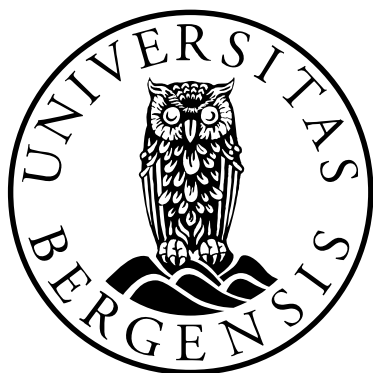


Developing a rodent model for antipsychotic-induced metabolic adverse effects

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Contents

CONTENTS.....	2
1. ACKNOWLEDGEMENTS.....	5
2. SUMMARY.....	6
3. LIST OF PUBLICATIONS.....	8
4. ABBREVIATIONS	9
5. INTRODUCTION.....	10
5.1 Schizophrenia	10
5.1.1. Historical aspects	10
5.1.2 Clinical manifestations.....	10
5.2 Epidemiological aspects of schizophrenia	11
5.2.1 Incidence and prevalence	11
5.2.2 Costs of schizophrenia	12
5.2.3 Risk factors for schizophrenia.....	13
5.2.4 Neurochemical paradigms of schizophrenia	13
5.3 Antipsychotic drugs.....	14
5.3.1. Early history of pharmacological therapy for psychiatric disorders.....	14
5.3.2 First-generation antipsychotics	15
5.3.3 Second-generation antipsychotic agents	17
5.3.4 Metabolic adverse effects of antipsychotic drugs	20
5.3.5 Clinical implications of metabolic adverse effects	21
5.3.6 Can receptor binding profiles explain metabolic adverse effects?.....	22
5.3.7 Animal models for antipsychotic-induced metabolic adverse effects.....	23
5.4 Lipid metabolism.....	27

5.4.1 General aspects of lipid metabolism	27
5.4.2 Free fatty acids and triglycerides.....	27
5.4.3 Cholesterol metabolism.....	29
5.4.4 Lipids in the brain	29
5.4.5 Regulatory factors in lipid biosynthesis.....	30
5.4.6 Mechanisms of fatty acid oxidation.....	32
5.4.7 Regulation of fatty acid oxidation and lipid storage	32
5.4.8 Tetradecylthioacetic acid (TTA).....	33
6. AIMS OF THE STUDY	34
7. SUMMARY OF RESULTS.....	35
8. DISCUSSION.....	37
8.1. Methodological aspects	37
8.1.1 Cell culture.....	37
8.1.2 RealTime PCR	38
8.1.3 MRI-based quantification of adipose tissue volume.....	40
8.1.4 Selecting a drug vehicle	41
8.2 Modelling metabolic adverse effects in rat.....	42
8.2.1 Divergent findings in human and rat.....	42
8.2.2 Challenge I: pharmacokinetics of antipsychotics in rat.....	43
8.2.3 Challenge II: dosing of antipsychotics in rats	43
8.2.4 Challenge III: administration of antipsychotics to rats.....	45
8.2.5 Challenge IV: the influence of diet	45
8.2.6 Steps towards increased reliability of rat models.....	46
8.4. Molecular mechanisms of metabolic adverse effects.....	47
8.4.1 Hyperphagia is the main cause of body weight gain.....	47
8.4.4 The role of energy expenditure in weight gain.....	47
8.4.5 The role of fatty acid oxidation in antipsychotic-induced metabolic adverse effects.....	49
8.4.6 “Uncoupling” of body weight and serum lipid levels	49
8.4.7 Antipsychotic-induced, SREBP-mediated lipogenic activation in cultured cells	50

8.4.8 Antipsychotic-induced lipogenic activation in rodents and humans.....	51
8.5. Clinical aspects related to lipogenic activation by antipsychotics	52
8.5.1 Do metabolically potent antipsychotic agents have superior clinical efficiency?.....	52
8.5.2 Are the differences in dysmetabolic potency between different antipsychotic agents as substantial as formerly thought?	53
8.5.3 Are clinical improvement and metabolic adverse effects correlated, independent of antipsychotic agent?	54
8.5.4 Lipogenesis as a possible therapeutic mechanism of action	56
8.5.5 Potential intervention strategies in patients with antipsychotic-induced dysmetabolism	57
9. CONCLUDING REMARKS.....	58
10. FUTURE PERSPECTIVES	59
11. REFERENCES.....	61

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2. Summary

Antipsychotic agents represent efficient therapy for serious psychiatric disorders, particularly schizophrenia, but also bipolar disorder, and are used by millions of patients worldwide. Metabolic adverse effects of antipsychotic drugs are thought to contribute significantly to the fact that life expectancy among schizophrenic patients is reduced with several decades. In particular, the so-called second-generation antipsychotics – most notably clozapine and olanzapine - significantly increase the prevalence of obesity, dyslipidemia, and type 2 diabetes. After initial cell culture experiments in our lab demonstrated that antipsychotic drugs activate lipid biosynthesis through the transcription factor SREBP, we set out to elaborate our findings in various preclinical model systems. Exposing glial-like and neuronal-like cultured cells to different antipsychotic agents, we showed that antipsychotics activated the expression of several SREBP-regulated genes encoding key enzymes in lipid synthesis with varying potency between the different drugs. The effects were much more potent in glial-derived than in neuron-derived cells, which is interesting in light of the fact that glial cells produce the bulk of lipids, essential in myelination and synaptic development, in the central nervous system.

We then treated female rats with the metabolically potent antipsychotic agent olanzapine or with aripiprazole, which is considered metabolically neutral in humans, for two weeks. Olanzapine induced marked increase in food intake and significant weight gain in rats. By including olanzapine-treated rats with restricted access to food, which did not gain weight, we demonstrated that weight gain primarily relies on increased food intake. Aripiprazole, included as a negative control, yielded significant increase in food intake and weight gain. Notably, increased serum triglyceride levels were detected in all olanzapine-treated rats, independent of weight gain, while serum triglyceride elevation was not present in rats treated with aripiprazole. In olanzapine-treated rats, serum triglyceride increase was accompanied by lipogenic activation in peripheral metabolic tissues, particularly in visceral adipose tissue.

In this 2-week experiment, we also included one treatment group receiving the modified fatty acid tetradecylthioacetic acid (TTA), a lipid-lowering agent, and one group treated with a combination of olanzapine and TTA. Despite olanzapine-induced weight gain in the olanzapine-TTA treatment group, TTA cotreatment led to significant reduction in lipid levels in serum and liver. In a follow-up experiment spanning 8 weeks, serum and lipid levels were similarly reduced in all rats receiving TTA, either as monotherapy or in combination with olanzapine or clozapine, in spite of weight-potentiating effects. In the liver, we found that TTA induced the transcription and activity of the key oxidative enzymes ACOX1 and CPT2, and downregulated transcription of HMGCR, the rate-limiting step in cholesterol synthesis. The effects of olanzapine monotherapy on food intake and weight gain wore off approximately three weeks into the experiment, and serum triglycerides were not elevated in olanzapine-treated after 8 weeks of treatment. Clozapine, unlike in humans, did not induce weight gain. We concluded that improved dosing regimens are necessary in order to maintain dysmetabolic effects of antipsychotic in rat in the long term and thus increase the relevance of this animal model. The concomitant weight gain potentiation and lipid-lowering effects of TTA, on the other hand, further supported the presence of independent mechanisms regulating body weight and lipid levels. These parameters may not be fully disconnected, however, as one potential mechanism suggested by us to underlie favourable lipid values was increased adipose tissue mass, providing storage capacity for surplus lipids.

3. List of publications

Paper I

Fernø J, Skrede S, Vik-Mo AO, Håvik B, Steen VM. Drug-induced activation of SREBP-controlled lipogenic gene expression in CNS-related cell lines: marked differences between various antipsychotic drugs. *BMC Neurosci.* 2006 Oct 20;7:69.

Paper II

Skrede S, Fernø J, Vázquez MJ, Fjær S, Pavlin T, Lunder N, Vidal-Puig A, Diéguez C, Berge RK, López M, Steen, VM. Olanzapine, but not aripiprazole, elevates serum triglycerides and activates lipogenic gene expression in female rats. *Int J Neuropsychopharmacol.* 2011 Aug 19:1-17.

Paper III

Skrede S, Fernø J, Bjørndal B, Brede WR, Bohov P, Berge RK, Steen VM. Antipsychotic-induced metabolic adverse effects and pharmacological intervention: challenges with the female rat model (Manuscript).

4. Abbreviations

ACC	Acetyl-CoA carboxylase
AMPK	AMP-activated protein kinase
bHLH-Zip	basic-helix-loop-helix leucine zipper
CVD	cardiovascular disorder
DAG	diacylglycerol
DGAT	diacylglycerol acetyltransferase
ER	endoplasmatic reticulum
FASN	fatty acid synthase
GPAT	glycerol-3-phosphate acyltransferase
HMGCR	hydroxymethylglutaryl-Coenzyme A reductase
HMGCS1	hydroxymethylglutaryl-Coenzyme A synthase 1
Insig	Insulin-induced gene
MGAT	monoacylglycerol acyltransferase
PPAR	peroxisome proliferator-activated receptor
PGC1	peroxisome proliferator activated receptor gamma coactivator 1
SCAP	SREBP cleavage activating protein
SCD	stearoyl-CoA desaturase
SOAT	sterol O-acyltransferase
SREBP	sterol regulatory element binding protein
TZD	thiazolidinedione
TTA	tetradecylthioacetic acid

5. Introduction

5.1 Schizophrenia

5.1.1. Historical aspects

Schizophrenia is probably the psychiatric diagnosis surrounded by the most persistent mythical beliefs and the most resistant prejudice. In Norway, the adjective “schizophrenic” is quite frequently used in order to describe equivocal or inconsistent actions, statements or situations, demonstrating that members of the public confuse schizophrenia with the far less common dissociative identity disorder (formerly known as multiple personality disorder). The fact that the term “schizophrenia” originally means “split mind” may have contributed to this misconception. When Eugene Bleuler introduced the term in 1908, he used the German words “Zerreiung” (tearing) and “Spaltung” (splitting) in order to describe the core concept of a group of “syndromes” (i.e., constellations of symptoms) characterized, among other pathological manifestations, by disintegrated psychological association processes ¹. He elaborated on the concept of “splitting” in his famed 1911 work on schizophrenic disorders, “Dementia praecox oder gruppe der schizophrenien”, and defined several other classic symptoms of schizophrenia, such as psychotic symptoms (“a predilection for fantasy”), disrupted affective abilities and “autism”, a severe loss of interest in the surroundings ¹.

5.1.2 Clinical manifestations

At present, the most commonly used diagnostic criteria for schizophrenia are found in the diagnostic manuals published by the American Psychiatric Association (Diagnostic and Statistical Manual of Mental Disorders, 4th edition - DSM-IV), or by

the World Health Organization (International Statistical Classification of Diseases and Related Health Problems, 10th revision - ICD-10) ^{2,3}. DSM-IV lists 5 diagnostic subgroups for schizophrenia, with the common features of “disturbances in thought, perception, affect, behaviour, and communication that last longer than 6 months.” In addition, patients must exhibit so-called “active phase symptoms” for at least 1 of these 6 months (unless successfully treated). Active phase symptoms include psychotic symptoms, such as hallucinations (often auditory) and delusions, odd beliefs, or bizarre perceptual experiences. Such symptoms, representing features not seen in healthy individuals, are often characterized as “positive symptoms”. In the other end of the spectrum, “negative symptoms” are also hallmarks of schizophrenia. This term is used to describe the absence of emotions, thoughts or behaviour desirably present in healthy individuals, and may manifest as social withdrawal, affective flattening, apathy, or anhedonia. Patients may present with additional symptoms such as disorganized speech and/or disorganized or catatonic behaviour. Impairment of cognitive capabilities is also recognized as an important aspect of schizophrenia. Most patients with schizophrenia experience recurring psychotic episodes throughout their lives.

5.2 Epidemiological aspects of schizophrenia

5.2.1 Incidence and prevalence

The lifetime risk of schizophrenia has traditionally been given at ~1% worldwide. Estimates of incidence (i.e., the number of new cases in a given population per year) depend on a large number of factors such as diagnostic criteria, the diagnostic methods used, the organisation of local health care systems, and demographic elements such as general mortality and migration ⁴. Thus, incidence estimates vary between studies. Stringent diagnostic criteria yielded incidence rates ranging from

6/100,000 to 14/100,000 in a large multinational WHO study (the so-called 10-country study) ⁵. One of the conclusions in this study was that the incidence of schizophrenia shows little variation across populations. However, the question of whether the 10-country study was designed in a way that would ensure the detection of differing incidence between populations has been raised, and the results sparked much debate ⁶. Recently, a meta-analysis indicated an overall median incidence of schizophrenia of 15,2 per 100,000, with an estimated 7 out of 1000 individuals diagnosed with the disorder at some point in their life ⁷. This and other studies indicate that average lifetime prevalence across all populations may be slightly lower than the conventional 1% estimate, and that schizophrenia may be less uniformly distributed than previously thought ⁷⁻⁹. Furthermore, contrasting former beliefs of even gender distribution, meta-analyses have revealed that the male:female risk ratio of developing schizophrenia may be ~ 1.4:1 ^{10,11}.

5.2.2 Costs of schizophrenia

Due to factors such as early symptom debut (i.e., early twenties), protracted course and complex treatment schemes, the economic burden of schizophrenia is overwhelming. An estimated 1,5-3% of health care and social spending of developed countries is accounted for partly by direct costs, such as expenses for treatment, and partly by indirect costs (e.g., lost productivity) of schizophrenia ¹². In addition, non-quantifiable losses of social and psychological character affect patients and family members alike. In statistics generated by the WHO, schizophrenia is listed as the 5th and 6th most significant cause of years lived with disability (YLD) in men and women, respectively ¹³.

5.2.3 Risk factors for schizophrenia

Twin and adoption studies have provided clear evidence that the heritability of schizophrenia is high, perhaps as high as 80% according to one meta-analysis¹⁴. Conventionally, a heritable, “intrinsic” vulnerability is thought to coincide with “external” risk factors to trigger the onset of the disorder in an individual. Generally accepted “external” risk factors include being born during the winter, high paternal age, obstetric complications, prenatal viral infections, and cannabis use⁸. The disorder is often characterized as “multifactorial”, meaning that several circumstances must coexist in order to trigger symptoms.

Conscious of the high heritability, researchers have attempted to identify susceptibility genes for schizophrenia, i.e. genes in which defects (mutations) could increase the risk of suffering from the disorder. The Schizophrenia Research Forum (<http://www.schizophreniaforum.org>) maintains a list of the genes presently found to have the strongest association with schizophrenia. The latest ranking (April 2011) is topped by the genes PRSS16 (PRSS16 protease, serine, 16), PGBD1 (piggyBac transposable element derived 1), and NRG1 (neurogranin (protein kinase C substrate, RC3))¹⁵.

5.2.4 Neurochemical paradigms of schizophrenia

The pathophysiology of schizophrenia, despite eager research, remains elusive. The most influential paradigm in neuromolecular schizophrenia research during the last 40 years has undoubtedly been the so-called dopamine hypothesis. Early versions of this theory were based on the fact that several drugs relieving schizophrenic symptoms bind to, and block, dopamine receptors (particularly D₂ receptors) in the brain, as discussed below¹⁶. Thus it was suggested that cerebral dopaminergic “overdrive” is an essential component of the pathophysiology of schizophrenia. Later versions of the dopamine hypothesis proposed differential dopaminergic dysfunction in neuronal

subpopulations, with elevated mesolimbic (subcortical) dopaminergic signalling possibly underlying positive symptoms, and reduced dopaminergic activity in the prefrontal cortex theoretically causing negative symptoms¹⁷. It has been suggested that the prefrontal hypodopaminergic state may actually cause an increase in striatal dopaminergic signalling¹⁷. More recently, dysfunction in other neurotransmitter systems, such as the glutamatergic and GABAergic systems, have also been implicated¹⁶. Possible defects in several aspects of neuronal signalling are integrated in the so-called neurodevelopmental hypothesis, which focuses on embryonic/developmental defects in synaptic density and other aspects of neuronal function¹⁶. Demyelination, i.e. loss of myelin, the primary component of white matter, affects neuronal connectivity and is thought to be of significance in the pathophysiology of schizophrenia^{18, 19}. In the CNS, myelin is synthesized by oligodendrocytes, a type of glial cells embedding neurons, to facilitate neuronal conductivity²⁰. Signs of impaired myelination have been demonstrated in patients with schizophrenia^{18, 21, 22}, and may either result from reduced myelination during late adulthood or degenerative processes during the course of the illness itself. Most relevant studies include schizophrenic patients receiving pharmacological treatment for schizophrenia, an important confounder which is difficult to avoid, but often deemphasized in the interpretation of results.

5.3 Antipsychotic drugs

5.3.1. Early history of pharmacological therapy for psychiatric disorders

Historically, medicine had little to offer patients suffering from psychosis or other severe psychiatric symptoms. Often, treatment was characterized by more or less desperate attempts to ameliorate suffering and prevent patients from inflicting injury on themselves or others. Methods of treatment such as lobotomy, insulin shocks (the

induction of a hypoglycaemic state leading to loss of consciousness) or “sleep cures” (prolonged comatose states induced by barbiturates or similar agents) were employed during the first half of the 20th century^{23, 24}. Treatment attempts such as these, which may seem primitive and ill-considered to us, must be viewed in light of the scarce options available at the time. Some patients actually appear to have improved, or at least to have experienced blunted positive symptoms, after receiving unspecific pharmacological treatment employed in order to achieve general sedation^{24, 25}. Nevertheless, the fact remains that many types of treatment administered to the mentally ill caused considerable harm or even fatal outcome; for instance, “sleep cures” had a 5% mortality rate²⁴.

Throughout the 1950s, several pharmacological agents specifically improving psychotic symptoms were introduced, and rapidly made their way into clinical practice. Reserpine, an alkaloid isolated from the dried root of the shrub *Rauwolfia serpentina*, predated the drugs presently regarded as antipsychotic agents. In India, *Rauwolfia serpentina* was reportedly used to treat “insanity” long before being introduced to the Western world as an antihypertensive agent in the late 1940s²⁶. Indeed, in addition to its antihypertensive properties, reserpine was found to possess antipsychotic properties²⁷. However, reserpine never gained widespread use as an antipsychotic agent due to unacceptable side effects (hypotension and, importantly, depression²⁸), and due to the introduction of other pharmacological agents with antipsychotic properties.

5.3.2 First-generation antipsychotics

The first specific antipsychotic agent was chlorpromazine, a phenothiazine synthesized in 1950²⁶. Commercially introduced as a treatment for psychiatric illnesses in 1953, chlorpromazine is considered the prototype antipsychotic agent, and the first of the so-called “typical”, or first-generation, antipsychotics²⁹. Its introduction has been described as a revolution by psychiatrists who, for the first time, observed specific treatment-induced regression of positive symptoms in patients with

schizophrenia²⁵. Haloperidol, synthesized in 1958 and commercially launched in Europe in 1959, belongs to a different chemical class than chlorpromazine, namely the butyrophenones²⁹. Several other antipsychotics were also introduced during the 1950s and 1960s (Table 5.1). During the 1970s, experiments revealed that all antipsychotic agents known thus far were characterized by high affinity for dopaminergic receptors, blocking such receptors in the brain and thereby inhibiting the binding of dopamine³⁰. In particular, dopamine D₂ receptor antagonism seemed essential in terms of antipsychotic effect; drugs lacking this property have later been demonstrated to have inferior effect on psychotic symptoms^{31,32}. Chlorpromazine is a relatively weak D₂ antagonist compared to other early antipsychotics, while haloperidol is a potent D₂ blocker^{33,34}.

Drug	Chemical group	Commercially introduced	Current trade names ¹
Chlorpromazine	Phenothiazine	1953	(Largactil) ²
Perphenazine	Phenothiazine	1957	Trilafon
Haloperidol	Butyrophenone	1959	Haldol
Zuclopenthixol	Thioxanthene	1962	Cisordinol

Table 5.1 Selected first-generation (typical) antipsychotics³⁵.

The correlation of D₂ affinity with antipsychotic effect is now well established; D₂ occupancy above a certain threshold is required in order to achieve clinical antipsychotic effect³⁶. However, several dopaminergic pathways with physiologically

¹ Norway

² Not for standard sale in Norway (2011)

distinct functions exist in the brain, and D₂ occupancy yields site-specific clinical effects³². During clinical trials and early clinical use, it became evident that both chlorpromazine, haloperidol and other typical antipsychotic agents induce severe dose-dependent extrapyramidal side effects, including akathisia (an intense feeling of restlessness or unease), Parkinsonism, dystonias, and tardive dyskinesia^{34, 37}. The propensity to induce these adverse effects is correlated with D₂ affinity in the striatum, and dopaminergic blockade in this area of the brain is thought to be the main cause of extrapyramidal side effects³².

5.3.3 Second-generation antipsychotic agents

The serious adverse effects associated with typical antipsychotic agents encouraged the search for new antipsychotics. Clozapine, the first of the so-called second-generation antipsychotic agents, was synthesized in 1958 (i.e., the same year as haloperidol) patented in Switzerland in 1960, but not introduced clinically in Europe until 1972, and in the USA in 1990 (reviewed in^{38, 39}). Second-generation antipsychotics are frequently designated “atypical” and viewed as a group, despite pharmacological heterogeneity. In general, the most distinct differences between first- and second-generation drugs result from variations in D₂ and serotonin (5-hydroxytryptamine, 5-HT) receptor affinity. Several second-generation agents occupy 90-100% of 5-HT₂ receptors, with 5-HT_{2A} antagonism not observed for typical agents, while the degree of D₂ blockade is generally lower than among the typical antipsychotics^{32, 36}. Accordingly, the risk of extrapyramidal side effects is significantly lower in patients treated with second-generation than in those treated with first-generation antipsychotics. For instance, clozapine binds D₂ receptors much more weakly than do first-generation drugs, while its affinity for serotonergic receptors (5-HT_{2A}, 5-HT_{2C}) is as much as 20 times higher than its D₂ affinity^{31, 36, 40}. Unfortunately, treatment with clozapine carries a risk of agranulocytosis (a sharp decline in the number of circulating white blood cells, with resultant risk of infection), a potentially lethal adverse effect⁴¹. After being withdrawn from the

market in 1975 due to the risk of agranulocytosis, clozapine was relaunched in the USA in 1990 after clinical studies demonstrated its superiority over typical antipsychotic agents in treatment-resistant cases of schizophrenia ⁴². At present, however, other atypical drugs are commonly regarded as primary choices in newly diagnosed psychoses.

Drug	Chemical group	Commercially introduced	Current trade names (Norway)
Clozapine	Dibenzodiazepine	1972	Leponex, Clozapin, Clozapine
Risperidone	Benzisoxazole	1994	Risperdal, Risperidon
Olanzapine	Thienobenzodiazepine	1996	Zyprexa, ZypAdhera, Olanzapin
Ziprasidone	Benzisothiazolyl	2001	Zeldox
Aripiprazole	Quinolone	2002	Abilify
Quetiapine	Dibenzothiazepine	1998	Seroquel, Quetiapin
Amisulpride	Benzamide	1990	Solian

Table 5.2 Second-generation (atypical) antipsychotics ³⁵. Aripiprazole has been called the first “third-generation” antipsychotic due to its properties as a partial D₂ agonist.

Olanzapine, approved by the American Food and Drug Administration (FDA) in 1996, is chemically related to clozapine ⁴³. Reminiscent of clozapine’s properties, olanzapine’s affinity for 5-HT₂ receptors exceeds its affinity for D₂ receptors, with an *in vitro* 5-HT₂/D₂ affinity ratio approximating 12 ⁴⁰. Imaging studies have

demonstrated that olanzapine's D₂ affinity is higher than that of clozapine³⁶. Agranulocytosis has rarely been reported in patients treated with olanzapine⁴⁴. However, relatively soon after the drug's introduction, reports of severe metabolic side effects surfaced; these effects are discussed below⁴⁵. Ziprasidone, marketed since 2001, is another atypical antipsychotic, with D₂ affinity comparable to that of risperidone as well as high affinity for several 5-HT receptors, combined with serotonin and noradrenaline reuptake inhibition⁴⁶. Aripiprazole, commercially available from 2002, is frequently referred to as the first "third-generation" antipsychotic. This agent's pharmacological properties deviate from that of prior antipsychotics in that it is a partial D₂ agonist (or possibly possessing "functionally selective" D₂ affinity) rather than a "traditional" D₂ antagonist⁴⁷. Aripiprazole also possesses partial agonism at 5-HT_{1A} receptors, as well as 5-HT₂ antagonism.

	5-HT _{1a}	5-HT _{2A}	5-HT _{2C}	D ₁	D ₂	α _{2C}	α _{1A}	α _{1B}	β ₂	M ₁	M ₃	H ₁
Chlorpromazine	(+)	+++	++	+	+++	++	++++	++++	+	++	++	++++
Haloperidol	(+)	++	0	++	+++	+	++	+++	0	0	0	(+)
Risperidone	+	++++	++	+	+++	+++	+++	+++	0	0	0	++
Olanzapine	(+)	+++	++	++	++	++	+	+	0	++	++	+++
Clozapine	+	++	++	+	+	++	+++	+++	0	++	++	+++
Quetiapine	+	+	(+)	+	+	++	++	++	(+)	+	(+)	+++
Aripiprazole	+++	+++	+	+	++++	++	++	++	0	(+)	(+)	++
Ziprasidone	++	+++	++	++	+++	++	++	+++	(+)	0	0	+

Table 5.3 Receptor binding profiles of various antipsychotic agents. 0: no affinity; (+) very weak affinity; + weak affinity; ++ intermediate affinity; +++ strong affinity; ++++ very strong affinity for the receptor subtype, reflected in darkening colour gradient. Adapted from³¹. 5-HT: 5-hydroxytryptaminergic (=serotonergic); D: dopaminergic; α,β: subtypes of adrenergic receptors; M: muscarinic, H: histaminergic.

5.3.4 Metabolic adverse effects of antipsychotic drugs

Metabolic disturbances, including weight gain, are recognized adverse effects of typical antipsychotic drugs, both phenothiazines and, to a moderate degree, haloperidol, as reviewed in ^{48, 49}. Glucose dysregulation, occasionally debuting as ketoacidosis, was also observed in patients treated with typical antipsychotics ^{50, 51}, and increased serum cholesterol was described in patients treated with chlorpromazine in 1967 ⁵². With increasing use of clozapine and olanzapine, however, it soon became evident that these antipsychotics induce more frequent and more serious metabolic dysfunction than older drugs, and this issue has gained increasing attention during the last decades. Early clinical studies on clozapine mention weight gain as an adverse event ^{53, 54}. Several years later, elevated serum triglyceride levels were reported in patients treated with clozapine ⁵⁵⁻⁵⁷. Reports on olanzapine's adverse effect profile published in the late 1990s, while describing low risk of dyskinesias and hematotoxicity, also mention the risk of weight gain ^{58, 59}, later demonstrated to occur due to increased adipose tissue mass ^{60, 61}. For olanzapine and clozapine, a frequently cited meta-analysis estimated an average short-term weight gain (10 weeks) in the range of 3.5-4 kg, with continued weight gain at least during the first year of treatment – in one study, 80% of first-episode psychotic patients receiving olanzapine gained >7% of pre-treatment body weight during the first 52 weeks of treatment ⁶².

During the early years of olanzapine availability, the implications of metabolic adverse effects remained unclear (“the significance of this [i.e., weight gain] beyond cosmetic effects is unknown” ⁶³), but the first reports of olanzapine-induced hypertriglyceridemia were published during the same period ⁶³⁻⁶⁵. Average olanzapine-induced increase in serum triglycerides is often given at 30-50%, while increase in serum cholesterol levels has also been reported during clozapine and olanzapine treatment ^{57, 65, 66}. Furthermore, both clozapine and olanzapine have been demonstrated to increase the risk of insulin resistance and type 2 diabetes ⁶⁶⁻⁶⁹. Consequently, atypical antipsychotics significantly increase the risk of developing the constellation of parameters often termed the metabolic syndrome (Table 5.4).

Parameter	Males	Females
Waist circumference	≥94 cm*	≥88 cm*
Serum triglycerides	>1,7 mmol/l	>1,7 mmol/l
Serum HDL	<1,03 mmol/l	<1,29 mmol/l
Blood pressure	Systolic >130 or diastolic >85 mmHg	
Fasting serum glucose	> 5,6 mmol/l or recognized type 2 diabetes	

Table 5.4. Patients with central obesity plus any two of the findings described above fulfil the criteria for the diagnosis of metabolic syndrome⁷⁰. * Europids.

The risk of weight gain, serum lipid increase and glucose dysregulation is generally regarded as intermediate for the second-generation agents risperidone and quetiapine, and low for aripiprazole and ziprasidone^{49, 62, 65, 69, 71-73}. In fact, replacing olanzapine with aripiprazole has been shown to significantly improve the metabolic status of patients⁷⁴.

5.3.5 Clinical implications of metabolic adverse effects

Mortality rates among patients with schizophrenia are markedly increased compared to those found in the general population, causing patients with serious mental disorders to lose 2-3 decades of life on average⁷. This is partly due to increased suicide rates and increased susceptibility to fatal accidents, but most importantly due to early death from somatic conditions, with cardiovascular disorders as the single most common cause of death^{7, 75, 76}. Compared to the general population, patients with psychiatric disorders may have a higher background risk of developing the metabolic syndrome, which may lead to cardiovascular disorders^{77, 78}. Failure to seek

medical care or attend screening programmes, life style issues among patients (smoking, sedentary life style), and inadequate attention from caregivers concerning somatic comorbidity are probably important causes^{75, 76, 79}. This complicates the interpretation of data regarding the contribution of antipsychotics to metabolic risk, particularly as some reports on metabolic dysfunction in schizophrenic patients include patients having received antipsychotic agents⁸⁰. However, numerous studies indicate that treatment with antipsychotics adds significantly to the mortality rates in patients with serious mental disorders^{69, 75, 81, 82}. Metabolic dysfunction, particularly weight gain, is also a potential cause of non-adherence, increasing the risk of psychotic relapse⁸³.

In addition to patients with schizophrenia, many individuals diagnosed with other psychiatric disorders, e.g. bipolar disorder, may respond well to antipsychotic drugs⁸⁴. According to Eli Lilly's 2008 sales figures, the company made \$4.7 billion from worldwide sales of olanzapine that year⁸⁵. In Norway, official sales figures show that 15,649,516 DDD (estimated average daily dose for an adult patient) of antipsychotic agents were prescribed in Norway in 2008⁸⁶. Consequently, a very large number of patients worldwide receive antipsychotic treatment and are thus at risk of developing metabolic adverse effects.

5.3.6 Can receptor binding profiles explain metabolic adverse effects?

Increased food intake, primarily due to impairment of satiety onset, is thought to be the main underlying cause of weight gain induced by antipsychotic agents, and has been demonstrated both in humans^{81, 87} and in rodents⁸⁸⁻⁹⁰. At present, no consensus exists in terms of the pharmacological properties underlying hyperphagia and other metabolic adverse effects, or the intracellular signalling pathways through which they are mediated. Several antipsychotic agents have antihistaminergic properties³¹, and affinity for histaminergic (H₁) receptors correlates with weight gain^{91, 92}. H₁ antagonism is linked to increased food intake⁹¹, suggestedly through H₁-mediated activation of AMP-activated protein kinase (AMPK) in the hypothalamus⁹³. The

involvement of several other receptors (serotonergic 5-HT_{2C}, adrenergic α 1 and α 2 receptors, and muscarinic M3 receptors) has also been implicated in antipsychotic-induced weight gain⁸⁹. The complex receptor binding profiles of antipsychotic drugs (Table 5.3) complicate the identification of one or several receptors primarily responsible for weight gain⁹⁴, and predictions of weight gain risk based on receptor binding profiles are sometimes unsuccessful. For instance, ziprasidone, which is recognized not to induce significant weight gain in humans, possesses both 5-HT_{2C} antagonism (high) and H₁ affinity (moderate)⁹⁵, and would thus be expected to induce weight gain. Notably, some antipsychotics with weak affinity for H₁ receptors, e.g. haloperidol, are known to cause moderate weight gain^{31, 49, 91}. Thus, a well-defined receptor binding profile resulting in increased risk of weight gain has yet to emerge. Regarding other dysmetabolic adverse effects, H₁, 5-HT_{2C}, and M₃ receptors has been linked to derangements in glucose metabolism, while no receptor binding profile has been defined as far as dyslipidemia is concerned^{96, 97}.

5.3.7 Animal models for antipsychotic-induced metabolic adverse effects

In the exploration of the molecular mechanisms underlying metabolic adverse effects, a reliable animal model is instrumental. Rodent models of antipsychotic treatment have been extensively explored, with two major challenges surfacing during the two last decades. Firstly, the degree to which each specific antipsychotic agent induces metabolic side effects differ, in some cases, between human and rodent, particularly with regard to weight gain. Secondly, in rodents, antipsychotic-induced weight gain is sex-dependent, i.e. observed almost exclusively in females (Table 5.5). Conclusive evidence for gender differences in the risk of developing antipsychotic adverse effects has not been found in humans^{98, 99}. As described above, olanzapine and clozapine are the antipsychotic agents most prone to induce massive weight gain and related dysmetabolic features, such as dyslipidemia, in patients^{49, 51, 100, 101}. In female rats, elevated food intake and weight gain through increased adipose tissue mass during

short-term treatment with olanzapine (1-10 mg/kg) are well characterized, even in animals receiving standard rodent chow with high carbohydrate and low fat content^{89, 102, 103}. Olanzapine has also been demonstrated to have hyperphagic effects in male rats^{88, 104, 105}. Furthermore, studies in male rats have shown that subchronic treatment with olanzapine increases adipose tissue mass, but not body weight, using diets with medium to high fat content^{106, 107}. One study in which olanzapine-treated male rats received standard laboratory chow also reported increased adipose tissue mass in the absence of hyperphagia and body weight gain after 20 days of olanzapine treatment¹⁰⁵.

As for clozapine, with a clinical metabolic profile similar to that of olanzapine⁶⁹, hyperphagia has been reported in male rats receiving a clozapine dose of 0.3 mg/kg⁸⁸. Weight gain has not been demonstrated in rats of either gender treated with 0.5-8 mg/kg^{108, 109}, but was reported in one 28-day study in female rats treated with 20 mg/kg clozapine¹¹⁰. In contrast, clozapine has somewhat unexpectedly been reported to induce weight reduction in rats at doses of 6-10 mg/kg^{109, 111}. Reminiscent of observations from olanzapine-treated male rats, clozapine treatment has been shown to induce adiposity in female rats, with no effect on weight gain, except in one study reporting weight gain in male rats receiving clozapine 20 mg/kg for 7 weeks^{111, 112}. Aripiprazole, considered metabolically neutral in patients, has been demonstrated in one study to induce moderate weight gain in female rats (8 mg/kg)¹⁰³, while apparently weight-neutral in a similar experiment using aripiprazole a dose of 2.25 mg/kg¹¹³. In agreement with the latter report, an aripiprazole dose of 2 mg/kg failed to induce hyperphagia in female rats¹¹⁴. Ziprasidone, also regarded metabolically neutral in patients, has not been demonstrated to possess hyperphagic effect in rat, although some groups have reported moderate weight gain in female rats at relatively low doses (2-10 mg/kg)¹¹⁵⁻¹¹⁷.

	Olanzapine		Clozapine		Aripiprazole		Ziprasidone	
	♂	♀	♂	♀	♂	♀	♂	♀
Weight gain	↔ ^{105-107, 118} ↑ ¹¹⁹	↑ ^{89, 102, 103, 118}	↔/↓ ^{108, 109, 118} ↑ ¹¹²	↔/↓ ^{108, 109, 111, 118} ↑ ¹¹⁰		↑ ¹⁰³ ↔ ¹¹³	↔ ¹⁰⁶	↑ ¹¹⁷
Adipose mass	↑ ¹⁰⁵⁻¹⁰⁷	↑ ¹⁰³		↑ ¹¹¹		↔ ¹¹³	↑ ¹⁰⁶	
Hyperphagia	↑ ^{88, 105} ↔ ¹⁰⁵	↑ ^{103, 120}	↑ ⁸⁸ ↔ ¹¹⁸	↔ ^{111, 118}		↔ ¹¹⁴	↔ ¹⁰⁶	
Serum triglycerides		↔ ^{103, 115, 121}					↔ ¹⁰⁶	↔ ¹¹⁵
Glucose dysmetabolism	↑ ^{122, 123}	↑ ¹²⁴	↑ ^{122, 123}				↔ ^{122, 123}	

Table 5.5 Overview of dysmetabolic features demonstrated in rats. ↑: increase observed relative to vehicle. ↔: no change observed relative to vehicle. ↓ decrease observed relative to vehicle.

Few studies have reported lipid levels in rodent experiments. Serum triglycerides have largely been reported as unaltered by olanzapine^{103, 115, 121}, while increased serum free fatty acids after treatment with this drug have been shown in one experiment¹⁰³. Derangements in glucose metabolism have been thoroughly demonstrated in rats treated with olanzapine and clozapine¹²²⁻¹²⁴. In female mice, the same pattern as in rats, with olanzapine-induced weight gain, has been demonstrated¹²⁵. A few studies have also shown olanzapine-induced increase in serum triglycerides in female mice^{126, 127}.

5.4 Lipid metabolism

5.4.1 General aspects of lipid metabolism

Lipids constitute a large group of molecules involved in numerous essential processes and structures in the human organism. Fatty acyls (fatty acids), mono-, di-, and triglycerides, phospholipids and sterol-containing molecules, such as cholesterol, all belong to this class of macromolecules. A short overview of relevant aspects of lipid metabolism is presented below.

5.4.2 Free fatty acids and triglycerides

In times of excess, energy is primarily stored as triglycerides in adipose depots¹²⁸. Lipids may be absorbed from the diet or synthesized *de novo* from pyruvate¹²⁸. *De novo* synthesis primarily occurs in the liver, from which triglycerides are exported to white adipose tissue. The committed step in fatty acid synthesis is the formation of malonyl-CoA from acetyl-CoA, synthesized by acetyl-CoA carboxylase 1 (ACC1) (Figure 5.1)¹²⁸. Malonyl-CoA then undergoes elongation in several steps catalyzed by fatty acid synthase (FASN), which possesses 7 enzymatic sites¹²⁸. FASN synthesises palmitate (16:0), a 16-carbon, saturated fatty acid (i.e., lacking double bonds). Palmitate may be further elongated by elongases, and/or desaturated by desaturases, enzymes introducing double bonds. The desaturase most relevant to this thesis is stearoyl-CoA desaturase (SCD1), a Δ^9 desaturase introducing double bond between C9 and C10 to yield, if palmitate is the substrate, palmitoleate [C16:1(Δ^9)]¹²⁸.

Three fatty acyl-CoA molecules linked to a glycerol-derived backbone (glycerol-3-phosphate) form a triglyceride molecule. Two of the three carbon sites of the glycerol backbone is acetylated in a reaction catalyzed by glycerol-3-phosphate acyltransferase (GPAT; Figure 5.1) and monoacylglycerol acyltransferase (MGAT), forming

phosphatidic acid¹²⁹. GPAT constitutes the committed step in triglyceride synthesis. After dephosphorylation of phosphatidic acid, yielding diacylglycerol (DAG), DAG is acetylated by diacylglycerol acetyltransferase (DGAT), producing triacylglyceride (triglyceride)¹²⁹.

The anabolic hormone insulin is necessary for both triglyceride synthesis and for energy uptake and storage in adipose depots; lipid uptake to adipose tissues depends on maintained insulin sensitivity¹²⁸. Abnormalities in lipid metabolism and glucose dysregulation are intimately related, and obesity is closely correlated with insulin resistance¹³⁰.

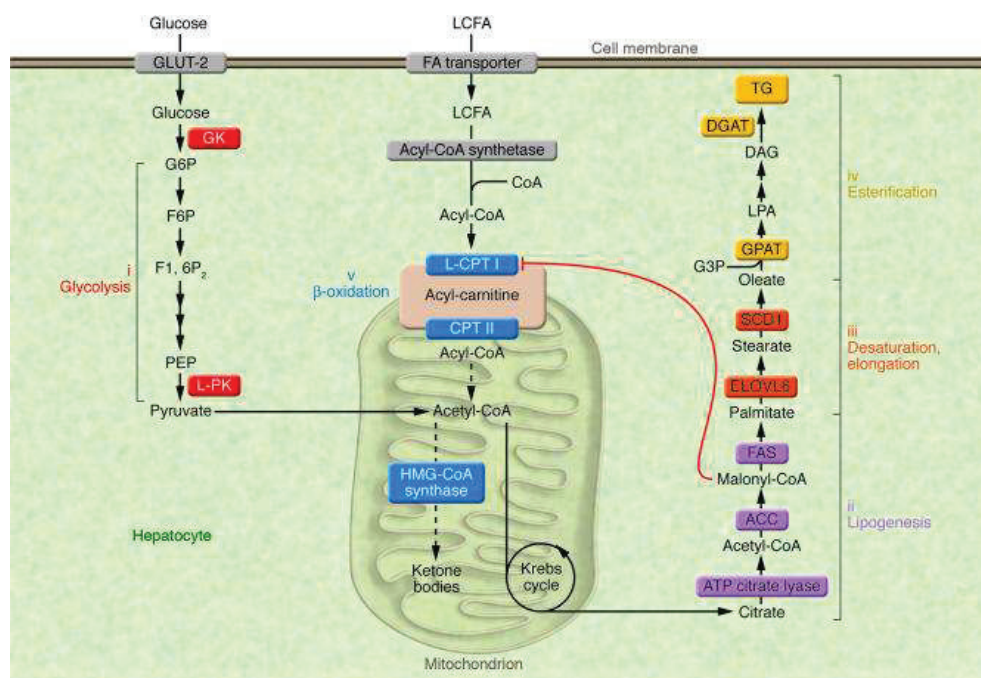


Figure 5.1 Important steps in fatty acid synthesis (purple), desaturation (orange), triglyceride biosynthesis (yellow) and fatty acid oxidation (blue). Taken from¹³¹, with permission. For full names of relevant enzymes, see text.

5.4.3 Cholesterol metabolism

The percentage of cholesterol in cellular membranes has significant influence on the physical properties and organization of the membrane^{132, 133}. In addition, cholesterol is a substrate for synthesis of complex sterols, such as steroid hormones (e.g. cortisol, testosterone and estradiol), and bile acids¹³⁴. Like fatty acids and triglycerides, cholesterol may be absorbed from the diet or synthesized *de novo* in the liver. *De novo* synthesis of cholesterol is a complex pathway, with hydroxymethylglutaryl-Coenzyme A reductase (HMGCR) as the rate-limiting enzyme¹²⁸. Statins, commonly used lipid-lowering drugs, are inhibitors of HMGCR¹²⁸. For transport and storage, cholesterol is esterified, i.e., linked to fatty acids through an ester binding, possible because cholesterol contains an –OH group. Esterification, which decreases cholesterol's lipophilic properties, is catalyzed by the enzyme sterol O-acyltransferase (SOAT, also known as ACAT).

5.4.4 Lipids in the brain

As mentioned in section 5.2.4, glial cells (oligodendrocytes) produce myelin embedding the axons of neurons in the CNS¹³⁵. Myelin is rich in cholesterol, which is synthesized *de novo* by glial cells, as cholesterol cannot be transported across the blood-brain barrier, and neurons have been thought to possess limited capacity for cholesterol synthesis^{136, 137}. Increasing amounts of data also support the idea that glial cells, previously regarded as passive cells whose only function is to maintain neurons, may be required for the formation and maintenance of interneuronal synapses in the brain^{138, 139}. Lipids, among them cholesterol, constitute key components in the efficient communication between neuronal and glial cells^{133, 138-140}. Glial cells secrete apolipoprotein E (ApoE)-bound cholesterol, which is taken up by neurons by means of low-density lipoprotein (LDL) receptors and acts as a growth factor for neurons^{138, 141, 142}.

5.4.5 Regulatory factors in lipid biosynthesis

As Figure 5.1 shows, a large number of enzymes are involved in the synthesis of fatty acids and triglycerides. Many of these are primarily regulated at the transcriptional level, meaning that transcription of the genes encoding them is regulated in a coordinated manner¹⁴³. The sterol regulatory element binding proteins (SREBPs) are transcription factors involved in numerous aspects of fatty acid, triglyceride, and cholesterol synthesis, and are frequently designated “master” transcription factors in lipogenesis, as they hold key positions in the coordinated transcription of lipogenic genes. Two main SREBP proteins, SREBP1 and SREBP2, are encoded by two distinct genes. The SREBF1 gene encodes two isoforms, SREBP1a and SREBP1c, of which SREBP1c is the isoform most extensively expressed in liver and adipose tissues in mice, while SREBP1a is primarily found in cultured cells¹⁴⁴. SREBP1c is a main regulator of genes encoding enzymes involved in fatty acid and triglyceride metabolism, e.g. the genes encoding ACC1, FASN, SCD1, and GPAM¹⁴⁵⁻¹⁴⁷. SREBP2, encoded by SREBF2, controls the transcription of enzymes synthesizing sterols, including the rate-limiting HMGCR as well as HMGCS and several of the enzymes catalyzing later steps in the cholesterol biosynthesis pathway¹⁴⁷.

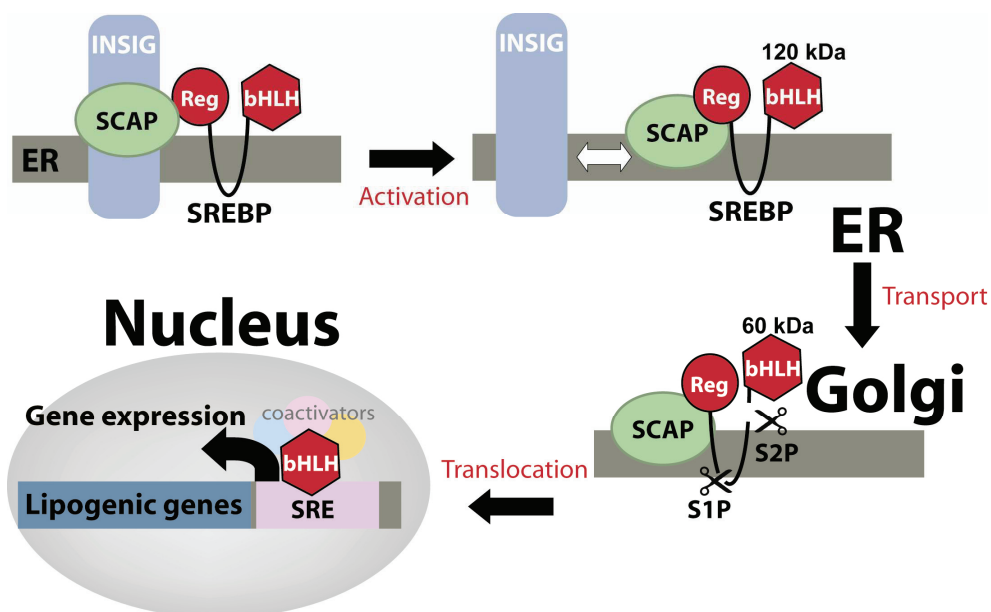


Figure 5.2 SREBP and its activation. SREBP, consisting of one regulatory domain and one domain containing a basic-helix-loop-helix leucine zipper (bHLH-Zip) protein¹⁴⁸. The inactive form of SBERP forms a complex with SREBP cleavage activating protein (SCAP), a lipid sensor. Insulin-induced gene (Insig) immobilizes the SREBP-SCAP complex in the ER when lipid levels are high. Upon cholesterol depletion, SREBP-SCAP is transported to the Golgi apparatus, where the bHLH-Zip domain is released, translocating to the nucleus to initiate transcription of its target genes. SREBP1 is regulated by numerous nutritional factors (e.g. carbohydrates)¹⁴⁹, while SREBP2 is primarily regulated by cholesterol levels. Illustration by Johan Fernø.

The SREBP proteins reside in the endoplasmic reticulum (ER) membrane as inactive precursor proteins of 120-130 kDa. Intracellular sterol depletion or other alterations in the cell's nutritional status result in translocation of the inactive SREBP protein to the Golgi apparatus, where proteolytic cleavage produces an active (nuclear) form of 60-70 kDa (Figure 5.2)¹⁴⁷. A large number of lipogenic gene promoters contain sterol regulatory elements (SRE) or an E-box motif with affinity for cleaved SREBPs. As a key regulator of anabolic processes, SREBPs are activated in states of energy surplus, such as increased energy intake (in the form of

carbohydrates or lipids). Both saturated fatty acids and insulin promotes SREBP1c-mediated lipogenesis^{147, 150, 151}.

5.4.6 Mechanisms of fatty acid oxidation

When energy mobilization is required, free fatty acids may be released from triglycerides by lipolysis, and oxidised in the mitochondria in a process releasing ATP¹²⁸. If energy reserves are depleted, free fatty acids are transported from white adipose tissues to skeletal muscle, heart, and liver, for oxidation¹⁵². Oxidation takes place in the mitochondrial matrix. Fatty acids, “activated” through linkage to CoA yielding fatty acyl-CoA, are linked to carnitine by carnitine palmitoyltransferase 1 (CPT1) before being transported across the outer mitochondrial membrane (Figure 5.1). In the mitochondrial matrix, the fatty acyl group is transferred from carnitine to a matrix-specific pool of CoA. The transient linkage to carnitine (carnitine shuttle) represents the rate-limiting steps of fatty acid oxidation. In the matrix, fatty acyl substrates are oxidised in a four-step process. Oxidation of one palmitoyl molecule (C16:0), which is broken down to 8 acetyl-CoA molecules, yields 28 ATP units (in addition, acetyl-CoA oxidation through the citric acid cycle yields further ATP)¹²⁸. Organelles other than mitochondria, namely peroxisomes, may also be the site of fatty acid β oxidation, catalyzed by different enzymes than those found in mitochondria. In particular, the enzyme acyl-CoA oxidase 1 (Acox1), catalyzing the first step in peroxisomal β oxidation, is important.

5.4.7 Regulation of fatty acid oxidation and lipid storage

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors sensing lipid levels and regulating a wide array of responses to altered lipid load. Three PPAR isoforms - PPAR α , PPAR δ , and PPAR γ - are recognized, all transcription factors with a large number of target genes¹⁵². PPAR α , highly expressed in the liver, induces β -oxidation in times of reduced energy access, e.g. in the fasting state. PPAR γ is predominantly expressed in adipose tissues, and activates pathways facilitating lipid

storage through biosynthesis and adipocyte differentiation. Increased lipid storage capacity resulting from PPAR γ activation is thought to be an important mechanism of action for the pharmacological PPAR γ agonists thiazolidinediones (TZDs), presently used as insulin-sensitizing agents in patients¹⁵².

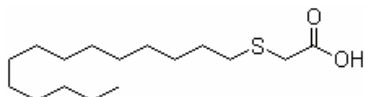


Figure 5.3 The structural formula of TTA.

5.4.8 Tetradecylthioacetic acid (TTA)

Tetradecylthioacetic acid (TTA) is an artificially synthesized fatty acid where the 3rd carbon atom is replaced with a sulphur atom, producing a non-oxidizable fatty acid derivative ($\text{CH}_3\text{-(CH}_2\text{)}_{13}\text{-S-CH}_2\text{-COOH}$). Acting as an agonist for all PPAR subspecies, with most potent effects on PPAR α , TTA nevertheless induces the mitochondrial β -oxidative apparatus, resulting in increased mitochondrial oxidation of naturally occurring substrates for β -oxidation¹⁵³. In male rats, TTA has been shown to prevent adiposity and insulin resistance induced by high-fat diet, decreasing plasma triacylglycerol and free fatty acid levels^{153, 154}. Fibrates, another type of PPAR α agonists used clinically in the management of hypertriglyceridemia¹⁵², have also been shown to improve insulin sensitivity in rodents¹⁵⁵⁻¹⁵⁷. Small clinical trials have indicated metabolically beneficial effects of TTA in patients¹⁵⁸, and TTA was therefore included in our rat experiments.

6. Aims of the study

The overall aim of this study was to identify new molecular mechanisms underlying metabolic adverse effects of antipsychotic drugs, and to confirm their relevance by means of a rat model.

Specific aims:

- To identify differential metabolic effects of different antipsychotic drugs in various cultured cell types modelling cell populations in the CNS
- To clarify the role of hyperphagia in antipsychotic-induced weight gain, in a rat model
- To examine the possible uncoupling of weight gain from alterations in lipid metabolism in rat exposed to antipsychotic drugs
- To explore the use of a non-invasive imaging technique (MRI) for quantification of antipsychotic-induced adiposity in rat
- To examine the development of food intake, weight gain and lipogenic alterations in long-term antipsychotic treatment in rat
- To investigate the lipid-lowering, modified fatty acid TTA as a potential pharmacological intervention strategy for metabolic adverse effects

7. Summary of results

Paper I

The experiments in Paper I were based on results from our initial microarray studies in antipsychotic-treated, cultured cells^{159, 160}. Examining the effects of a number of antipsychotics on lipogenic gene expression in human neuron-like and glial-like cell types we found that clozapine, one of the two most metabolically potent antipsychotics in humans, and chlorpromazine, the “prototype” first-generation antipsychotic, activated the transcription of lipogenic genes with most pronounced effects in glial-like cells. Lipogenic activation was mediated by the SREBP transcription factor, a master regulator of lipogenesis.

Paper II

Rats were treated with olanzapine or aripiprazole for 13 days. As expected, olanzapine-treated rats increased their food intake and gained weight in the form of increased adipose tissue mass, demonstrated by weighing and MRI-based quantification of adipose tissue. Aripiprazole, included as a negative control due to its clinical status as metabolically neutral, induced a similar pattern. In an olanzapine-treated group of rats with limited food access, weight gain was absent. However, serum triglyceride levels were increased in both olanzapine treatment groups, as was lipogenic gene expression in visceral adipose tissue. Aripiprazole-treated rats did not develop these features. We concluded that factors other than weight gain may significantly contribute to antipsychotic-induced metabolic derangements.

Paper III

Seeking to elaborate our findings from Paper II, we extended the treatment period to 8 weeks, included two new antipsychotics and slightly lower drug doses. The weight gain-inducing effects of olanzapine wore off ~ 3 weeks into the experiment, while treatment with clozapine failed to induce weight gain. The modified fatty acid TTA potentiated weight gain both in combination with olanzapine and clozapine, with concomitant reduction in plasma and liver lipid levels. The lipid-lowering effects of TTA were accompanied by substantial increase in the transcription and enzymatic activity of the key oxidative enzymes ACOX1 and CPT2 in the liver, as well as reduced transcription of the rate-limiting enzyme in cholesterol, HMGCR. While calling the relevance of the female rat model into question, the results supported the concept of weight-lipid uncoupling.

8. Discussion

8.1. Methodological aspects

8.1.1 Cell culture

In paper I, we examined potential lipogenic effects of antipsychotic drugs in five different cell cultures. The use of cell cultures is extremely widespread in biological research. Among the numerous advantages of using cultured cells are the easily controlled environment, e.g. availability of nutrients, and flexible experimental setups, e.g. regarding drug doses. Furthermore, working with cell culture circumvents most ethical considerations. However, even though cultured cells interact, traditional cell cultures represent a considerable oversimplification, since interaction between different cell types usually present in an organ, as well as tissue-tissue interaction, are absent. In addition, cells are usually transformed, usually malignantly, in order to enable division and growth in culture. In many instances, such “non-physiological” conditions are necessary in order to discriminate relevant molecular processes from feedback responses and other compensatory events normally present in a complete organism. Using two neuron-like (HCN2 and SH-SY5Y) and two glial-like (GaMg and CCF-STTG1) human cell lines, as well as one hippocampal primary culture from rat (R-Hi-501), we found corresponding upregulation of SREBP target genes in all cell types, but with minor effects in cells derived from neurons. The common pattern observed across cell lines indicated that antipsychotic-induced SREBP activation observed in GaMg cells in our previously published article ¹⁵⁹ is not limited to one type of cultured cells, and could represent a generalized drug effect.

8.1.2 RealTime PCR

In order to quantify expression levels of potentially relevant genes in both cell culture and in rat tissues, we have extensively used RealTime PCR. This method is based on the concept of a fluorescent probe or a DNA-binding dye being released as mRNA is replicated throughout 35-40 PCR cycles. Continuous quantification of released probe or dye permits “real-time” quantification of mRNA levels¹⁶¹. The PCR cycle during which the level of probe or dye is first detected at a higher level than the sample background represents the gene’s cycle threshold (Ct) value¹⁶¹. The RealTimePCR method is highly sensitive to alterations in gene expression across a wide range of expression levels, i.e. even for genes with particularly high or low expression levels in a certain tissue¹⁶¹. We used SYBR® Green, a DNA-binding dye, and primers designed in-house, for all RealTime reactions. For analysis of results, we used the comparative Ct method ($2^{-\Delta\Delta Ct}$ method), with normalization towards one or more endogenous control genes. Here, the relative difference in gene expression between antipsychotic-treated samples and vehicle-treated samples, with the latter used as calibrator, was calculated based on the genes’ Ct values. This method assumes similar replication efficiencies between genes, controlled by means of serial dilutions, and is more reliable if PCR products are kept below 150 bp. We therefore designed utilized primers such that the PCR product size was kept below this size for all genes.

In order to control for differences in RNA input in the reverse transcription stage, Ct values for all target genes were normalised to Ct values for genes thought to be stably expressed in target tissues (i.e. housekeeping genes). Selection of endogenous control genes is a major challenge when using RealTime PCR, and the expression of several housekeeping genes have been shown to be affected by drug exposure in cell culture

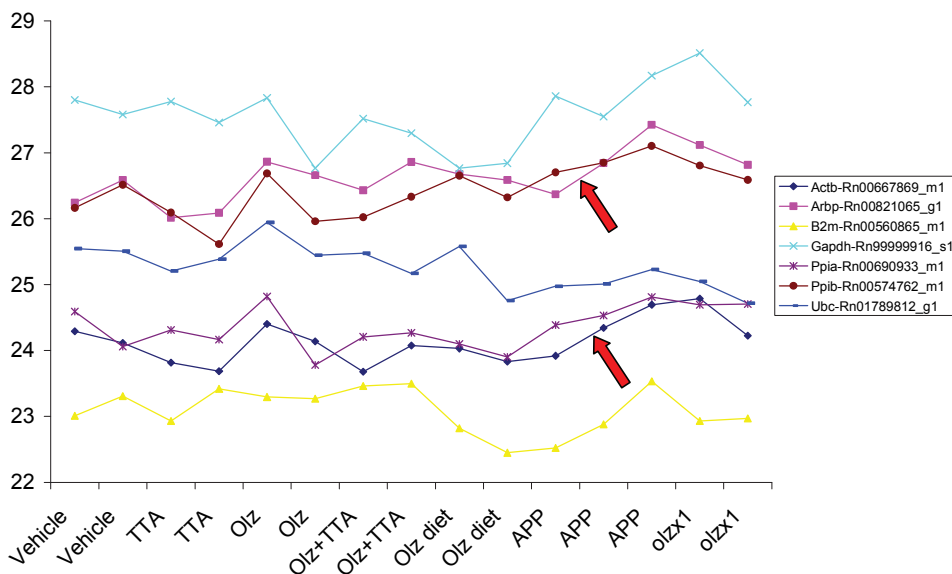


Figure 8.1 Ct values (Y axis) for 7 potential endogenous control genes in white adipose tissue from rats exposed to different pharmacological treatments. Each data point represents one sample. Samples were taken from rats exposed to different pharmacological treatments. Each coloured curve represents one potential reference gene. Arrows signify the two endogenous control genes used in our rat experiments.

Preparing to analyze tissues not previously examined in our lab (particularly white adipose tissues), we ran selected samples on predesigned panels (low density arrays, LDA; Applied Biosystems) containing several pre-selected endogenous control genes commonly used (Figure 8.1; unpublished data), in order to select stably expressed housekeeping genes. Based on LDA results, candidate genes for further use as endogenous controls were chosen and further evaluated using different RealTime assays. The reference gene used in Paper I, ribosomal protein, large, P0 (Rplp0; designated P0 in Paper I-III) in addition to the commonly used β -actin, were selected for use in further analyses, and these two reference genes were run in each new batch of cDNA in the rat experiments. In order to detect potential systematic treatment effects on endogenous control genes, Ct values in vehicle- and antipsychotic-treated rats were habitually compared for all examined genes during analysis of RealTime

PCR data. Results from several biological replicates have confirmed our initial patterns of transcriptional regulation by antipsychotic agents, increasing the validity of results. Furthermore, several key findings were validated at the protein level using Western blots.

8.1.3 MRI-based quantification of adipose tissue volume

Paper II includes tissue imaging data collected using a 7T MRI scanner. Olanzapine- and aripiprazole-exposed rats included in a 2-week experiment underwent MRI scanning prior to the initiation of treatment, and by the end of the treatment period. The protocol used for image analysis was developed by in-house collaborators. In the attempt to extract quantitative data (adipose tissue volumes) from MRI images, two major challenges deserve mention. Firstly, in order to quantify alterations in white adipose tissue mass between two time points, one needs reliable anatomical landmarks. Scanning the entire animal was not an option, as this would require an unreasonable amount of time (considering both time spent in anaesthesia and experimental logistics). As MRI is not a sensitive method for imaging of skeletal parts, the early idea of using lumbar vertebrae as landmarks was dismissed. Instead, we chose to use the easily visible kidneys. A second challenge was the distinction between white adipose tissue and artefacts, particularly in the intestine. The segmentation protocol developed to distinguish and quantify adipose tissue yielded a relevant impression of increased adipose tissue volume in olanzapine-treated rats, but numerical estimates were not significantly correlated with dissected adipose tissue mass (Paper II). The reasons behind this discrepancy remain unclear. In further developing MRI acquisition and analysis, a natural first step would be to increase the anatomical area examined, e.g. by scanning the entire abdominal area. Alternative methods (e.g. dual-energy X-ray absorptiometry [DXA] scans) are available for quantification of total adipose tissue mass in rodents (reviewed in ¹⁶³). MRI images permit the distinction of different adipose depots (visceral, subcutaneous), and as demonstrated by us, rodents could be examined at several time points during the same

experiment, with no significant mortality among examined rats. These are among the advantages that should encourage further use and development of MRI protocols and analysis tools in studies centred on metabolic adverse effects.

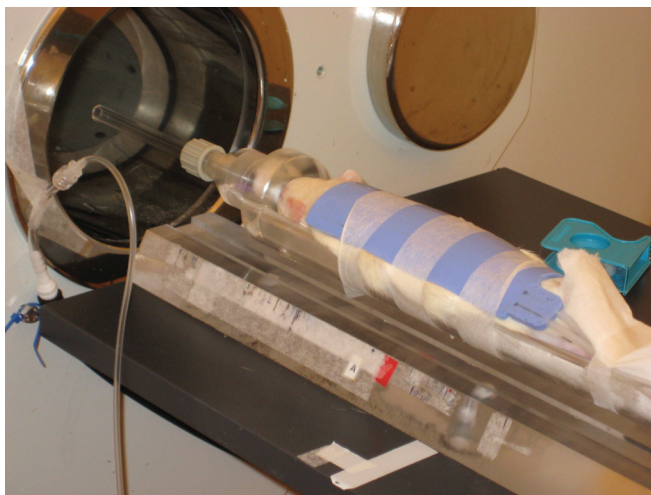


Figure 8.2. Deeply anaesthetized rat mounted for MRI examination, covered by a heating mat (blue). Photograph by Silje Skrede.

8.1.4 Selecting a drug vehicle

Most antipsychotic agents are close to insoluble in water, but readily dissolved in DMSO or alcohol. Concerns over toxicity, vehicle-induced biochemical effects confounding results, and palatability issues led us to search for an appropriate vehicle for use in rat studies involving oral administration of antipsychotics. Eventually, based on our own experiments and relevant literature, 4% carboxymethyl cellulose (CMC) was chosen^{114, 117}. Antipsychotics were suspended, not dissolved, in the CMC solution. As the drugs rapidly precipitated, frequent resuspension was necessary in order to maintain the correct drug concentration during administration to rats, potentially introducing dosing inaccuracy. Measurements of serum drug levels

showed modest variation across antipsychotic-treated rats at the time of sacrifice, indicating adequate dosing of drug suspended in CMC.

8.2 Modelling metabolic adverse effects in rat

8.2.1 Divergent findings in human and rat

As mentioned in the Introduction (see Table 5.5), several aspects of metabolic adverse effects diverge in rodent and human, primarily considering gender differences (observed in rat, not convincingly demonstrated in humans) and dysmetabolic potencies between different antipsychotic agents. In particular, the near absence of metabolic alterations in clozapine-treated rats remains puzzling.

The question of gender specificity raises the question of whether endocrine factors may play a more significant role than has been demonstrated thus far. Antipsychotics, as recognized dopamine receptor antagonists, are known to increase prolactin levels in patients^{164, 165}. Olanzapine has been demonstrated to increase serum prolactin levels both in female^{117, 124} and in male rat^{105, 166}, indicating that hyperprolactinemia *per se* cannot provide a straightforward explanation for the gender pattern observed in rat. Oestrogens are closely linked with distribution of adipose tissue as well as several other aspects of energy metabolism¹⁶⁷, and may be relevant to the gender differences observed in rat. One study found unaltered serum oestradiol levels in female, olanzapine-treated rats¹²⁴. In ovariectomized rats, with negligible oestradiol levels, olanzapine has still been demonstrated to induce food intake and weight gain¹⁶⁸. In patients, olanzapine treatment had no effect on oestradiol levels, neither in males¹⁶⁹ nor in females¹⁷⁰. Even though other endocrine factors could be relevant, there are no obvious candidates in the relatively limited number of studies examining hormonal effects of antipsychotics with high dysmetabolic potential¹⁷¹.

During the last few years, a quite differentiated picture of antipsychotic-induced alterations in rodents has emerged. For instance, increased adiposity in spite of constant body weight after subchronic olanzapine treatment of male rats has been described in several studies^{105, 106}. Through these, and our subchronic experiments demonstrating olanzapine-induced serum triglyceride elevation, an increased number of shared features in human and rodent may emerge, adding to the relevance of rodent models. Nevertheless, in further development of animal models for antipsychotic-induced metabolic adverse effects, several important challenges remain.

8.2.2 Challenge I: pharmacokinetics of antipsychotics in rat

In rat, metabolism of antipsychotic agents differs significantly from human metabolism of these drugs. For instance, the half-life ($t_{1/2}$) of clozapine in rat serum is 1.5 hours, while the average $t_{1/2}$ is 10 hours in human^{172, 173}. Similar differences are found for olanzapine, with a $t_{1/2}$ of 2.5-3 hours in rat, compared to an average of 30 hours in human¹⁷⁴⁻¹⁷⁶. The rapid metabolism of antipsychotics in rat complicates the selection of appropriate drug doses in rat experiments, as serum drug concentrations rapidly reach negligible levels.

8.2.3 Challenge II: dosing of antipsychotics in rats

Defining a “correct” dose for antipsychotics in rats based on clinical observation is difficult. A commonly used approach when selecting doses for rat experiment is direct transfer of doses used in patients. In many countries, the maximum olanzapine dose approved for use in patients is 20 mg, i.e. ~0.3 mg/kg in a person weighing 70 kg¹⁷⁷. As for clozapine, patients may receive 600 mg, i.e. ~8.6 mg/kg¹⁷⁷. In rats, these doses are too low to induce weight gain^{108, 109, 111, 178}. Based on the significant differences in human and rodent drug metabolism, a dosing strategy based on the percentage of D_2 receptor occupancy in the CNS has been suggested¹⁷⁹. In order to achieve D_2

occupancy comparable with that observed in humans (65-80% for olanzapine and 45-65% for clozapine) after a single dose of antipsychotic agents in rat, olanzapine doses in the range of 1-2 mg/kg and clozapine doses of 5-15 mg/kg have been recommended¹⁷⁹. As mentioned in the Introduction, raising clozapine doses in rats to this level has so far not yielded weight gain. In fact, in one subchronic study rats received 30 mg/kg clozapine, with absence of weight gain¹⁸⁰. Notably, in male rats receiving 20 or 40 mg/kg clozapine, D₂ occupancy in the CNS was far below the levels observed in humans at clinically relevant doses; body weight was not measured¹⁸¹. High doses (olanzapine: ~ 20 mg/kg; clozapine: 10-20 mg/kg?) may cause sedation^{108, 182}, and this fact has influenced the doses selected for studies in rodents. Some studies, however, reported absence of sedation in rats in the 20-40 mg/kg clozapine dose interval^{180, 181}. Thus, underdosing may represent an explanation for the lack of weight gain in clozapine-treated rats.

A second dosing-related issue is drug tolerability. In patients, weight gain is typically considered to continue for approximately one year^{183, 184} before body weight reaches a plateau, although this issue is debated (reviewed in^{100, 185}). Weight gain in rats treated with low to moderate doses of olanzapine (1-6 mg/kg) seems liable to dwindle during treatment, as indicated by our results, with dampened effects on weight gain after 2-3 weeks of treatment (Papers II, III), although one group showed continued weight-inducing effect of olanzapine 1,5 mg/kg for 8 weeks¹¹³. A stepwise increase of olanzapine doses, from 4 to 20 mg/kg, potentiated weight gain compared to our results, but at 33 days, cumulative weight gain for rats treated with control substance and olanzapine (end dose 20 mg/kg) was nevertheless relatively similar¹⁰⁸. Stepwise dose increase may, however, circumvent sedation, and should be considered in long-term rat experiments.

8.2.4 Challenge III: administration of antipsychotics to rats

Administration of antipsychotics to rats is commonly achieved by means of subcutaneous or intraperitoneal injections^{124, 186}, oral administration through gavage¹⁰², through food¹⁰⁶ or through drinking water^{105, 181}. As the number of daily injections is, for practical reasons, limited, and food/water intake is unevenly distributed throughout the light/dark phases, all these approaches are likely to result in fluctuating serum concentrations during a 24-hour period¹⁸¹. Several antipsychotic agents degrade when exposed to light, further complicating the approach of mixing drugs with food or drinking water. In order to ensure constant delivery of non-degraded antipsychotics, some researchers have utilized osmotic minipumps, with subcutaneous or intraperitoneal implantation of a syringe constantly delivering a fixed dose of dissolved drug. As pumps previously needed to be refilled frequently due to drug degradation, the use of osmotic minipumps could be more labour-intensive than anticipated¹⁸⁷. Implantation of syringes carries a risk for infection, and minipumps are subject to mechanical failure. In carefully controlled conditions, using light-protected minipumps, dissolved olanzapine has been demonstrated to remain stable for 42 days¹⁸⁸. When successful, serum drug concentrations reached by means of minipumps are higher and more stable than those achieved through other means of administration^{118, 188}. The use of minipumps, therefore, may be preferable to intermittent oral dosing or injections. Other potential strategies include pellets designed for subcutaneous implantation, followed by constant drug release, which are available, but have not been extensively used¹⁸⁹.

8.2.5 Challenge IV: the influence of diet

Standard rat chow used in laboratories is very high in carbohydrate (40-50%), with a low fat content (~5%). In male, olanzapine-treated rats and in female, clozapine-treated rats, the limited number of studies demonstrating weight gain included chow

with high fat content^{110, 119, 190}. Some studies with very similar setups, however, have not yielded significant weight gain^{106, 190, 191}. Finding the “ultimate” dietary composition may not be sufficient to achieve weight gain in male rats treated with olanzapine or in clozapine-treated rats, but is likely to represent one of several necessary conditions.

8.2.6 Steps towards increased reliability of rat models

The possibility remains that a certain constellation of drug dose, mode of administration, and dietary fat content will lead to successful replication of a more “human-like” pattern of antipsychotic-induced metabolic adverse effects than has previously been achieved. In two recently described experiments, male rats were fed high-fat chow and treated with 1.5-10 mg/kg olanzapine administered by minipumps; still, only marginal effects on body weight gain were observed, in spite of increased adipose tissue mass^{188, 192}. Experiments including even higher antipsychotic doses than previously administered to rats may be a natural next step. Of course, one can ask whether the need to “fine-tune” several parameters will yield a sufficiently robust model - even if clozapine-induced weight gain is successfully modelled, the fact may remain that some as yet unrevealed property distinguishes metabolic effects of olanzapine in rat from that of clozapine.

8.4. Molecular mechanisms of metabolic adverse effects

8.4.1 Hyperphagia is the main cause of body weight gain

Weight gain may be caused by increased energy intake, reduced energy expenditure, or a combination of the two. In order to investigate the role of food intake in olanzapine-induced weight gain, we included olanzapine-treated, pair-fed treatment groups in our rat experiments. Pair-fed rats were offered an amount of chow corresponding with the average amount consumed by control animals during the preceding 24 hours, typically 15-16 grams per rat. Freely fed olanzapine-treated rats may consume 20-24 g of chow during 24 hours. We observed that olanzapine-treated rats with free access to chow rapidly gained weight, while pair-fed rats did not gain weight, or even gained less weight than control animals (Paper II). This led us to conclude that increased food intake (hyperphagia) is the main driving force behind the observed weight gain, in agreement with previous rat studies^{88, 121} as well as clinical studies^{193, 194}. Our MRI studies, coupled with dissection and weighing of adipose tissues, demonstrated that weight gain occurred primarily in the form of increased adipose tissue mass, also in agreement with previous results in rat (Table 5.5)^{103, 124} and in patients^{60, 61}. The pair-fed rats in our experiment did not show signs of increased adipose tissue mass. In an article not included in this thesis, our group has explored the possible mechanisms of antipsychotic-induced hyperphagia, demonstrating increased levels of appetite-stimulating neuropeptides in hypothalamus¹⁹⁵.

8.4.4 The role of energy expenditure in weight gain

In addition to increased energy intake, reduced energy expenditure may contribute to weight gain. As previously mentioned, sedation resulting in reduced physical activity is a recognized adverse effect of antipsychotic agents. We did not, however, observe

significant sedation in the experiments described in Paper II. If sedation contributed significantly to weight gain, the obese phenotype would have been expected to be present in the olanzapine pair-fed treatment group.

Rodents possess brown adipose tissue, which is able to convert surplus energy to heat through a process termed thermogenesis¹⁹⁶. Reduced thermogenesis, measured as reduced protein levels of uncoupling protein 1 (UCP1), has been reported in pair-fed, olanzapine-treated rats which gained weight, in contrast to pair-fed rats in our experiments¹⁹⁷. In a previously mentioned study, olanzapine treatment did not induce altered thermogenic rates in brown adipose tissue from rats with stable body weight, but increased adiposity¹⁷⁸. Based on these conflicting findings, we examined the expression of UCP1, PPAG γ , and peroxisome proliferator activated receptor gamma coactivator 1 α (PGC1 α), important markers of thermogenesis, in brown adipose tissue¹⁹⁸. Several of these markers were downregulated in both *ad libitum*-fed and pair-fed olanzapine groups, possibly signifying reduced thermogenesis. Although synergistic effects of hyperphagia and reduced thermogenesis in the olanzapine *ad libitum*-group cannot be ruled out, the lack of weight gain in the olanzapine pair-fed group demonstrated that reduced thermogenesis alone did not significantly contribute to weight gain in our experiments. In our 8-week experiment (Paper III), TTA monotherapy downregulated PGC1 α and UCP1, while no weight gain was observed in this treatment group. In olanzapine-TTA treated rats, PGC1 α was also downregulated, possibly contributing to the weight gain observed in this treatment group.

Adult humans were formerly thought to lack brown adipose tissue. In recent years, however, the presence of brown adipose tissue in adults has been demonstrated by means of PET scans¹⁹⁹⁻²⁰¹. Brown adipose tissue may play a significant role in human metabolism, and should not be ignored when examining energy balance²⁰¹.

8.4.5 The role of fatty acid oxidation in antipsychotic-induced metabolic adverse effects

Reduced capacity for fatty oxidation could, theoretically, induce energy surplus and thus obesity. Examining the expression of ACOX1, CPTs, and PPARs in liver and white adipose tissues from subchronically treated rats (Paper II) or from chronically treated rats (Paper III), we found no evidence for reduced oxidative capacity induced by olanzapine or clozapine. The lipid-lowering effects of TTA, on the other hand, were likely related to the sharp increase observed in hepatic ACOX1 and CPT1 transcription and activity.

8.4.6 “Uncoupling” of body weight and serum lipid levels

Hypertriglyceridemia is known to correlate with obesity, in particular with the amount of visceral adipose tissue. However, the existence of both “obese, but metabolically healthy” and “metabolically obese” populations is now recognized²⁰². The latter group is characterized by normal BMI, but still present with dyslipidemia and reduced insulin sensitivity²⁰³. In paper II, through the introduction of pair-fed treatment groups, we found that olanzapine-treated rats developed elevated levels of serum triglycerides without concomitant adiposity. These results were further supported by results in paper III, in which olanzapine-TTA treated rats gained weight, while nevertheless developing lowered serum and liver levels of cholesterol and triglycerides, resulting in lipid profiles not expected in rats with this degree of obesity.

Interestingly, early clinical findings, both of chlorpromazine-induced increase in serum cholesterol levels and olanzapine-induced increase in serum triglyceride levels, indicated that serum lipid levels in patients were not simply raised secondary to weight gain^{52, 65}. Several recent clinical studies have also presented evidence for increased serum triglycerides independent of weight gain²⁰⁴⁻²⁰⁶. In the CAFE study, patients treated with quetiapine experienced the least increase in weight gain; in spite

of this, increase in serum triglycerides and total cholesterol were most pronounced in this treatment group ²⁰⁷. Of note, in a “drug switching” study in which patients changed from olanzapine to aripiprazole treatment, a significant reduction in serum triglycerides occurred rapidly after the switch, while weight loss occurred more gradually, indicating that triglyceride levels did not simply decrease in parallel with body weight ²⁰⁸. To our knowledge, no animal studies other than our own have investigated the potential uncoupling between weight gain and increased serum lipids.

8.4.7 Antipsychotic-induced, SREBP-mediated lipogenic activation in cultured cells

In addition to our papers describing antipsychotic-induced SREBP activation in GaMg cells, our research group published a work demonstrating similar effects in human liver cell lines (THLE-3 and HepG2) ¹⁶⁰. Several studies in cell culture have subsequently supported our finding of antipsychotic-induced SREBP activation in cells, including adipocyte cultures and primary hepatocytes ²⁰⁹⁻²¹². In paper I, we demonstrated that antipsychotics induce both SREBP1 and SREBP2 target genes. We showed increased proteolytic cleavage of SREBP2, i.e. activation at the protein level, and upregulation of the genes encoding the SREBP1a and SREBP2. The exact nature of the interaction between antipsychotic agents and the SREBP system remains unclear, but may be related to hampered intracellular cholesterol transport through direct interaction between drugs and intracellular membranes ^{213,214}. Chlorpromazine, which was demonstrated by us to induce cholesterol biosynthesis genes in GaMg cells, is a tricyclic cationic amphipathic drug with both lipophilic and hydrophilic chemical properties ²¹⁵. Several antipsychotics, among them clozapine, also possess a tricyclic structure, and have been demonstrated to directly interact with the cholesterol biosynthesis pathway ^{212, 215-217}. Possibly, reduced ER cholesterol causes “imaginary” lack of intracellular cholesterol, inducing SREBP translocation, maturation and thus target gene transcription. Recently, an antipsychotic-induced ER stress response (unfolded protein response) has been suggested to contribute to SREBP activation,

offering a potential explanation for the activation of both SREBP isoforms, which are differentially regulated in the physiological setting²¹⁸.

8.4.8 Antipsychotic-induced lipogenic activation in rodents and humans

Prior to our subchronic studies, we performed acute experiments on clozapine and olanzapine treatment in rats. We found short-term lipogenic effects, most notably significant hepatic lipid accumulation, accompanied by an early transcriptional activation followed by sustained downregulation of lipogenic genes in liver and adipose tissues^{219, 220}. These observations illustrate the presence of potent negative feedback mechanisms complicating interpretation of direct pharmacological effects of antipsychotics in rat. In our two-week studies with moderate olanzapine doses (Paper II), feedback mechanisms were partly circumvented through the reduction of drug doses and repeated exposure, and we demonstrated weight-independent upregulation of several SREBP1c target genes in liver and in visceral adipose tissue. Corresponding upregulation of *Fasn* has previously been demonstrated in adipocytes from male, olanzapine-treated rats that did not gain significantly more weight than control animals during 5 weeks of treatment, but developed increased adipose tissue mass¹⁰⁷. Furthermore, in our subchronic study (Paper II), we found signs of SREBP1c activation in the liver, both at the transcriptional and at the protein level. In agreement with other studies, serum levels of monodesaturated fatty acids were increased, indicating elevated desaturase activity^{221, 222}. Upregulation of *Scd1* transcription is notable, as this desaturase has been suggested to represent a key branch point in lipid biosynthesis, directing lipids towards triglyceride or cholesterol ester formation (i.e., lipid storage)²²³.

In visceral adipose tissue, in spite of upregulation of several recognized SREBP1c target genes, *Srebp1c* transcription or SREBP1c activation at the protein level were absent. In fact, there are indications that in adipocytes and adipose tissues, SREBP1c exerts less profound effects on the transcription of its classic target genes than in the liver²²⁴⁻²²⁷. Other, as of yet unrevealed, mechanisms may contribute to olanzapine-

induced lipogenic activation in adipose tissues. As lipogenic upregulation was present in olanzapine pair-fed rats, this induction is likely to represent a pharmacological effect of olanzapine, not a consequence of hyperphagia and obesity.

In conclusion, we found partial overlap of results in cell culture and animal models, underlining the complexity of lipid metabolism in the “real-life” setting. SREBP-mediated lipogenesis may be relevant for dyslipidemic adverse effects of antipsychotics, independent of hyperphagia and weight gain. Further experiments are required in order to examine potential subchronic effects of clozapine on lipogenesis, the relationship between lipogenic activation in metabolic tissues and the elevation of serum triglycerides, to identify the factors mediating lipogenic effects of antipsychotics in adipose tissues, and to perform relevant clinical studies. Interestingly, a clinical pilot study demonstrated elevated transcription of the genes encoding FASN and SDC1 in blood cells from olanzapine-treated patients²²⁸.

8.5. Clinical aspects related to lipogenic activation by antipsychotics

8.5.1 Do metabolically potent antipsychotic agents have superior clinical efficiency?

Whether some antipsychotic agents are more efficient than others in relieving either positive or negative symptoms of schizophrenia - in particular, whether second-generation antipsychotics are more efficient than first-generation drugs - has been extensively debated²²⁹⁻²³⁷. Results from several large clinical studies, among them the CATIE and CUtLASS studies, indicate that despite trends towards superior efficacy of olanzapine on certain outcomes (primarily time to treatment discontinuation), atypical antipsychotics are not convincingly superior to first-generation antipsychotics in terms of overall clinical efficiency or quality of life scores^{177, 233, 238, 239}. As

previously mentioned, several reports have documented the superior efficacy of clozapine in treatment-resistant schizophrenia^{42, 177}. In fact, a large population-based Finnish study demonstrated significantly reduced mortality in patients treated with clozapine compared to patients treated with other antipsychotics, in spite of clozapine's metabolic risk profile²⁴⁰; this study subsequently received criticism for strongly confounded analyses²⁴¹.

Comparisons of typical and atypical antipsychotics are complicated, among other issues, by differences in dosing and adverse effect profiles. For instance, motor effects of typical antipsychotics could result in the impression that patients suffer from more severe negative symptoms²⁴². Potential interests of the pharmaceuticals industry must also be kept in mind when evaluating treatment effects²⁴³. In general, issues such as adverse effect profile, former response to medication, patient satisfaction, cost-effectiveness, and clinicians' former experiences are significant issues when patients with suspected schizophrenia are assigned to a certain pharmacological treatment^{177, 231, 244}. Naturalistic, non-sponsored studies are required to further facilitate evidence-based choice of treatment of psychosis²⁴⁵.

8.5.2 Are the differences in dysmetabolic potency between different antipsychotic agents as substantial as formerly thought?

As mentioned above, adverse effect profile constitutes an important consideration when selecting an antipsychotic, particularly for patients experiencing their first episode of symptoms. Therefore, a balanced image of adverse effect profiles is required. In paper II, as expected, we found a significant stimulatory effect of olanzapine on food intake and weight gain in female rats. Aripiprazole, believed to be metabolically neutral in humans, was included as a negative control, and both in our subchronic and in our chronic (8-week) experiment (Paper III), aripiprazole showed hyperphagic and weight-inducing potential. One former experiment in female rat

resulted in aripiprazole-induced weight gain (aripiprazole dose 4-8 mg/kg)¹⁰³, while a long-term experiment yielded no weight gain (aripiprazole dose 2.25 mg/kg)¹¹³. This could indicate that body weight in female rats is more easily affected by pharmacological treatment than body weight in human subjects. On the other hand, could these results be transferred back to the clinical setting, helping to define a more differentiated picture of the dysmetabolic potential of individual antipsychotics? It is quite obvious that in patients, some antipsychotics - particularly clozapine and olanzapine - induce more potent weight gain, dyslipidemia and insulin resistance than other agents. Furthermore, the switching of treatment from olanzapine, quetiapine, or risperidone to aripiprazole in patients led to significant improvements in body weight and lipid parameters^{74, 208}. However, it is obligatory to keep in mind the fact that a majority of clinical data is obtained through studies involving previously medicated patients, which may significantly influence results. The value of data collected in previously unmedicated patients is gaining increasing attention²⁴⁶. For instance, quetiapine and risperidone, have been shown to cause significant weight gain ($\geq 7\%$ of pre-treatment body weight) after 1 year of treatment in 50% of treatment-naïve patients receiving quetiapine, and in 58% of treatment-naïve patients receiving risperidone^{62, 207}. Similarly, aripiprazole, quetiapine and risperidone all caused weight gain in adolescent patients²⁴⁷. As agents such as quetiapine and aripiprazole have been in clinical use for an increasing number of years, more data concerning their metabolic adverse effects will accumulate, and results from rat experiments may turn out to be more relevant than they presently appear.

8.5.3 Are clinical improvement and metabolic adverse effects correlated, independent of antipsychotic agent?

As discussed in paragraph 8.5.1, no sound conclusion has been reached regarding superior efficacy of individual antipsychotics. The idea of a link between symptom relief and metabolic adverse effects has been a recurrent issue for decades of research on antipsychotics. In 1967, an article concerning patients treated with chlorpromazine

stated that serum cholesterol levels were positively correlated with clinical improvement⁵². A number of studies, both in patients treated with olanzapine and in clozapine-treated patients, have pointed out a similar link between metabolic adverse effects, commonly weight gain, and improvement of positive or negative schizophrenic symptoms^{205, 248-251}. One study found a relation between therapeutic response and weight gain both in olanzapine and haloperidol treatment groups, most pronounced in olanzapine-treated patients⁹⁸. Another study, which has received criticism for prophylactically administering the anticholinergic drug to benztropine to all patients receiving haloperidol, reported positive correlation of serum cholesterol and improved cognition in schizophrenic patients across clozapine, olanzapine and haloperidol treatment groups²⁵².

Findings such as these are highly interesting considering that antipsychotics recognized to have the highest incidence of weight gain, hyperlipidemia and glucose dysregulation remain widely used⁸⁵. However, some studies have failed to detect correlation of clinical state and metabolic adverse effects; for instance, analyses of data from the CATIE study resulted in drug-independent association between increased BMI and improvement, but the effect size was deemed too subtle to be clinically significant^{205, 253}. Furthermore, lack of correlation between clinical improvement and weight gain has been reported in clozapine-treated patients^{254, 255}. A recent study in hospitalized psychotic patients found that quetiapine, which is less metabolically potent than olanzapine, was more efficient than olanzapine in reducing several treatment outcomes²⁴⁵. Several issues complicate correlation analyses of metabolic adverse effects and clinical improvement. For instance, regain of self-care (including increase in food intake), could precede weight gain. No mechanistic link has yet been suggested between clinical response and metabolic adverse effects, but antipsychotic-induced lipogenesis is highly interesting in this context.

8.5.4 Lipogenesis as a possible therapeutic mechanism of action

As mentioned in the Introduction, demyelination (decreased volume or disrupted function of white matter) is presently thought to play a significant role in the development of schizophrenia, and may be of particular importance with regard to negative symptoms^{18, 256-258}. Oligodendrocytes, the cells primarily responsible for myelin production, synthesize cholesterol, an essential component of myelin, as cholesterol is not imported to the CNS^{133, 137}. Interestingly, a physiological role of the SREBP transcription factors in the CNS is emerging (reviewed in²⁵⁹). Lipid synthesis, controlled by both SREBP1 and SREBP2, in different subtypes of glial cells is thought to be important both in myelination and various aspects of neuronal development, such as synaptic plasticity. For instance, SREBP1 and SCD1 activation, correlated with oleic acid synthesis in astrocytes, may be involved in neuronal growth and differentiation²⁵⁹. Thus, alterations in lipid metabolism in the CNS may be highly relevant in light of the demyelination, as well as other aspects of neuronal dysfunction, observed in patients with schizophrenia. Indeed, myelin-related genes have been shown to be downregulated in prefrontal cortex from patients^{260, 261}. In paper I, we demonstrated that SREBP2-activating effects of antipsychotics were more potent in glial-derived cells than in cells derived from neurons. A similar study on antidepressant drugs yielded comparable results²⁶². Consequently, our results demonstrating antipsychotic-induced induction of lipid metabolism may be relevant both to the clinical and adverse effects of these drugs. In fact, the potential effects of antipsychotic agents on myelination have been investigated in a rodent model for demyelination, where the copper chelator cuprizone was used to pharmacologically induce demyelination. In rats treated with cuprizone, which developed phenotypic features reminiscent of negative symptoms of schizophrenia, downregulation of oligodendrocyte markers was demonstrated in the prefrontal cortex²⁶³. In another experiment on mice, clozapine and quetiapine was found to prevent loss of myelin induced by cuprizone²⁶⁴. In male patients with schizophrenia, treatment with risperidone led to increased myelination, as quantified by means of MRI^{265, 266}. A

recent review by the first author of the latter article states that “[...] widely used psychotropic treatments have under-appreciated CNS metabolic and neurotransmitter effects on myelination, its plasticity, and repair that may substantially contribute to their mechanisms of action”²⁶⁷. The possible relation between clinical efficacy and metabolic adverse effects is intriguing, and the results discussed above underline the fact that avoiding all use of antipsychotics with metabolically unfavourable adverse profiles does not provide an easy solution to the huge clinical challenge represented by metabolic adverse effects.

8.5.5 Potential intervention strategies in patients with antipsychotic-induced dysmetabolism

88% of patients with dyslipidemia identified in the CATIE study population received no lipid-lowering treatment⁷⁹. In general, secondary prevention of metabolic complications of antipsychotic treatment appears to have received little attention. Clinical studies have suggested that adjunctive treatment with metformin may have beneficial effect on insulin sensitivity and body weight²⁶⁸⁻²⁷⁰. Statins, i.e. inhibitors of HMGCR, have been demonstrated as efficient in antipsychotic-induced dyslipidemia²⁷¹. Notably, metformin indirectly inhibits HMGCR through the activation of AMPK²⁷², meaning that both classes of agents regarded as candidates for efficient intervention act on the rate-limiting step in cholesterol synthesis.

Prior to pharmacological intervention strategies, information regarding hyperphagia and the risk of developing metabolic adverse effects should be given; patients may be surprisingly accessible to educational measures²⁷³. A few relatively small studies have addressed the effect of healthy dietary habits and exercise, and found promising effects²⁷⁴⁻²⁷⁶. Combining behavioural and pharmacological intervention strategies could probably reduce the risk for cardiovascular disease in patients receiving antipsychotic agents, but it is difficult to imagine that other measures than newly developed, clinically efficient drugs with negligible adverse effects will reduce risk levels to those found in the general population.

9. Concluding remarks

In selecting the correct treatment for psychotic, delusional or cognitively impaired patients, a substantial responsibility rests on the carer. Patient compliance is more crucial and challenging in schizophrenia than in many somatic conditions, and facilitating compliance greatly reduces the risk of relapse. Risk-benefit considerations must rely on solid evidence for clinical efficiency and adverse effects. A survey of the massive body of literature published regarding clinical effects of antipsychotic agents leaves no definite conclusion with regard to superior clinical efficacy of individual antipsychotics. In terms of adverse effect profiles, though, some antipsychotics are clearly more metabolically potent than others. Quite a few authors have pointed to a correlation of clinical improvement with the degree of metabolic adverse effects. Despite several important confounders, this apparent link is highly interesting in light of the essential role of lipid synthesis in the brain. Thus, understanding the mechanisms underlying adverse effects is not simply a step towards elimination of side effects, but may be necessary in order to develop more efficient antipsychotics. Judging by the lack of progress in such development during the last 60 years, the most realistic short-term aim will be to develop well-founded strategies for the prevention of weight gain, diabetes and dyslipidemia in patients treated with the drugs in question. As a complement to dietary measures and physical activity, metformin and statins appear to be the best prophylactic candidates.

10. Future perspectives

As mentioned in the Discussion, we recently published an article detailing the hypothalamic mechanisms underlying olanzapine-induced hyperphagia. An important question is whether the antipsychotics' lipogenic effects and negative effects on glucose metabolism are mediated via the CNS, or whether they occur due to direct effects of antipsychotics on peripheral metabolic tissues, such as the liver or adipose tissues. Distinguishing primary effects from feedback effects is challenging, and specific pharmacological and/or genetic inhibition seems a natural step forward in this regard. Such strategies may also strengthen the rationale for prophylactic pharmacological intervention for antipsychotic-induced metabolic adverse effects.

Further work is also necessary in order to increase the scientific and clinical relevance of rodent models in this field. In particular, issues such as drug dosing and administration should be carefully considered in order to reduce the gap between effects of olanzapine and clozapine, as well as gender differences, in rat. An improved rat model would facilitate *in vivo* investigations of the potential significance of antipsychotic-induced lipogenesis in myelination.

Regarding the translational aspects of our work, we plan to search for biological markers associated with the use antipsychotic drugs and metabolic adverse effects, in patient materials including several hundred patients who will be thoroughly examined clinically and biochemically. In one study, patients will be offered follow-up appointments for at least a year after starting randomized treatment with either olanzapine, amisulpride, or aripiprazole. We will have the opportunity to examine DNA and RNA profiles from these patients and, among other outcomes, investigate alterations in RNA expression during treatment initiation. Searching for alterations in the transcripts described in this thesis, as well as new candidates for further research, will be highly exciting. Correlating gene expression with clinical parameters may

enable the identification of biomarkers for clinical response, or for increased risk of metabolic adverse effects.

11. References

1. McNally K. Eugene Bleuler's Four As. *History of Psychology* 2009;12:43-59.
2. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*(4th ed., text revision). Washington, DC: American Psychiatric Association 2000.
3. World Health Organization. *International Statistical Classification of Diseases and Related Health Problems*, 10th revision, Geneva. 1992;2.
4. Jablensky A. Epidemiology of schizophrenia: the global burden of disease and disability. *Eur Arch Psychiatry Clin Neurosci* 2000;250:274-85.
5. Jablensky A, Sartorius N, Ernberg G, et al. Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization ten-country study. *Psychol Med Monogr Suppl* 1992;20:1-97.
6. McGrath JJ. Myths and plain truths about schizophrenia epidemiology--the NAPE lecture 2004. *Acta Psychiatr Scand* 2005;111:4-11.
7. McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. *Epidemiol Rev* 2008;30:67-76.
8. Messias EL, Chen CY, Eaton WW. Epidemiology of schizophrenia: review of findings and myths. *Psychiatr Clin North Am* 2007;30:323-38.
9. Perala J, Suvisaari J, Saarni SI, et al. Lifetime prevalence of psychotic and bipolar I disorders in a general population. *Arch Gen Psychiatry* 2007;64:19-28.
10. McGrath J, Saha S, Welham J, El Saadi O, MacCauley C, Chant D. A systematic review of the incidence of schizophrenia: the distribution of rates and the influence of sex, urbanicity, migrant status and methodology. *BMC Med* 2004;2:13.
11. Aleman A, Kahn RS, Selten JP. Sex differences in the risk of schizophrenia: evidence from meta-analysis. *Arch Gen Psychiatry* 2003;60:565-71.
12. Knapp M, Mangalore R, Simon J. The global costs of schizophrenia. *Schizophr Bull* 2004;30:279-93.
13. World Health Organization. *The Global Burden of Disease 2004 Update*. 2004.
14. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 2003;60:1187-92.
15. Allen NC, Bagade S, McQueen MB, et al. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat Genet* 2008;40:827-34.
16. Lang UE, Puls I, Muller DJ, Strutz-Seebohm N, Gallinat J. Molecular mechanisms of schizophrenia. *Cell Physiol Biochem* 2007;20:687-702.
17. Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry* 1991;148:1474-86.
18. Davis KL, Stewart DG, Friedman JI, et al. White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Arch Gen Psychiatry* 2003;60:443-56.

19. Hof PR, Haroutunian V, Copland C, Davis KL, Buxbaum JD. Molecular and cellular evidence for an oligodendrocyte abnormality in schizophrenia. *Neurochem Res* 2002;27:1193-200.
20. Miller RH. Regulation of oligodendrocyte development in the vertebrate CNS. *Prog Neurobiol* 2002;67:451-67.
21. Sigmundsson T, Suckling J, Maier M, et al. Structural abnormalities in frontal, temporal, and limbic regions and interconnecting white matter tracts in schizophrenic patients with prominent negative symptoms. *Am J Psychiatry* 2001;158:234-43.
22. Flynn SW, Lang DJ, Mackay AL, et al. Abnormalities of myelination in schizophrenia detected in vivo with MRI, and post-mortem with analysis of oligodendrocyte proteins. *Mol Psychiatry* 2003;8:811-20.
23. Lerner BH. Last-ditch medical therapy - revisiting lobotomy. *N Engl J Med* 2005;353:119-21.
24. Lopez-Munoz F, Ucha-Udabe R, Alamo C. The history of barbiturates a century after their clinical introduction. *Neuropsychiatr Dis Treat* 2005;1:329-43.
25. Cancro R. The introduction of neuroleptics: a psychiatric revolution. *Psychiatr Serv* 2000;51:333-5.
26. Curzon G. How reserpine and chlorpromazine act: the impact of key discoveries on the history of psychopharmacology. *Trends Pharmacol Sci* 1990;11:61-3.
27. Bennett MR. *History of the Synapse*. Harwood Academic Publishers, Amsterdam 2001.
28. Quetsch RM, Achor RW, Litin EM, Faucett RL. Depressive reactions in hypertensive patients; a comparison of those treated with Rauwolfia and those receiving no specific antihypertensive treatment. *Circulation* 1959;19:366-75.
29. Lopez-Munoz F, Alamo C. The consolidation of neuroleptic therapy: Janssen, the discovery of haloperidol and its introduction into clinical practice. *Brain Res Bull* 2009;79:130-41.
30. Anden NE, Butcher SG, Corrodi H, Fuxe K, Ungerstedt U. Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. *Eur J Pharmacol* 1970;11:303-14.
31. Roth BL, Sheffler DJ, Kroeze WK. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev Drug Discov* 2004;3:353-9.
32. Stahl SM. Describing an atypical antipsychotic: receptor binding and its role in pathophysiology. *Primary Care Companion J Clin Psychiatry* 2003;5:9-13.
33. Marder SR, Van Putten T. *Antipsychotic medications*. The American Psychiatric Press Textbook of Psychopharmacology American Psychiatric Press, Inc 1995.
34. Schatzberg A, Nemeroff C. *The American Psychiatric Press Textbook of Psychopharmacology*. American Psychiatric Press, Inc 1995.
35. Sethi S. *Textbook of Psychiatry*: Elsevier India; 2008.
36. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT₂ and D₂ receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am J Psychiatry* 1999;156:286-93.

37. Dayalu P, Chou KL. Antipsychotic-induced extrapyramidal symptoms and their management. *Expert Opin Pharmacother* 2008;9:1451-62.
38. Sneader W. *Drug Discovery: a history*. Wiley 2005.
39. Crilly J. The history of clozapine and its emergence in the US market: a review and analysis. *Hist Psychiatry* 2007;18:39-60.
40. Schotte A, Janssen PF, Gommeren W, et al. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacology (Berl)* 1996;124:57-73.
41. Idanpaan-Heikkila J, Alhava E, Olkinuora M, Palva IP. Agranulocytosis during treatment with chlozapine. *Eur J Clin Pharmacol* 1977;11:193-8.
42. Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch Gen Psychiatry* 1988;45:789-96.
43. Mullen J, Jibson MD, Sweitzer D. A comparison of the relative safety, efficacy, and tolerability of quetiapine and risperidone in outpatients with schizophrenia and other psychotic disorders: the quetiapine experience with safety and tolerability (QUEST) study. *Clin Ther* 2001;23:1839-54.
44. Ruhe HG, Becker HE, Jessurun P, Marees CH, Heeringa M, Vermeulen HD. Agranulocytosis and granulocytopenia associated with quetiapine. *Acta Psychiatr Scand* 2001;104:311-3; discussion 3-4.
45. Wirshing DA, Spellberg BJ, Erhart SM, Marder SR, Wirshing WC. Novel antipsychotics and new onset diabetes. *Biol Psychiatry* 1998;44:778-83.
46. Goodnick PJ. Ziprasidone: profile on safety. *Expert Opin Pharmacother* 2001;2:1655-62.
47. Mailman RB, Murthy V. Third generation antipsychotic drugs: partial agonism or receptor functional selectivity? *Curr Pharm Des* 2010;16:488-501.
48. Baptista T, Kin NM, Beaulieu S, de Baptista EA. Obesity and related metabolic abnormalities during antipsychotic drug administration: mechanisms, management and research perspectives. *Pharmacopsychiatry* 2002;35:205-19.
49. Allison DB, Mentore JL, Heo M, et al. Antipsychotic-induced weight gain: a comprehensive research synthesis. *Am J Psychiatry* 1999;156:1686-96.
50. Korenyi C, Lowenstein B. Chlorpromazine induced diabetes. *Dis Nerv Syst* 1968;29:827-8.
51. Haupt DW, Newcomer JW. Hyperglycemia and antipsychotic medications. *J Clin Psychiatry* 2001;62 Suppl 27:15-26; discussion 40-1.
52. Clark ML, Ray TS, Paredes A, et al. Chlorpromazine in women with chronic schizophrenia: the effect on cholesterol levels and cholesterol-behavior relationships. *Psychosom Med* 1967;29:634-42.
53. Lindstrom LH. The effect of long-term treatment with clozapine in schizophrenia: a retrospective study in 96 patients treated with clozapine for up to 13 years. *Acta Psychiatr Scand* 1988;77:524-9.
54. Lieberman JA, Safferman AZ. Clinical profile of clozapine: adverse reactions and agranulocytosis. *Psychiatr Q* 1992;63:51-70.
55. Ghaeli P, Dufresne RL. Serum triglyceride levels in patients treated with clozapine. *Am J Health Syst Pharm* 1996;53:2079-81.

-
56. Spivak B, Roitman S, Vered Y, et al. Diminished suicidal and aggressive behavior, high plasma norepinephrine levels, and serum triglyceride levels in chronic neuroleptic-resistant schizophrenic patients maintained on clozapine. *Clin Neuropharmacol* 1998;21:245-50.
 57. Gaulin BD, Markowitz JS, Caley CF, Nesbitt LA, Dufresne RL. Clozapine-associated elevation in serum triglycerides. *Am J Psychiatry* 1999;156:1270-2.
 58. Beasley CM, Jr., Tollefson GD, Tran PV. Safety of olanzapine. *J Clin Psychiatry* 1997;58 Suppl 10:13-7.
 59. Tran PV, Hamilton SH, Kuntz AJ, et al. Double-blind comparison of olanzapine versus risperidone in the treatment of schizophrenia and other psychotic disorders. *J Clin Psychopharmacol* 1997;17:407-18.
 60. Eder U, Mangweth B, Ebenbichler C, et al. Association of olanzapine-induced weight gain with an increase in body fat. *Am J Psychiatry* 2001;158:1719-22.
 61. Graham KA, Perkins DO, Edwards LJ, Barrier RC, Jr., Lieberman JA, Harp JB. Effect of olanzapine on body composition and energy expenditure in adults with first-episode psychosis. *Am J Psychiatry* 2005;162:118-23.
 62. McEvoy JP, Lieberman JA, Perkins DO, et al. Efficacy and tolerability of olanzapine, quetiapine, and risperidone in the treatment of early psychosis: a randomized, double-blind 52-week comparison. *Am J Psychiatry* 2007;164:1050-60.
 63. Sheitman BB, Bird PM, Binz W, Akinli L, Sanchez C. Olanzapine-induced elevation of plasma triglyceride levels. *Am J Psychiatry* 1999;156:1471-2.
 64. Osser DN, Najarian DM, Dufresne RL. Olanzapine increases weight and serum triglyceride levels. *J Clin Psychiatry* 1999;60:767-70.
 65. Meyer JM. A retrospective comparison of weight, lipid, and glucose changes between risperidone- and olanzapine-treated inpatients: metabolic outcomes after 1 year. *J Clin Psychiatry* 2002;63:425-33.
 66. Lindenmayer JP, Czobor P, Volavka J, et al. Changes in glucose and cholesterol levels in patients with schizophrenia treated with typical or atypical antipsychotics. *Am J Psychiatry* 2003;160:290-6.
 67. Lindenmayer JP, Patel R. Olanzapine-induced ketoacidosis with diabetes mellitus. *Am J Psychiatry* 1999;156:1471.
 68. Henderson DC, Cagliero E, Gray C, et al. Clozapine, diabetes mellitus, weight gain, and lipid abnormalities: A five-year naturalistic study. *Am J Psychiatry* 2000;157:975-81.
 69. American Diabetes Association APA, American Association of Clinical Endocrinologists, North American Association for the Study of Obesity. Consensus development conference on antipsychotic drugs and obesity and diabetes. *J Clin Psychiatry* 2004;65:267-72.
 70. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006;23:469-80.
 71. Newcomer JW, Meyer JM, Baker RA, et al. Changes in non-high-density lipoprotein cholesterol levels and triglyceride/high-density lipoprotein cholesterol ratios among patients randomized to aripiprazole versus olanzapine. *Schizophr Res* 2008;106:300-7.

-
72. Lieberman JA, Stroup TS, McEvoy JP, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N Engl J Med* 2005;353:1209-23.
 73. Greenberg WM, Citrome L. Ziprasidone for schizophrenia and bipolar disorder: a review of the clinical trials. *CNS Drug Rev* 2007;13:137-77.
 74. Newcomer JW, Campos JA, Marcus RN, et al. A multicenter, randomized, double-blind study of the effects of aripiprazole in overweight subjects with schizophrenia or schizoaffective disorder switched from olanzapine. *J Clin Psychiatry* 2008;69:1046-56.
 75. Colton CW, Manderscheid RW. Congruencies in increased mortality rates, years of potential life lost, and causes of death among public mental health clients in eight states. *Prev Chronic Dis* 2006;3:A42.
 76. Newcomer JW, Hennekens CH. Severe mental illness and risk of cardiovascular disease. *JAMA* 2007;298:1794-6.
 77. Allison DB, Fontaine KR, Heo M, et al. The distribution of body mass index among individuals with and without schizophrenia. *J Clin Psychiatry* 1999;60:215-20.
 78. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998;97:1837-47.
 79. Nasrallah HA, Meyer JM, Goff DC, et al. Low rates of treatment for hypertension, dyslipidemia and diabetes in schizophrenia: data from the CATIE schizophrenia trial sample at baseline. *Schizophr Res* 2006;86:15-22.
 80. McEvoy JP, Meyer JM, Goff DC, et al. Prevalence of the metabolic syndrome in patients with schizophrenia: baseline results from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) schizophrenia trial and comparison with national estimates from NHANES III. *Schizophr Res* 2005;80:19-32.
 81. Fontaine KR, Heo M, Harrigan EP, et al. Estimating the consequences of antipsychotic induced weight gain on health and mortality rate. *Psychiatry Res* 2001;101:277-88.
 82. Daumit GL, Goff DC, Meyer JM, et al. Antipsychotic effects on estimated 10-year coronary heart disease risk in the CATIE schizophrenia study. *Schizophr Res* 2008;105:175-87.
 83. Perkins DO. Predictors of noncompliance in patients with schizophrenia. *J Clin Psychiatry* 2002;63:1121-8.
 84. Derry S, Moore RA. Atypical antipsychotics in bipolar disorder: systematic review of randomised trials. *BMC Psychiatry* 2007;7:40.
 85. Eli Lilly and Company. 2008 Annual Report. <http://fileshareholdercom/downloads/LLY/1333246824x0x296463/611E167A-61C9-4C03-8866-ACF5FA7C8953/EnglishPDF> 2008.
 86. Reseptregisteret Nf. Accessed August 14, 2009. <http://www.reseptregisteretno/Prevalensaspx>.
 87. Kluge M, Schuld A, Himmerich H, et al. Clozapine and olanzapine are associated with food craving and binge eating: results from a randomized double-blind study. *J Clin Psychopharmacol* 2007;27:662-6.

-
88. Hartfield AW, Moore NA, Clifton PG. Effects of clozapine, olanzapine and haloperidol on the microstructure of ingestive behaviour in the rat. *Psychopharmacology (Berl)* 2003;167:115-22.
 89. Pouzet B, Mow T, Kreilgaard M, Velschow S. Chronic treatment with antipsychotics in rats as a model for antipsychotic-induced weight gain in human. *Pharmacol Biochem Behav* 2003;75:133-40.
 90. Coccorello R, D'Amato FR, Moles A. Chronic administration of olanzapine affects Behavioral Satiety Sequence and feeding behavior in female mice. *Eat Weight Disord* 2008;13:e55-60.
 91. Kroeze WK, Hufeisen SJ, Popadak BA, et al. H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology* 2003;28:519-26.
 92. Wirshing DA, Wirshing WC, Kysar L, et al. Novel antipsychotics: comparison of weight gain liabilities. *J Clin Psychiatry* 1999;60:358-63.
 93. Kim SF, Huang AS, Snowman AM, Teuscher C, Snyder SH. From the Cover: Antipsychotic drug-induced weight gain mediated by histamine H1 receptor-linked activation of hypothalamic AMP-kinase. *Proc Natl Acad Sci U S A* 2007;104:3456-9.
 94. Casey DE, Zorn SH. The pharmacology of weight gain with antipsychotics. *J Clin Psychiatry* 2001;62 Suppl 7:4-10.
 95. Daniel DG, Copeland LF. Ziprasidone: comprehensive overview and clinical use of a novel antipsychotic. *Expert Opin Investig Drugs* 2000;9:819-28.
 96. Silvestre JS, Prous J. Research on adverse drug events. I. Muscarinic M3 receptor binding affinity could predict the risk of antipsychotics to induce type 2 diabetes. *Methods Find Exp Clin Pharmacol* 2005;27:289-304.
 97. Nasrallah HA. Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. *Mol Psychiatry* 2008;13:27-35.
 98. Ascher-Svanum H, Stensland M, Zhao Z, Kinon BJ. Acute weight gain, gender, and therapeutic response to antipsychotics in the treatment of patients with schizophrenia. *BMC Psychiatry* 2005;5:3.
 99. Zipursky RB, Gu H, Green AI, et al. Course and predictors of weight gain in people with first-episode psychosis treated with olanzapine or haloperidol. *Br J Psychiatry* 2005;187:537-43.
 100. Gentile S. Long-term treatment with atypical antipsychotics and the risk of weight gain : a literature analysis. *Drug Saf* 2006;29:303-19.
 101. Koro CE, Meyer JM. Atypical antipsychotic therapy and hyperlipidemia: a review. *Essent Psychopharmacol* 2005;6:148-57.
 102. Arjona AA, Zhang SX, Adamson B, Wurtman RJ. An animal model of antipsychotic-induced weight gain. *Behav Brain Res* 2004;152:121-7.
 103. Kalinichev M, Rourke C, Daniels AJ, et al. Characterisation of olanzapine-induced weight gain and effect of aripiprazole vs olanzapine on body weight and prolactin secretion in female rats. *Psychopharmacology (Berlin)* 2005;182:220-31.
 104. Davoodi N, Kalinichev M, Clifton PG. Comparative effects of olanzapine and ziprasidone on hypophagia induced by enhanced histamine neurotransmission in the rat. *Behav Pharmacol* 2008;19:121-8.

-
105. Cooper GD, Pickavance LC, Wilding JP, Harrold JA, Halford JC, Goudie AJ. Effects of olanzapine in male rats: enhanced adiposity in the absence of hyperphagia, weight gain or metabolic abnormalities. *J Psychopharmacol* 2007;21:405-13.
 106. Minet-Ringuet J, Even PC, Goubern M, Tome D, de Beaurepaire R. Long term treatment with olanzapine mixed with the food in male rats induces body fat deposition with no increase in body weight and no thermogenic alteration. *Appetite* 2006;46:254-62.
 107. Minet-Ringuet J, Even PC, Valet P, et al. Alterations of lipid metabolism and gene expression in rat adipocytes during chronic olanzapine treatment. *Mol Psychiatry* 2007;12:562-71.
 108. Albaugh VL, Henry CR, Bello NT, et al. Hormonal and metabolic effects of olanzapine and clozapine related to body weight in rodents. *Obesity (Silver Spring)* 2006;14:36-51.
 109. Baptista T, Mata A, Teneud L, de Quijada M, Han HW, Hernandez L. Effects of long-term administration of clozapine on body weight and food intake in rats. *Pharmacol Biochem Behav* 1993;45:51-4.
 110. Sondhi S, Castellano JM, Chong VZ, et al. cDNA array reveals increased expression of glucose-dependent insulinotropic polypeptide following chronic clozapine treatment: role in atypical antipsychotic drug-induced adverse metabolic effects. *Pharmacogenomics J* 2006;6:131-40.
 111. Cooper GD, Harrold JA, Halford JC, Goudie AJ. Chronic clozapine treatment in female rats does not induce weight gain or metabolic abnormalities but enhances adiposity: implications for animal models of antipsychotic-induced weight gain. *Prog Neuropsychopharmacol Biol Psychiatry* 2008;32:428-36.
 112. Schleimer SB, Johnston GA, Henderson JM. Novel oral drug administration in an animal model of neuroleptic therapy. *J Neurosci Methods* 2005;146:159-64.
 113. Han M, Deng C, Burne TH, Newell KA, Huang XF. Short- and long-term effects of antipsychotic drug treatment on weight gain and H1 receptor expression. *Psychoneuroendocrinology* 2008;33:569-80.
 114. Snigdha S, Thumbi C, Reynolds GP, Neill JC. Ziprasidone and aripiprazole attenuate olanzapine-induced hyperphagia in rats. *J Psychopharmacol* 2008;22:567-71.
 115. Fell MJ, Anjum N, Dickinson K, et al. The distinct effects of subchronic antipsychotic drug treatment on macronutrient selection, body weight, adiposity, and metabolism in female rats. *Psychopharmacology (Berlin)* 2007;194:221-31.
 116. Fell MJ, Gibson R, McDermott E, Sisodia G, Marshall KM, Neill JC. Investigation into the effects of the novel antipsychotic ziprasidone on weight gain and reproductive function in female rats. *Behav Brain Res* 2005;160:338-43.
 117. Kalinichev M, Rourke C, Jones DN. Body weights and plasma prolactin levels in female rats treated subchronically with ziprasidone versus olanzapine. *Behav Pharmacol* 2006;17:289-92.
 118. Choi S, DiSilvio B, Unangst J, Fernstrom JD. Effect of chronic infusion of olanzapine and clozapine on food intake and body weight gain in male and female rats. *Life Sci* 2007;81:1024-30.

-
119. Minet-Ringuet J, Even PC, Lacroix M, Tome D, de Beaurepaire R. A model for antipsychotic-induced obesity in the male rat. *Psychopharmacology (Berl)* 2006;187:447-54.
 120. Hartfield AW, Moore NA, Clifton PG. Effects of atypical antipsychotic drugs on intralipid intake and cocaine-induced hyperactivity in rats. *Neuropsychopharmacology* 2006;31:1938-45.
 121. Davoodi N, Kalinichev M, Korneev SA, Clifton PG. Hyperphagia and increased meal size are responsible for weight gain in rats treated sub-chronically with olanzapine. *Psychopharmacology (Berl)* 2009;203:693-702.
 122. Chintoh AF, Mann SW, Lam L, et al. Insulin resistance and secretion in vivo: effects of different antipsychotics in an animal model. *Schizophrenia Research* 2009;108:127-33.
 123. Houseknecht KL, Robertson AS, Zavadoski W, Gibbs EM, Johnson DE, Rollema H. Acute effects of atypical antipsychotics on whole-body insulin resistance in rats: implications for adverse metabolic effects. *Neuropsychopharmacology* 2007;32:289-97.
 124. Cooper GD, Pickavance LC, Wilding JP, Halford JC, Goudie AJ. A parametric analysis of olanzapine-induced weight gain in female rats. *Psychopharmacology (Berl)* 2005;181:80-9.
 125. Coccorello R, Caprioli A, Ghirardi O, et al. Chronic administration of olanzapine induces metabolic and food intake alterations: a mouse model of the atypical antipsychotic-associated adverse effects. *Psychopharmacology (Berl)* 2006;186:561-71.
 126. Coccorello R, Caprioli A, Conti R, et al. Olanzapine (LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5] benzodiazepine), but not the novel atypical antipsychotic ST2472 (9-piperazin-1-ylpyrrolo[2,1-b][1,3]benzothiazepine), chronic administration induces weight gain, hyperphagia, and metabolic dysregulation in mice. *Journal of Pharmacology and Experimental Therapeutics* 2008;326:905-11.
 127. Coccorello R, Caprioli A, Ghirardi O, et al. Chronic administration of olanzapine induces metabolic and food intake alterations: a mouse model of the atypical antipsychotic-associated adverse effects. *Psychopharmacology (Berlin)* 2006;186:561-71.
 128. Nelson D, Cox M. *Lehninger Principles of Biochemistry*. Worth Publishers 2000.
 129. Shi Y, Burn P. Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. *Nat Rev Drug Discov* 2004;3:695-710.
 130. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 2002;23:201-29.
 131. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest* 2008;118:829-38.
 132. Mouritsen OG, Zuckermann MJ. What's so special about cholesterol? *Lipids* 2004;39:1101-13.

-
133. Korade Z, Kenworthy AK. Lipid rafts, cholesterol, and the brain. *Neuropharmacology* 2008;55:1265-73.
 134. Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. *Endocr Rev* 2004;25:947-70.
 135. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci* 2005;6:626-40.
 136. Turley SD, Burns DK, Rosenfeld CR, Dietschy JM. Brain does not utilize low density lipoprotein-cholesterol during fetal and neonatal development in the sheep. *J Lipid Res* 1996;37:1953-61.
 137. Dietschy JM, Turley SD. Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J Lipid Res* 2004;45:1375-97.
 138. Mauch DH, Nagler K, Schumacher S, et al. CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 2001;294:1354-7.
 139. Goritz C, Thiebaut R, Tessier LH, et al. Glia-induced neuronal differentiation by transcriptional regulation. *Glia* 2007;55:1108-22.
 140. Stevens B. Neuron-astrocyte signaling in the development and plasticity of neural circuits. *Neurosignals* 2008;16:278-88.
 141. Pfrieger FW. Cholesterol homeostasis and function in neurons of the central nervous system. *Cell Mol Life Sci* 2003;60:1158-71.
 142. Ullian EM, Sapperstein SK, Christopherson KS, Barres BA. Control of synapse number by glia. *Science* 2001;291:657-61.
 143. Guillou H, Martin PG, Pineau T. Transcriptional regulation of hepatic fatty acid metabolism. *Subcell Biochem* 2008;49:3-47.
 144. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J Clin Invest* 1997;99:838-45.
 145. Edwards PA, Tabor D, Kast HR, Venkateswaran A. Regulation of gene expression by SREBP and SCAP. *Biochim Biophys Acta* 2000;1529:103-13.
 146. Guha P, Aneja KK, Shilpi RY, Haldar D. Transcriptional regulation of mitochondrial glycerophosphate acyltransferase is mediated by distal promoter via ChREBP and SREBP-1. *Arch Biochem Biophys* 2009;490:85-95.
 147. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002;109:1125-31.
 148. Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. *Prog Lipid Res* 2001;40:439-52.
 149. Kim JB, Sarraf P, Wright M, et al. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J Clin Invest* 1998;101:1-9.
 150. Jump DB, Botolin D, Wang Y, Xu J, Christian B, Demeure O. Fatty acid regulation of hepatic gene transcription. *J Nutr* 2005;135:2503-6.
 151. Le Lay S, Lefrere I, Trautwein C, Dugail I, Krief S. Insulin and sterol-regulatory element-binding protein-1c (SREBP-1C) regulation of gene expression in

-
- 3T3-L1 adipocytes. Identification of CCAAT/enhancer-binding protein beta as an SREBP-1C target. *J Biol Chem* 2002;277:35625-34.
152. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. *Nat Med* 2004;10:355-61.
153. Madsen L, Guerre-Millo M, Flindt EN, et al. Tetradecylthioacetic acid prevents high fat diet induced adiposity and insulin resistance. *J Lipid Res* 2002;43:742-50.
154. Wensaas AJ, Rustan AC, Rokling-Andersen MH, et al. Dietary supplementation of tetradecylthioacetic acid increases feed intake but reduces body weight gain and adipose depot sizes in rats fed on high-fat diets. *Diabetes Obes Metab* 2009;11:1034-49.
155. Chou CJ, Haluzik M, Gregory C, et al. WY14,643, a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipoatrophic A-ZIP/F-1 mice. *J Biol Chem* 2002;277:24484-9.
156. Guerre-Millo M, Gervois P, Raspe E, et al. Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J Biol Chem* 2000;275:16638-42.
157. Kim H, Haluzik M, Asghar Z, et al. Peroxisome proliferator-activated receptor-alpha agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. *Diabetes* 2003;52:1770-8.
158. Lovas K, Rost TH, Skorve J, et al. Tetradecylthioacetic acid attenuates dyslipidaemia in male patients with type 2 diabetes mellitus, possibly by dual PPAR-alpha/delta activation and increased mitochondrial fatty acid oxidation. *Diabetes Obes Metab* 2009;11:304-14.
159. Ferno J, Raeder MB, Vik-Mo AO, et al. Antipsychotic drugs activate SREBP-regulated expression of lipid biosynthetic genes in cultured human glioma cells: a novel mechanism of action? *Pharmacogenomics J* 2005;5:298-304.
160. Raeder MB, Ferno J, Vik-Mo AO, Steen VM. SREBP activation by antipsychotic- and antidepressant-drugs in cultured human liver cells: relevance for metabolic side-effects? *Mol Cell Biochem* 2006;289:167-73.
161. Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. *Biotechniques* 2005;39:75-85.
162. Schmittgen TD, Zakrajsek BA. Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. *J Biochem Biophys Methods* 2000;46:69-81.
163. Nixon JP, Zhang M, Wang C, et al. Evaluation of a quantitative magnetic resonance imaging system for whole body composition analysis in rodents. *Obesity (Silver Spring)* 2010;18:1652-9.
164. Citrome L, Stauffer VL, Chen L, et al. Olanzapine plasma concentrations after treatment with 10, 20, and 40 mg/d in patients with schizophrenia: an analysis of correlations with efficacy, weight gain, and prolactin concentration. *J Clin Psychopharmacol* 2009;29:278-83.
165. Montejo AL. Prolactin awareness: an essential consideration for physical health in schizophrenia. *Eur Neuropsychopharmacol* 2008;18 Suppl 2:S108-14.

-
166. Rourke C, Starr KR, Reavill C, Fenwick S, Deadman K, Jones DN. Effects of the atypical antipsychotics olanzapine and risperidone on plasma prolactin levels in male rats: a comparison with clinical data. *Psychopharmacology (Berl)* 2006;184:107-14.
167. Shi H, Seeley RJ, Clegg DJ. Sexual differences in the control of energy homeostasis. *Front Neuroendocrinol* 2009;30:396-404.
168. Park S, Hong SM, Ahn IL, Kim da S, Kim SH. Estrogen replacement reverses olanzapine-induced weight gain and hepatic insulin resistance in ovariectomized diabetic rats. *Neuropsychobiology* 2010;61:148-61.
169. Konarzewska B, Wolczynski S, Szulc A, Galinska B, Poplawska R, Waszkiewicz N. Effect of risperidone and olanzapine on reproductive hormones, psychopathology and sexual functioning in male patients with schizophrenia. *Psychoneuroendocrinology* 2009;34:129-39.
170. Canuso CM, Goldstein JM, Wojcik J, et al. Antipsychotic medication, prolactin elevation, and ovarian function in women with schizophrenia and schizoaffective disorder. *Psychiatry Res* 2002;111:11-20.
171. Knegtering H, van der Moolen AE, Castelein S, Kluiters H, van den Bosch RJ. What are the effects of antipsychotics on sexual dysfunctions and endocrine functioning? *Psychoneuroendocrinology* 2003;28 Suppl 2:109-23.
172. Baldessarini RJ, Centorrino F, Flood JG, Volpicelli SA, Huston-Lyons D, Cohen BM. Tissue concentrations of clozapine and its metabolites in the rat. *Neuropsychopharmacology* 1993;9:117-24.
173. Cheng YF, Lundberg T, Bondesson U, Lindstrom L, Gabrielsson J. Clinical pharmacokinetics of clozapine in chronic schizophrenic patients. *Eur J Clin Pharmacol* 1988;34:445-9.
174. Mattiuz E, Franklin R, Gillespie T, et al. Disposition and metabolism of olanzapine in mice, dogs, and rhesus monkeys. *Drug Metab Dispos* 1997;25:573-83.
175. Aravagiri M, Teper Y, Marder SR. Pharmacokinetics and tissue distribution of olanzapine in rats. *Biopharm Drug Dispos* 1999;20:369-77.
176. Callaghan JT, Bergstrom RF, Ptak LR, Beasley CM. Olanzapine. Pharmacokinetic and pharmacodynamic profile. *Clin Pharmacokinet* 1999;37:177-93.
177. Buchanan RW, Kreyenbuhl J, Kelly DL, et al. The 2009 schizophrenia PORT psychopharmacological treatment recommendations and summary statements. *Schizophr Bull* 2010;36:71-93.
178. Weston-Green K, Huang XF, Deng C. Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats. *Behav Brain Res* 2011;217:337-46.
179. Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN. Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. *J Pharmacol Exp Ther* 2003;305:625-31.
180. von Wilmsdorff M, Bouvier ML, Henning U, Schmitt A, Gaebel W. The impact of antipsychotic drugs on food intake and body weight and on leptin levels in blood and hypothalamic ob-r leptin receptor expression in wistar rats. *Clinics (Sao Paulo)* 2010;65:885-94.

181. Perez-Costas E, Guidetti P, Melendez-Ferro M, Kelley JJ, Roberts RC. Neuroleptics and animal models: feasibility of oral treatment monitored by plasma levels and receptor occupancy assays. *J Neural Transm* 2008;115:745-53.
182. Zhao C, Li M. Sedation and disruption of maternal motivation underlie the disruptive effects of antipsychotic treatment on rat maternal behavior. *Pharmacol Biochem Behav* 2009;92:147-56.
183. Kinon BJ, Kaiser CJ, Ahmed S, Rotelli MD, Kollack-Walker S. Association between early and rapid weight gain and change in weight over one year of olanzapine therapy in patients with schizophrenia and related disorders. *J Clin Psychopharmacol* 2005;25:255-8.
184. Bai YM, Chen JY, Chen TT, et al. Weight gain with clozapine: 8-year cohort naturalistic study among hospitalized Chinese schizophrenia patients. *Schizophr Res* 2009;108:122-6.
185. Haddad P. Weight change with atypical antipsychotics in the treatment of schizophrenia. *J Psychopharmacol* 2005;19:16-27.
186. Fell MJ, Neill JC, Rao C, Marshall KM. Effects of sub-chronic antipsychotic drug treatment on body weight and reproductive function in juvenile female rats. *Psychopharmacology (Berl)* 2005;182:499-507.
187. van der Zwaal EM, Luijendijk MC, Adan RA, la Fleur SE. Olanzapine-induced weight gain: chronic infusion using osmotic minipumps does not result in stable plasma levels due to degradation of olanzapine in solution. *Eur J Pharmacol* 2008;585:130-6.
188. Shobo M, Yamada H, Koakutsu A, et al. Chronic treatment with olanzapine via a novel infusion pump induces adiposity in male rats. *Life Sci* 2011;88:761-5.
189. de Leeuw van Weenen JE, Parlevliet ET, Schroder-van der Elst JP, et al. Pharmacological modulation of dopamine receptor D2-mediated transmission alters the metabolic phenotype of diet induced obese and diet resistant C57Bl6 mice. *Exp Diabetes Res* 2011;2011:928523.
190. Smith GC, Vickers MH, Shepherd PR. Olanzapine effects on body composition, food preference, glucose metabolism and insulin sensitivity in the rat. *Arch Physiol Biochem* 2011.
191. Guesdon B, Denis RG, Richard D. Additive effects of olanzapine and melanin-concentrating hormone agonism on energy balance. *Behav Brain Res* 2010;207:14-20.
192. Shobo M, Yamada H, Mihara T, et al. Two models for weight gain and hyperphagia as side effects of atypical antipsychotics in male rats: validation with olanzapine and ziprasidone. *Behav Brain Res* 2011;216:561-8.
193. Blouin M, Tremblay A, Jalbert ME, et al. Adiposity and eating behaviors in patients under second generation antipsychotics. *Obesity (Silver Spring)* 2008;16:1780-7.
194. Theisen FM, Linden A, Konig IR, Martin M, Remschmidt H, Hebebrand J. Spectrum of binge eating symptomatology in patients treated with clozapine and olanzapine. *J Neural Transm* 2003;110:111-21.
195. Ferno J, Varela L, Skrede S, et al. Olanzapine-induced hyperphagia and weight gain associate with orexigenic hypothalamic neuropeptide signaling without concomitant AMPK phosphorylation. *PLoS One* 2011;6:e20571.

-
196. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;84:277-359.
 197. Stefanidis A, Verty AN, Allen AM, Owens NC, Cowley MA, Oldfield BJ. The role of thermogenesis in antipsychotic drug-induced weight gain. *Obesity (Silver Spring)* 2009;17:16-24.
 198. Richard D, Picard F. Brown fat biology and thermogenesis. *Front Biosci* 2011;16:1233-60.
 199. Cypess AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009;360:1509-17.
 200. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab* 2007;293:E444-52.
 201. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009;360:1500-8.
 202. Karelis AD, St-Pierre DH, Conus F, Rabasa-Lhoret R, Poehlman ET. Metabolic and body composition factors in subgroups of obesity: what do we know? *J Clin Endocrinol Metab* 2004;89:2569-75.
 203. Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S. The metabolically obese, normal-weight individual revisited. *Diabetes* 1998;47:699-713.
 204. Birkenaes AB, Birkeland KI, Engh JA, et al. Dyslipidemia independent of body mass in antipsychotic-treated patients under real-life conditions. *J Clin Psychopharmacol* 2008;28:132-7.
 205. Procyshyn RM, Wasan KM, Thornton AE, et al. Changes in serum lipids, independent of weight, are associated with changes in symptoms during long-term clozapine treatment. *J Psychiatry Neurosci* 2007;32:331-8.
 206. Verma SK, Subramaniam M, Liew A, Poon LY. Metabolic risk factors in drug-naive patients with first-episode psychosis. *J Clin Psychiatry* 2009;70:997-1000.
 207. Patel JK, Buckley PF, Woolson S, et al. Metabolic profiles of second-generation antipsychotics in early psychosis: findings from the CAFE study. *Schizophr Res* 2009;111:9-16.
 208. Stroup TS, McEvoy JP, Ring KD, et al. A Randomized Trial Examining the Effectiveness of Switching From Olanzapine, Quetiapine, or Risperidone to Aripiprazole to Reduce Metabolic Risk: Comparison of Antipsychotics for Metabolic Problems (CAMP). *Am J Psychiatry* 2011.
 209. Polymeropoulos MH, Licamele L, Volpi S, et al. Common effect of antipsychotics on the biosynthesis and regulation of fatty acids and cholesterol supports a key role of lipid homeostasis in schizophrenia. *Schizophr Res* 2009;108:134-42.
 210. Yang LH, Chen TM, Yu ST, Chen YH. Olanzapine induces SREBP-1-related adipogenesis in 3T3-L1 cells. *Pharmacol Res* 2007;56:202-8.
 211. Laouressgues E, Staels B, Valeille K, et al. Antipsychotic drug action on SREBPs-related lipogenesis and cholesterologenesis in primary rat hepatocytes. *Naunyn Schmiedebergs Arch Pharmacol* 2010.
 212. Wiklund ED, Catts VS, Catts SV, et al. Cytotoxic effects of antipsychotic drugs implicate cholesterol homeostasis as a novel chemotherapeutic target. *Int J Cancer* 2010;126:28-40.

-
213. De Filippi L, Fournier M, Cameroni E, et al. Membrane stress is coupled to a rapid translational control of gene expression in chlorpromazine-treated cells. *Curr Genet* 2007;52:171-85.
 214. Kristiana I, Sharpe LJ, Catts VS, Lutze-Mann LH, Brown AJ. Antipsychotic drugs upregulate lipogenic gene expression by disrupting intracellular trafficking of lipoprotein-derived cholesterol. *Pharmacogenomics J* 2009.
 215. Adams CM, Goldstein JL, Brown MS. Cholesterol-induced conformational change in SCAP enhanced by Insig proteins and mimicked by cationic amphiphiles. *Proc Natl Acad Sci U S A* 2003;100:10647-52.
 216. Lange Y, Ye J, Rigney M, Steck TL. Regulation of endoplasmic reticulum cholesterol by plasma membrane cholesterol. *J Lipid Res* 1999;40:2264-70.
 217. Lange Y, Steck TL. Cholesterol homeostasis. Modulation by amphiphiles. *J Biol Chem* 1994;269:29371-4.
 218. Lauressegues E, Bert E, Duriez P, et al. Does endoplasmic reticulum stress participate in APD-induced hepatic metabolic dysregulation? *Neuropharmacology* 2011.
 219. Ferno J, Vik-Mo AO, Jassim G, et al. Acute clozapine exposure in vivo induces lipid accumulation and marked sequential changes in the expression of SREBP, PPAR, and LXR target genes in rat liver. *Psychopharmacology (Berl)* 2009;203:73-84.
 220. Jassim G, Skrede S, Vazquez MJ, et al. Acute effects of orexigenic antipsychotic drugs on lipid and carbohydrate metabolism in rat. *Psychopharmacology (Berl)* 2011.
 221. Kaddurah-Daouk R, McEvoy J, Baillie RA, et al. Metabolomic mapping of atypical antipsychotic effects in schizophrenia. *Molecular Psychiatry* 2007;12:934-45.
 222. McNamara RK, Jandacek R, Rider T, Tso P, Cole-Strauss A, Lipton JW. Atypical antipsychotic medications increase postprandial triglyceride and glucose levels in male rats: relationship with stearoyl-CoA desaturase activity. *Schizophr Res* 2011;129:66-73.
 223. Ntambi JM, Miyazaki M, Stoehr JP, et al. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci U S A* 2002;99:11482-6.
 224. Bertile F, Raclot T. mRNA levels of SREBP-1c do not coincide with the changes in adipose lipogenic gene expression. *Biochem Biophys Res Commun* 2004;325:827-34.
 225. Palmer DG, Rutter GA, Tavare JM. Insulin-stimulated fatty acid synthase gene expression does not require increased sterol response element binding protein 1 transcription in primary adipocytes. *Biochem Biophys Res Commun* 2002;291:439-43.
 226. Sekiya M, Yahagi N, Matsuzaka T, et al. SREBP-1-independent regulation of lipogenic gene expression in adipocytes. *J Lipid Res* 2007;48:1581-91.
 227. Yahagi N, Shimano H, Hasty AH, et al. Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in *Lep(ob)/Lep(ob)* mice. *J Biol Chem* 2002;277:19353-7.

-
228. Vik-Mo AO, Birkenaes AB, Ferno J, Jonsdottir H, Andreassen OA, Steen VM. Increased expression of lipid biosynthesis genes in peripheral blood cells of olanzapine-treated patients. *Int J Neuropsychopharmacol* 2008;11:679-84.
229. Geddes J, Freemantle N, Harrison P, Bebbington P. Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. *BMJ* 2000;321:1371-6.
230. Leucht S, Wahlbeck K, Hamann J, Kissling W. New generation antipsychotics versus low-potency conventional antipsychotics: a systematic review and meta-analysis. *Lancet* 2003;361:1581-9.
231. Stahl SM. Selecting an atypical antipsychotic by combining clinical experience with guidelines from clinical trials. *J Clin Psychiatry* 1999;60 Suppl 10:31-41.
232. Chakos M, Lieberman J, Hoffman E, Bradford D, Sheitman B. Effectiveness of second-generation antipsychotics in patients with treatment-resistant schizophrenia: a review and meta-analysis of randomized trials. *Am J Psychiatry* 2001;158:518-26.
233. Swartz MS, Stroup TS, McEvoy JP, et al. What CATIE found: results from the schizophrenia trial. *Psychiatr Serv* 2008;59:500-6.
234. Lehman AF, Kreyenbuhl J, Buchanan RW, et al. The Schizophrenia Patient Outcomes Research Team (PORT): updated treatment recommendations 2003. *Schizophr Bull* 2004;30:193-217.
235. Krakowski MI, Czobor P, Nolan KA. Atypical antipsychotics, neurocognitive deficits, and aggression in schizophrenic patients. *J Clin Psychopharmacol* 2008;28:485-93.
236. Lindenmayer JP, Khan A, Iskander A, Abad MT, Parker B. A randomized controlled trial of olanzapine versus haloperidol in the treatment of primary negative symptoms and neurocognitive deficits in schizophrenia. *J Clin Psychiatry* 2007;68:368-79.
237. Murphy BP, Chung YC, Park TW, McGorry PD. Pharmacological treatment of primary negative symptoms in schizophrenia: a systematic review. *Schizophr Res* 2006;88:5-25.
238. Jones PB, Barnes TR, Davies L, et al. Randomized controlled trial of the effect on Quality of Life of second- vs first-generation antipsychotic drugs in schizophrenia: Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS 1). *Arch Gen Psychiatry* 2006;63:1079-87.
239. Kahn RS, Fleischhacker WW, Boter H, et al. Effectiveness of antipsychotic drugs in first-episode schizophrenia and schizophreniform disorder: an open randomised clinical trial. *Lancet* 2008;371:1085-97.
240. Tiihonen J, Lonnqvist J, Wahlbeck K, et al. 11-year follow-up of mortality in patients with schizophrenia: a population-based cohort study (FIN11 study). *Lancet* 2009;374:620-7.
241. De Hert M, Correll CU, Cohen D. Do antipsychotic medications reduce or increase mortality in schizophrenia? A critical appraisal of the FIN-11 study. *Schizophr Res* 2010;117:68-74.
242. Murphy BP. Inconclusive evidence for the efficacy of olanzapine in the treatment of negative symptoms in schizophrenia. *J Clin Psychiatry* 2008;69:164; author reply -5.

-
243. Davis JM, Chen N, Glick ID. Issues that may determine the outcome of antipsychotic trials: industry sponsorship and extrapyramidal side effect. *Neuropsychopharmacology* 2008;33:971-5.
 244. Lehman AF, Lieberman JA, Dixon LB, et al. Practice guideline for the treatment of patients with schizophrenia, second edition. *Am J Psychiatry* 2004;161:1-56.
 245. Johnsen E, Kroken RA, Wentzel-Larsen T, Jorgensen HA. Effectiveness of second-generation antipsychotics: a naturalistic, randomized comparison of olanzapine, quetiapine, risperidone, and ziprasidone. *BMC Psychiatry* 2010;10:26.
 246. Correll CU, Lencz T, Malhotra AK. Antipsychotic drugs and obesity. *Trends Mol Med* 2010;17:97-107.
 247. Correll CU, Manu P, Olshanskiy V, Napolitano B, Kane JM, Malhotra AK. Cardiometabolic risk of second-generation antipsychotic medications during first-time use in children and adolescents. *JAMA* 2009;302:1765-73.
 248. Bai YM, Lin CC, Chen JY, Lin CY, Su TP, Chou P. Association of initial antipsychotic response to clozapine and long-term weight gain. *Am J Psychiatry* 2006;163:1276-9.
 249. Leadbetter R, Shutty M, Pavalonis D, Vieweg V, Higgins P, Downs M. Clozapine-induced weight gain: prevalence and clinical relevance. *Am J Psychiatry* 1992;149:68-72.
 250. Meltzer HY, Perry E, Jayathilake K. Clozapine-induced weight gain predicts improvement in psychopathology. *Schizophr Res* 2003;59:19-27.
 251. Garyfallos G, Dimelis D, Kouniakakis P, et al. Olanzapine versus risperidone: weight gain and elevation of serum triglyceride levels. *Eur Psychiatry* 2003;18:320-1.
 252. Krakowski M, Czobor P. Cholesterol and cognition in schizophrenia: A double-blind study of patients randomized to clozapine, olanzapine and haloperidol. *Schizophr Res* 2011.
 253. Hermes E, Nasrallah H, Davis V, et al. The association between weight change and symptom reduction in the CATIE schizophrenia trial. *Schizophr Res* 2011;128:166-70.
 254. Hummer M, Kemmler G, Kurz M, Kurzthaler I, Oberbauer H, Fleischhacker WW. Weight gain induced by clozapine. *Eur Neuropsychopharmacol* 1995;5:437-40.
 255. Bustillo JR, Buchanan RW, Irish D, Breier A. Differential effect of clozapine on weight: a controlled study. *Am J Psychiatry* 1996;153:817-9.
 256. Dwork A, Mancevski M, Rosoklija G. White matter and cognitive function in schizophrenia. *Int J Neuropsychopharmacol* 2007;10:513-36.
 257. Herring NR, Konradi C. Myelin, copper, and the cuprizone model of schizophrenia. *Front Biosci (Schol Ed)* 2011;3:23-40.
 258. Kyriakopoulos M, Perez-Iglesias R, Woolley JB, et al. Effect of age at onset of schizophrenia on white matter abnormalities. *Br J Psychiatry* 2009;195:346-53.
 259. Camargo N, Smit AB, Verheijen MH. SREBPs: SREBP function in glia-neuron interactions. *FEBS J* 2009;276:628-36.
 260. Hakak Y, Walker JR, Li C, et al. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proc Natl Acad Sci U S A* 2001;98:4746-51.

-
261. Tkachev D, Mimmack ML, Ryan MM, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet* 2003;362:798-805.
 262. Raeder MB, Ferno J, Glambek M, Stansberg C, Steen VM. Antidepressant drugs activate SREBP and up-regulate cholesterol and fatty acid biosynthesis in human glial cells. *Neurosci Lett* 2006;395:185-90.
 263. Gregg JR, Herring NR, Naydenov AV, Hanlin RP, Konradi C. Downregulation of oligodendrocyte transcripts is associated with impaired prefrontal cortex function in rats. *Schizophr Res* 2009;113:277-87.
 264. Xu H, Yang HJ, McConomy B, Browning R, Li XM. Behavioral and neurobiological changes in C57BL/6 mouse exposed to cuprizone: effects of antipsychotics. *Front Behav Neurosci* 2010;4:8.
 265. Bartzokis G, Lu PH, Amar CP, et al. Long acting injection versus oral risperidone in first-episode schizophrenia: Differential impact on white matter myelination trajectory. *Schizophr Res* 2011;132:35-41.
 266. Bartzokis G, Lu PH, Nuechterlein KH, et al. Differential effects of typical and atypical antipsychotics on brain myelination in schizophrenia. *Schizophr Res* 2007;93:13-22.
 267. Bartzokis G. Neuroglialpharmacology: white matter pathophysiologies and psychiatric treatments. *Front Biosci* 2011;17:2695-733.
 268. Wu RR, Zhao JP, Jin H, et al. Lifestyle intervention and metformin for treatment of antipsychotic-induced weight gain: a randomized controlled trial. *JAMA* 2008;299:185-93.
 269. Praharaj SK, Jana AK, Goyal N, Sinha VK. Metformin for olanzapine-induced weight gain: a systematic review and meta-analysis. *Br J Clin Pharmacol* 2011;71:377-82.
 270. Ehret M, Goethe J, Lanosa M, Coleman CI. The effect of metformin on anthropometrics and insulin resistance in patients receiving atypical antipsychotic agents: a meta-analysis. *J Clin Psychiatry* 2010;71:1286-92.
 271. Ojala K, Repo-Tiihonen E, Tiihonen J, Niskanen L. Statins are effective in treating dyslipidemia among psychiatric patients using second-generation antipsychotic agents. *J Psychopharmacol* 2008;22:33-8.
 272. Wong AK, Howie J, Petrie JR, Lang CC. AMP-activated protein kinase pathway: a potential therapeutic target in cardiometabolic disease. *Clin Sci (Lond)* 2009;116:607-20.
 273. Archie SM, Goldberg JO, Akhtar-Danesh N, Landeen J, McColl L, McNiven J. Psychotic disorders, eating habits, and physical activity: who is ready for lifestyle changes? *Psychiatr Serv* 2007;58:233-9.
 274. Evans S, Newton R, Higgins S. Nutritional intervention to prevent weight gain in patients commenced on olanzapine: a randomized controlled trial. *Aust N Z J Psychiatry* 2005;39:479-86.
 275. Kwon JS, Choi JS, Bahk WM, et al. Weight management program for treatment-emergent weight gain in olanzapine-treated patients with schizophrenia or schizoaffective disorder: A 12-week randomized controlled clinical trial. *J Clin Psychiatry* 2006;67:547-53.

276. Menza M, Vreeland B, Minsky S, Gara M, Radler DR, Sakowitz M. Managing atypical antipsychotic-associated weight gain: 12-month data on a multimodal weight control program. *J Clin Psychiatry* 2004;65:471-7.