabolic syndrome?

What are the crosstalk mechanisms between cellular trafficking and metabolism in peripheral tissues and brain–gut levels regulating metabolism? How is the action of APs mediated by the crosstalk between AMPK and SREBPs?

What is the relationship between lipid metabolism alterations and weight gain? A thorough appraisal of the relationships between effects occurring at the central (psychopharmacological, endocrinological, integrative, i.e., hypothalamic AMPK) and peripheral (lipid and cholesterol trafficking and metabolism, peripheral AMPK) levels is necessary to produce a useful interpretation of the effect of APs in patients.

Non-antipsychotic weak base amphiphiles have been shown to influence cholesterol trafficking and metabolism to a similar extent as APs *in vitro*. What are the effects of these compounds on AMPK? Can these weak base amphiphiles be useful to test the relationship between SREBPs and AMPK as effectors of the adverse metabolic effects of APs?

The weight-neutral AP ziprasidone has been reported to have no effect on SREBPs but also to induce the SREBPs like other APs associated with weight gain; furthermore, it has psychopharmacological activities superimposable to those of other APs. Basic research should fill knowledge gaps regarding ziprasidone and other weight-neutral APs such as molindone. It would be important to verify whether they have a peculiar effect on lipid metabolism, that is, whether not promoting lipogenesis is sufficient to avoid weight gain even in the presence of disturbed cholesterol metabolism and trafficking, or whether

reduces treatment adherence and effectiveness [130]. For this topic to be properly appraised and acquire clinical relevance, some unsolved issues must be pointed out (see Outstanding Questions). Future basic research should focus on clarifying these aspects above all others:

- (i) The effect of APs on weight and SREBPs is not always consistent. In patients, clozapine and olanzapine lead to the worst adverse metabolic effects and are the most potent inducers of weight gain [6,104]. These effects, observed in patients, are not always reproduced in experiments *in vitro/in vivo*. Indeed, in cell lines clozapine is reported to be the strongest inducer, while olanzapine seems to have limited effects, on SREBP target genes [60,61]. In rats, olanzapine activates the SREBP system to a much larger extent than clozapine [74,77], and SREBP pathway activation in clozapine-treated animals does not result in weight gain [83,131]. Moreover, olanzapine-induced SREBP activation is associated with weight gain only in *ad libitum*-fed animals, indicating that hyperphagia is also involved in the overall effect of APs. Finally, while in rats APs induce SREBP activation independently of sex, they induce weight gain only in females, due to the involvement of other factors, such as estradiol and AMPK. Another inconsistency concerns the effects of the 'weight-neutral' AP ziprasidone that has been reported to induce SREBP activation *in vitro* like clozapine, olanzapine, and risperidone, known inducers of weight gain in patients [28,29,63]. Ziprasidone was also highlighted as the most potent suppressor of cholesterol synthesis. In some experiments, ziprasidone did not potentiate lipid synthesis [28], an aspect that, if confirmed, may be the key to interpreting its uniqueness. Unfortunately, to date ziprasidone was excluded from most analyses. Data regarding molindone, the other 'weight-neutral' strong-base AP, are currently limited to one observation that indicates an activity similar to other APs [63]. Future studies should include ziprasidone and/or molindone.
- (ii) AP-induced metabolic changes are currently associated with negative cardiovascular consequences, especially in patients undergoing long-term psychiatric rehabilitation. However, some studies reported a positive link between weight gain and clinical efficacy of APs on psychosis [132]. Similarly, AP-induced hypertriglyceridemia was associated with improvements in positive and overall psychotic symptoms [133]. Recent lipidomic research linked changes in cell membrane composition to treatment response, implying non-receptor-linked therapeutic effects of APs and stressing the multifaceted role of lipids in mental disorders [134]. The central role of lipids in psychopathology is supported by the presence of polymorphisms in SREBF genes, which have been associated with the incidence of schizophrenia [135]. Therefore, SREBP-SCAP-INSIG may also be evaluated as psychopharmacological targets in future research.
- (iii) Genetic variations in the actors of the SREBP-INSIG pathway should be considered when studying how APs modulate lipid metabolism. In a clinical perspective, the presence of SREBP/INSIG variants should be evaluated upfront, before beginning an AP therapy. This information may be useful to direct preventive measures, such as special diets and/or exercise.
- (iv) Although the excessive activation of SREBPs is connected with undesirable metabolic effects, moderate SREBP activity is physiologically important and beneficial. For example, SREBPs can contribute, to a minor extent compared with ChREBPs, to activate *de novo* lipogenesis in adipocytes [46]. As a direct consequence of *de novo* lipogenesis, glucose toxicity is reduced by controlling hyperglycemia; moreover, *de novo* lipogenesis triggers the production of lipokines, such as C16:1n7-palmitoleate from white adipocytes or 12,13-dihydroxy-9Z-octadecenoic acid from brown adipocytes, that promote insulin sensitivity in liver and muscle [136], protecting from metabolic disorders. Future studies should investigate whether acting indirectly on SREBP transcripts, for example via AMPK modulation, rather than acting directly on SREBPs, may yield better results thanks to the preservation of *de novo* lipogenesis induction by SREBPs.

other confounding mechanisms may intervene (e.g., neurotransmitter receptor mediated). To have comparable data to interpret, *in vitro* and maybe *in vivo* experiments could test a standardized panel of APs corresponding to the most common clinical uses, not forgetting new APs entering the market for both acute and rehabilitation uses.

Polymorphisms in the SREBP/SCAP/INSIG genes should be further investigated, as well as polymorphisms of AMPK, as they may contribute to the individual degree of susceptibility to AP-induced weight gain and dysmetabolism.

Metabolic alterations could lead to the production of atypical substrates, possibly released in the circulation. Could these be used as blood biomarkers of AP-mediated metabolic disruptions?

- (v) The disrupting effects of APs on cholesterol biosynthesis and inward trafficking have been observed also with non-antipsychotic weak base amphiphiles, and metabolic effects of antipsychotics may also be mediated by central actions of antipsychotics on the hypothalamic AMPK system. Therefore, future research must also evaluate the effects of non-antipsychotic weak base amphiphiles. This may help disentangle the relationship between SREBPs and AMPK, which is currently under debate due to the emergence of several inconsistencies between different experimental and clinical observations.

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