MECHANISMS OF THE METABOLIC SIDE EFFECTS OF ATYPICAL ANTIPSYCHOTIC DRUGS

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by
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ABSTRACT

Second generation ‘atypical’ antipsychotics are a popular and effective treatment for schizophrenia and other psychiatric illnesses. These drugs revolutionized the treatment of psychotic disorders because they lacked the debilitating movement side effects, sedation and hypotension that plagued first generation compounds. However, post-marketing studies revealed unexpected side effects of these atypical drugs, including obesity and diabetes. The aim of this project was to develop a rodent model of these metabolic effects in order to identify potential mechanisms involved in the diabetes and obesity observed clinically.

Animal models of the obesity and insulin resistance side effects were developed that showed similarities to the clinical situation, including hyperphagia, weight gain, increased adiposity and insulin resistance. Sexually dimorphic effects were observed in rats; for example, whereas females became obese, males accumulated adiposity without increased body weight gain. In female rats, the mechanism underlying this hyperphagia appears to be an acute lowering of the circulating concentration of several satiety factors, including glucose, insulin and leptin.

Chronic olanzapine did not alter food intake or body weight in male rats, although they exhibited increased adiposity and insulin resistance. Several factors likely contribute to the increased fat mass. First, male rats provided olanzapine displayed decreased physical activity but maintained food intake. Thus, food intake did not decline to compensate for the level of activity. This relative hyperphagia was associated with lowering of plasma leptin. Other metabolic changes in these animals that favor increased adiposity include increased nutrient partitioning to fat and decreased fat mobilization in response to fasting or isoproterenol. Insulin resistance occurred acutely in male rats, suggesting that it was a direct drug effect and not secondary to
adiposity. It was also tissue-specific, only affecting the heart and skeletal muscles and not fat, which exhibited either unaffected or improved insulin sensitivity.

A recent retrospective study of patients revealed a major new side effect of atypical antipsychotics, increased risk of sudden cardiac death due to a ventricular arrhythmia that is frequently associated with metabolic disorders. Plasma free fatty acids, the heart’s preferred fuel, were lowered by olanzapine in our model. Acute studies revealed that this was due not only to impaired \textit{in vivo} lipolysis, but also to a robust shift in the use of lipid fuels. This shift was confirmed by alterations in plasma nutrients after food deprivation, rapid and robust declines in respiratory exchange ratio, improved triglyceride tolerance without changes in hepatic secretion, and elevated FFA uptake (~2-fold or greater) into most tissues. Notably, the heart appeared to be more severely insulin resistant than other tissues and olanzapine did not stimulate uptake of FFAs as it did in other tissues. Furthermore, olanzapine increased heart rate without changing blood pressure or stroke volume. These results suggest that olanzapine may elevate cardiac risk by simultaneously causing cardiac insulin resistance, repartitioning of fatty fuels and tachycardia, while decreasing availability of free fatty acids from lipolysis. The olanzapine-induced metabolic inflexibility may explain its glucose sparing and glucose intolerance effects.
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<th>Full Form</th>
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<tbody>
<tr>
<td>2-DOG</td>
<td>2-deoxyglucose</td>
</tr>
<tr>
<td>ACC</td>
<td>Acetyl-CoA Carboxylase</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated Protein Kinase</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>b.i.d.</td>
<td>Twice a day</td>
</tr>
<tr>
<td>BMIPP</td>
<td>β-methyl-iodo-phenyl-pentadecanoic acid</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CPT-1</td>
<td>Carnitine Palmitoyl Transferase-1</td>
</tr>
<tr>
<td>DLS</td>
<td>Dorsolateral Striatum</td>
</tr>
<tr>
<td>DPM</td>
<td>Disintegrations Per Minute</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EGTA</td>
<td>Ethylene glycol tetraacetic acid</td>
</tr>
<tr>
<td>EPS</td>
<td>Extrapyramidal Symptoms</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle Stimulating Hormone</td>
</tr>
<tr>
<td>GIR</td>
<td>Glucose Infusion Rate</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>HGO</td>
<td>Hepatic Glucose Output</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
</tr>
</tbody>
</table>
i.v. Intravenous
LH Luteinizing Hormone
ITT Insulin Tolerance Test
MCD Malonyl-CoA Decarboxylase
NAcc Shell Nucleus Accumbens, Shell Region
NAcc Core Nucleus Accumbens, Core Region
NPY Neuropeptide Y
OGTT Oral Glucose Tolerance Test
OLTT Oral Lipid Tolerance Test
PDH Pyruvate Dehydrogenase
PMSF Phenylmethylsulphonyl Fluoride
POMC Pro-opiomelanocortin
PVDF Polyvinylidene Fluoride
p.o. By Mouth
R_a Rate of Appearance
R_d Rate of Disappearance
RER Respiratory Exchange Ratio
R_g Rate of Glucose Uptake
R_f Rate of FFA Uptake
RT Room Temperature
SDS Sodium Dodecyl Sulfate
SNpc Substantia Nigra pars compacta
SPECT Single Photon Emission Computed Tomography
TK Tardive Dyskinesia
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
</tr>
<tr>
<td>VCO₂</td>
<td>Rate of Carbon Dioxide Expiration</td>
</tr>
<tr>
<td>VO₂</td>
<td>Rate of Oxygen Consumption</td>
</tr>
<tr>
<td>VTA (r)</td>
<td>Ventral Tegmental Area, rostral expanse</td>
</tr>
<tr>
<td>VTA (c)</td>
<td>Ventral Tegmental Area, caudal expanse</td>
</tr>
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</table>
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If I have seen further, it is only by standing on the shoulders of Giants.

– Sir Isaac Newton, February 1676
Chapter 1

Overview of the Metabolic Side Effects of Atypical Antipsychotic Drugs

1.1 Schizophrenia & Antipsychotics

Antipsychotic medications comprise several groups of drugs used to treat a variety of psychiatric disorders; including schizophrenia, bipolar disorder, acute mania, major depression, delirium as well as other psychoses. Collectively these disorders will affect at least 3-5% of the population at some point during their lives (1, 2), potentially exposing a significant portion of these individuals to antipsychotic treatment. Schizophrenia, in particular, is a complex disease marked by combinations of positive symptoms (e.g. visual hallucinations, thought disorders) and negative symptoms (e.g. blunted affect, anhedonia). The pathophysiology of schizophrenia is unknown, however, the positive symptoms are thought to result from excessive dopaminergic stimulation in the brain. Accordingly, compounds that cause release and/or prevent the re-uptake of dopamine at presynaptic neurons (e.g. amphetamines) induce psychoses in otherwise healthy individuals and are alleviated by drugs (e.g. reserpine) that deplete CNS dopamine (3, 4). Until the 1950s, treatment options for schizophrenia were limited and the advent of antipsychotics (e.g. chlorpromazine, haloperidol) represented a significant advancement in psychopharmacology. These first generation compounds possessed good efficacy against the positive symptoms of schizophrenia, predominately because they are dopaminergic antagonists. Unfortunately, these drugs are non-selective and exhibit severe extrapyramidal symptoms, tardive dyskinesia and hypotension that decrease quality of life and patient adherence. The extrapyramidal symptoms and tardive dyskinesia are due to blockage of dopaminergic stimulation in the nigrostriatal tracts.
of the brain, an expected effect of dopaminergic antagonists like antipsychotic drugs (5). Aside from these adverse effects, first generation antipsychotics also caused moderate weight gain, though this problem was far outweighed by the debilitating extrapyramidal symptoms and tardive dyskinesia (6-10).

Second generation antipsychotics came onto the U.S. market in 1989 with the introduction of clozapine (Clozaril®), a powerful antipsychotic that alleviated both positive and negative symptoms of schizophrenia. Currently, clozapine therapy is limited to treatment-resistant patients due to the incidence of life-threatening agranulocytosis (~1%). However, the development of other second-generation antipsychotics followed, including Lilly’s olanzapine (Zyprexa®) and Johnson & Johnson’s risperidone (Risperdal®) in the mid-1990s. These newer drugs revolutionized the treatment of psychotic disorders because they possessed equal or better efficacy against the positive and negative symptoms of schizophrenia and, more importantly, lacked the extrapyramidal symptoms of first generation drugs, which is the reason they are referred to as ‘atypical’. Pharmacologically, almost all the atypical compounds have lower affinities for D2-like dopamine receptors and higher affinities for serotonin-2A (5-HT_2A) receptors. As the popularity and off-label uses of olanzapine and other new ‘atypical’ drugs increased, so did evidence suggesting possible metabolic side effects; including ketoacidosis (11-16), dyslipidemia (17, 18), severe weight gain (19-23), hyperglycemia (24-28) and even death (29-31). Interestingly, the weight gain side effect of atypical antipsychotics appears to exceed that of the first generation compounds. Numerous meta-analyses have confirmed that olanzapine and clozapine cause the most significant weight gain and other metabolic effects. The clinical impact of these metabolic side effects is underscored by FDA-mandated package warnings about the potential for hyperglycemia and/or hyperosmolar coma, as well as a consensus statement addressing the treatment and management of patients on atypical antipsychotics (32). Despite these significant risks, atypical antipsychotics continue to be widely prescribed because of their
unmatched efficacy, which outweighs their metabolic risks. The mechanisms of these metabolic side effects remain elusive, however, identifying the drug targets could provide a useful strategy to reduce adverse events and also to develop drugs with fewer side effects.

Given the immense clinical impact and breadth of this topic, a review of all the literature examining the basic scientific investigation, clinical, and epidemiological studies is impractical. Excellent comprehensive literature reviews that discuss the usage and clinical impact of these drugs (33-44), as well as recent reviews discussing potential insights from receptor profiles and in vitro studies (45-47) have been published. This review will focus primarily on the studies of animal models that have led to our current understanding of the metabolic effects of the atypical antipsychotics; including hyperphagia, weight gain, insulin resistance, and β-cell dysfunction. Specifically, the effects of sulpiride, risperidone, clozapine and olanzapine will be reviewed and compared to give insight into the potential mechanisms of these metabolic side effects.

1.2 Typical and Atypical Antipsychotic Drugs

1.2.1 Basic Pharmacology of Antipsychotics

Antipsychotic drugs can be broadly divided into groups by chemical class or by lack of extrapyramidal (i.e. movement) side effects. The first generation antipsychotics (also called typical antipsychotics) have non-specific binding profiles but had a shared ability to inhibit D2-dopamine receptors. These compounds fall into one of three subclasses based on their chemical structure: (1) phenothiazines, (2) thioxanthenes or (3) butyrophenones. Chlorpromazine (Thorazine®) is the prototypical phenothiazine, with a low clinical potency and more prevalent extrapyramidal, sedative and hypotensive effects. Thiothixene (Navane®), the prototypical thioxanthene, has a much greater clinical potency while carrying a smaller risk of sedative and
hypotensive effects. The risk of extrapyramidal symptoms is approximately the same as with chlorpromazine. Finally, the prototypical butyrophenone is haloperidol (Haldol®), which has a very high clinical potency and much lower sedating and hypotensive effects. Currently, haloperidol is the most commonly prescribed of the typical antipsychotic drugs, most commonly for acute agitation or psychiatric emergencies. However, with prolonged haloperidol treatment, the risk of extrapyramidal symptoms is much greater than either chlorpromazine or thiothixene.

![Chemical structures of chlorpromazine and haloperidol](image)

**Figure 1-1:** First Generation ‘Typical’ Antipsychotics

The second generation compounds, so-called atypical antipsychotics, are also strong dopaminergic antagonists, however, they exhibit lower affinity for dopaminergic receptors and much greater affinity for serotonergic receptors relative to the typical antipsychotics. In terms of chemical class, most of the atypical antipsychotics do not fit into the above-mentioned three
chemical categories and are lumped into a fourth category. Unlike all of the other second-
generation antipsychotics, sulpiride is unique because it is a selective, D2 receptor antagonist
that is prescribed as an antipsychotic in Europe and Japan; it has not been approved for use in the
United States and Canada. Sulpiride has a short half-life, typically between 6-8h in healthy
individuals, and is slowly absorbed from the gastrointestinal tract. Sulpiride is considered an
atypical antipsychotic because it lacks the extrapyramidal (i.e. movement) side effects.

Risperidone (Risperdal®), a prodrug, is a benzisoxazole derivative that entered the U.S. market
in the early 1990s and, as with many of the other atypical drugs, weight gain was identified as a
significant risk of therapy. However, risperidone does not carry as significant a risk of weight
gain and type II diabetes as either olanzapine or clozapine (35), though risk still exists and many
studies have examined risperidone alone or in comparison with either typical antipsychotics, like
haloperidol, or other atypical compounds. Like other atypical antipsychotics, risperidone has a
much greater anti-serotonergic affinity than anti-dopaminergic affinity. However, risperidone
has a much greater affinity for dopamine receptors, which explains its greater incidence of
extrapyramidal symptoms and hyperprolactinemia.

Olanzapine (Zyprexa®), a thienobenzodiazepine, along with clozapine (Clozaril®), a
dibenzodiazepine, are the most body weight gain promoting of the atypical antipsychotics.
Unlike clozapine, olanzapine treatment does not carry the risk of agranulocytosis (1%) that
requires frequent blood monitoring in clozapine-treated patients. This difference contributes to
olanzapine’s clinical popularity compared to clozapine. Nevertheless, clozapine is still one of the
only effective therapies for treatment-resistant patients, the mainstay of its clinical usefulness.
Unfortunately, almost one-third of patients taking these two drugs gain a “clinically significant”
amount of body weight (defined as greater than seven percent of baseline body weight) and some
patients gain much more. Given the extreme weight gain that has been reported and its prevalent
use, olanzapine has been the focus of many animal studies examining potential mechanisms of the metabolic effects of this drug class.

**Figure 1-2**: Second Generation ‘Atypical’ Antipsychotics

1.2.2 Clinical Observations and Potential Mechanisms of Metabolic Effects

Along with the typical antipsychotics, the atypical antipsychotics are largely thought to exert their ‘anti-schizophrenic’ actions through antagonism of dopamine receptors, though the potential role of these dopaminergic effects in the mechanism of antipsychotic-induced obesity is
not well defined. Dopamine, one of the endogenous catecholamines, plays important roles both centrally and peripherally. In the CNS, dopamine is a critical neurotransmitter in the basal ganglia, especially in the subthalamic nucleus, which receives dopaminergic transmission from the substantia nigra important in voluntary movement. Additionally, dopamine is important in neuroendocrine signaling and spatial waking memory (48). Mechanistically, dopaminergic signaling mediates the effects of other dopamine-mediated effects on calcium and potassium channels. Peripherally, dopamine acts on its own peripheral dopamine receptors (DA1 and DA2), but also signals through α- and β-adrenergic receptors as well. DA1 receptors are located in several arteries (e.g. renal afferent/efferent, coronary, mesenteric, cerebral), brain, kidney tubules and mesangial cells, eye, stomach and adrenal gland. DA2 receptors are located on peripheral adrenergic nerve terminals, in sympathetic ganglia, as well as in the kidney, stomach and adrenal gland like DA1 receptors. With this wide distribution of dopamine receptors as well as signaling through α- and β-adrenergic receptors there are several dose-dependent effects of dopamine (reviewed in 49); including (low dose) increasing splanchnic and renal perfusion, lowering diastolic blood pressure, (intermediate dose) inotropic and chronotropic effects on the heart as well as (high dose) vasoconstriction and hypertensive effects.

Given the rich pharmacology of the atypical antipsychotics, it has been challenging to identify the receptor or, more likely, the receptors responsible for the drug-induced hyperphagia and subsequent weight gain. Studies have compared weight gain liability and receptor affinity to identify the receptor responsible for the drug-induced hyperphagia (50), though these studies have not been conclusive. Observational and retrospective studies have suggested that human weight gain appears to be due to increased caloric intake and low habitual physical activity level. Increased appetite in patients does not appear to be associated with so-called ‘carbohydrate cravings’ (51).
In the search for a mechanism explaining the weight gain induced by these drugs, several hypotheses have been proposed. One of these ideas that has been examined is the possibility that antipsychotics induce a state of ‘anhedonia’, literally meaning ‘the inability to feel pleasure’ (52). This is referred to as the anhedonia hypothesis, so-called because dopamine is well recognized as an important substrate in reward-guided behaviors, including eating behavior, and has been implicated in human obesity (53-55). Thus, it is reasonable to posit that antipsychotics may potentially predispose patients to overeating by blocking the ‘rewarding’ feeling of food intake, which could lead to increased intake to counter the antipsychotic effect. Even though central dopaminergic signaling appears to be involved in determining meal size and food intake behavior in mice and rats (e.g. 56, 57), the exact mechanisms of dopaminergic signaling in individual brain regions in meal size, hyperphagia and satiety are not completely understood. Thus, dopamine agonists and antagonists may modulate the CNS reward circuitry (i.e. the reward-behavior system) and may underlie antipsychotic-induced obesity.

Hyperprolactinemia is another potential weight-promoting effect that has been implicated in antipsychotic-induced weight gain clinically and experimentally in rodents. Normally, prolactin secretion from the anterior pituitary is under tonic dopaminergic-mediated inhibition from the tubero-infundibular tract in the brain. In pathologic states, elevated plasma prolactin concentrations result in atrophy of the hypothalamic-pituitary-gonadal axis by inhibiting the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. The decrease in hormone-stimulated production of sex steroids results in a hypogonadal state. Numerous drugs that antagonize dopamine receptors (e.g. antipsychotics) are known to elevate circulating prolactin by lifting this tonic inhibition. Moreover, pituitary adenomas that result in hypersecretion of prolactin are generally associated with weight gain (58). The role of prolactin in human antipsychotic-induced weight gain, however, is not entirely clear since weight gain also occurs with antipsychotics (e.g. olanzapine) that are marketed for their
‘prolactin sparing’ effects (5, 59). Regardless, with the complexity and variability in observational or prospective studies in healthy individuals and also patients, animal studies have provided a wealth of knowledge and insight into the potential mechanisms behind these intriguing effects.

1.3 Animal Models of the Metabolic Effects of Atypical Antipsychotics

Even though the atypical antipsychotic drugs have revolutionized the treatment of schizophrenia and other psychoses, it is widely accepted that the non-specific nature of these compounds leads to the development of their metabolic side effects – namely (1) hyperphagia and body weight gain and (2) insulin resistance and β−cell dysfunction. The receptor(s) responsible for each of these metabolic effects are unknown, and it is likely that all these effects are not mediated through one target receptor. Given the complex nature of body weight regulation and metabolism, one must remember that each of these side effects likely contributes to an integrated phenotype that is observed clinically and experimentally.

1.3.1 Hyperphagia and Body Weight Gain

The body weight gain effect of the typical and atypical antipsychotics has long been recognized (e.g. 60). With the development of atypical antipsychotics, the weight gain-promoting effect of these drugs was brought to the forefront, as the extrapyramidal symptoms, tardive dyskinesia and sedative effects were much less apparent in the case of the atypical compounds. Table 1-1 highlights previous studies examining changes in body weight and/or adiposity induced by the atypical drugs sulpiride, risperidone, clozapine and olanzapine.
1.3.1.1 Sulpiride

Baptista, Parada and coworkers published many of the early studies examining sulpiride-induced body weight gain in rats. At the time it was well-accepted that other neuroleptics, such as thioridazine, trifluoperazine, haloperidol and sulpiride caused body weight gain in humans (7). Interestingly, the typical antipsychotics chlorpromazine and fluphenazine actually decreased body weight in male rats (61). Regardless, a series of studies demonstrated that many of these same antipsychotic drugs caused hyperphagia and subsequent weight gain in rats, though this effect was unexpectedly sex-dependent (61). That is, female rats showed a dose-dependent increase in body weight gain that was accompanied by hyperphagia, while male rats either lost or had little or no change in body weight with peripheral drug administration (i.p. injection). However, direct infusion of sulpiride into the lateral hypothalamus induces feeding and drinking in satiated male rats (62); thus, sulpiride may increase ‘meal size’ without changing daily food consumption in males. Even though sulpiride (20 mg/kg) causes obesity that is largely due to increased adiposity, increases in plasma leptin have not been observed (63).

Subsequent studies focused on determining why weight gain was sexually dimorphic in rats, an effect that had not be described clinically. Central infusions, but not peripheral administration of sulpiride, increased short-term food intake in males, and therefore one might think that sulpiride might not cross the blood-brain-barrier in rats. However, this is inconsistent with the clinical site of action of antipsychotics and additional findings that clearly show that sulpiride crosses the blood-brain-barrier as well as increases dopamine metabolites in the lateral hypothalamus of female rats (64).

Unlike other atypical antipsychotics, sulpiride is a specific D2-receptor antagonist, suggesting that the hyperphagia and body weight gain effects are largely mediated through D2 antagonism. Accordingly, co-administration of the dopamine agonist, bromocriptine, prevents
sulpiride-induced weight gain in female rats. Bromocriptine, a non-specific dopamine agonist, commonly causes anorexia and weight loss in humans, and whether bromocriptine specifically counteracts D2-mediated effect of sulpiride or causes anorexia through excessive dopaminergic stimulation is unknown (61).

The sexually dimorphic nature of this effect argues strongly for the involvement of prolactin and/or the sex steroids (i.e. androgens and estrogens) in the pathogenesis of sulpiride-induced obesity. In the rat, prolactin concentrations are typically highest in the nursing female, with the prolactin concentration increasing with increasing number of pups. Prolactin has been implicated in the hyperphagia that accompanies female rats, when food intake can easily increase by more than 2-fold (65, 66). Moreover, increases associated with prolactin elevation appear to be sex-dependent, with dose-dependent increases in food intake observed only in female rats, not males (67). Even though antipsychotic-induced increases in prolactin make sense physiologically, these results have been challenged and thus prolactin may not play as important a role in female food intake. Many of these studies were conducted using prolactin isolated from pituitary preparations, and thus contamination from other pituitary hormones (i.e. growth hormone, vasopressin) has been a concern of those who have not been able to reproduce prolactin-induced body weight gain findings (68, 69). Furthermore, data suggest that even though prolactin may be orexigenic, other hormones (e.g. progesterone) likely contribute to the increased body weight gain observed in previous studies (70).

Consistent with the involvement of the sex steroids, animal age (i.e. pubertal status) plays an important role, as sulpiride causes weight gain in pre-pubertal, peri-pubertal and adult female rats as well as pre-pubertal male rats. Interestingly, sulpiride does not cause weight gain in peri-pubertal or adult male rats (71); suggesting that a pubertal effect in the rat that nullifies the drug-induced hyperphagia. In another study, sulpiride-induced hyperphagia and weight gain were absent in ovariectomized female rats (72). Weight gain in this study, however, might be limited
by a ‘ceiling effect’, since ovariectomy by itself already produces potentially maximal rates of weight gain.

Antipsychotics are strong dopamine antagonists that increase secretion of prolactin from the anterior pituitary (72). The resultant hyperprolactinemia should lead to a ‘functional gonadectomy’ (i.e. decreased estrogen and/or testosterone) via inhibition of LH and FSH secretion. This hypothesis, which posits that weight gain results from a ‘functional gonadectomy’, is consistent with reports that ovariectomized female rats demonstrate marked hyperphagia and weight gain that is prevented by estradiol administration (73, 74); as well as other reports demonstrating that unlike female rats, male rats do not display hyperphagia following gonadectomy (75). In further supporting of this hypothesis were three additional observations: 1) sulpiride induced a diestrus state that persists for the duration of treatment, 2) ovariectomized female rats treated with sulpiride did not gain more weight than ovariectomized controls, and 3) estradiol co-administration prevented sulpiride-induced weight gain (72).

Collectively, these data suggest that at least two mechanisms appear to contribute to sulpiride-induced obesity: 1) hypogonadism related to the drug-induced hyperprolactinemia and 2) other central effects of the drug that favor female weight gain more so than that of males. Studies attempting to more directly demonstrate a sulpiride-induced ‘functional gonadectomy’ have demonstrated that sulpiride elevates prolactin in male and female rats. Counter to this hypothesis, though, sulpiride does not decrease serum estrogen levels in females (76); nor does it decrease serum testosterone levels in male rats (77), which also have elevated serum prolactin concentrations following chronic (>2wk) sulpiride administration. Indeed, basal concentrations of circulating estradiol and testosterone remain unchanged in these animals (77). Thus, it appears that the diestrus is secondary to inhibition of physiologic ‘cycling’ of LH and FSH, without affecting basal sex-steroid levels (76).
1.3.1.2 Risperidone

Similar to central (males) or peripheral administration (females) of sulpiride, peripherally administered risperidone (3 mg/kg) increases ‘meal size’ (4h consumption) in male rats without changing 24h food-consumption (78). Peripheral injection of risperidone in male rats increases dopamine metabolites in the lateral hypothalamus (77), though risperidone also induces a sexually dimorphic body weight gain in female rats that is less severe than sulpiride-induced weight gain. Male weight gain has been reported, though this potentially small effect has not been verified by others (79). Female rats treated for 12 days with risperidone (0.5 mg/kg) or sulpiride (20 mg/kg) showed similar increases in food intake, food conversion efficiency, body weight gain and adiposity, which was detectable as an increase in fat pad weight and adipocyte hyperplasia (80). Similar to findings in sulpiride-induced obesity in female rats, plasma leptin, which usually reflects the degree of total body fat, remained unchanged. Similar to findings in risperidone-treated male rats (81, 82), chronic risperidone in dogs (5 mg/day, 6 weeks) did not alter the body weight gain or food intake. Abdominal adipose tissue, as measured with magnetic resonance imagine (MRI), did not differ between placebo and risperidone treatment (83).

As expected from its known clinical effects, which includes a greater propensity for elevated prolactin, risperidone caused marked hyperprolactinemia and also increased circulating corticosterone in males and females (81). Counter to the ‘functional gonadectomy’ hypothesis, though, risperidone-treated female rats actually had increased concentrations of estradiol, even though they exhibited hyperphagia and weight gain. Similar to sulpiride, risperidone clearly elevates plasma prolactin concentrations that likely explain the diestrus state of female rats that resolves with cessation of drug treatment (81).
1.3.1.3 Clozapine

Studies examining whether or not clozapine has short-term effects on food intake in rats and mice have been conflicting (78, 84, 85), though clozapine (1-10 mg/kg) has consistently been shown not affect 24h food intake, even during chronic infusion (86-89). In fact, at higher doses, clozapine reduces food intake food intake in male (86, 90) and female rats (87), an effect that is likely due to sedation at higher doses (>10-20 mg/kg). Recently, it has been shown that clozapine activates AMPK in the hypothalamus (91), which is known to increase food intake (92), in mice through an H1R-mediated mechanism. Not surprisingly, histaminergic receptors have been implicated in antipsychotic-induced obesity (50), as histaminergic blockade and activation increases or decreases food intake and body weight gain, respectively (93-95). As observed with sulpiride and risperidone, clozapine also induces a state of diestrus in female rats for the duration of drug treatment that is most likely secondary to hyperprolactinemia (90), further suggesting that hyperprolactinemia per se is not sufficient to explain the antipsychotic-induced body weight gain.

1.3.1.4 Olanzapine

With its clinical propensity to cause severe weight gain, olanzapine has been extensively studied in animal models. Unlike risperidone, peripherally injected olanzapine (0.1-10 mg/kg) does not increase 4h or 24h food intake in male rats (78, 84). Feeding behavior (i.e. measured activity) following peripherally administered olanzapine is greater at a lower dose, suggesting that sedation may precluded increased food intake over a short-term (<4h) period (84, 96). Wurtman and colleagues have confirmed this drug-induced sedation and further demonstrated that olanzapine decreases gross motor activity in female rats at doses as low as 1.2 mg/kg/day, but motor activity returns to normal values with the cessation of drug treatment (97). With acute or
chronic treatment, olanzapine also does not alter food preference between carbohydrates and protein (97), even though olanzapine is a known antagonist of serotonergic neurons that are known to affect food preference (98, 99).

To better understand the mechanism of olanzapine-induced hyperphagia, neuropeptide expression levels following sub-chronic olanzapine administration (1 mg/kg b.i.d.) have been examined. No changes in hypothalamic neuropeptide Y (NPY), hypocretin/orexin (HCRT), melanin concentrating hormone and pro-opiomelanocortin (POMC) mRNA levels (100) were detectable after sub-chronic treatment. However, as mentioned with clozapine, olanzapine has also been shown to activate hypothalamic AMPK via an H1R-mediated mechanism, which may contribute to the drug-induced hyperphagia (91).

Similar to sulpiride and risperidone, olanzapine-induced weight gain is also sex-dependent. Olanzapine (1.2-8.0 mg/kg/day) increases food consumption, feed efficiency, body weight gain and adipose tissue mass in female rats (97, 101, 102). Further similarities include significant increases in adipose tissue mass without corresponding increases in plasma leptin (103). Another characteristic shared between animals and humans, similar to other antipsychotics as well, is that olanzapine-induced body weight reaches a plateau (101), even with ‘ramped-dosing’ (102). Pair-feeding eliminates olanzapine-induced obesity, demonstrating that hyperphagia is necessary for increased body weight gain (100). Importantly, when drug-administration is ceased the hyperphagia and weight gain are reversed an animals tend to lose weight, indicating that these effects are reversible.

Studies in male rats have been limited, given that increases in body weight originally appeared to be limited to female rats. However, Minet-Ringuet and coworkers showed that by administering olanzapine with food in a diet that more resembles a human diet (i.e. higher fat, 20%), male rats exhibit increased body weight gain and hyperphagia over a three week period, though the weight gain is clearly not as severe as in female rats (104). Indeed, a follow-up five-
week study using the same diet and drug-dosing protocol reported no significant effect on body weight (105). Similar to females, the small degree of weight gain in this model is associated with increased body fat. Interestingly, when broken down into components only subcutaneous adipose tissue tended to be increased, though this did not quite reach statistical significance (106). In two studies in which the higher fat diet was not used, body weight gain was not observed in male rats, though animals showed a trend for an increased percentage of body fat (106, 107). Follow-up studies have demonstrated that the increase in fat mass in males may be due to adipocyte hypertrophy and apparent decreases in adipocyte lipolysis (*in vitro*), changes that were accompanied by decreased and increased expression of hormone sensitive lipase and fatty acid synthase, respectively (105).

As clozapine increases visceral adiposity independent of weight gain (87), this same observation has been made following olanzapine treatment (1-8 mg/kg, 3-6 weeks) in male rats fed a standard chow diet (108). Moreover, 6-week olanzapine administration (10 mg/kg) in dogs increased visceral adiposity as well, without changes in body weight (83). Interestingly, data suggest the increased adiposity may be greater in subcutaneous compared to visceral adipose tissue (104).

Olanzapine has a much shorter half-life in rats than in humans (109, 110), as well as the other atypical antipsychotics, and this has been a point of concern when evaluating the literature. Chronic infusion studies (11 days) of olanzapine (5 mg/kg/day) also did not affect body weight gain or food intake in male rats. While olanzapine-treated females displayed a mild hyperphagia and increase in body weight gain (88). Chronic (4 week) olanzapine treatment (2.5 or 7 mg/kg/day) in another study using constant infusion pumps showed no change in body weight in female rats. However, chronic infusion studies should be interpreted with caution, as olanzapine is not stable in solution (111).
Finally, as with other atypical antipsychotics, the involvement of hyperprolactinemia has been implicated in olanzapine-induced obesity. However, Cooper et al (103) concluded that prolactin elevation is not implicated in antipsychotic-induced obesity because the dose-response of weight gain seemed to be disconnected from the ‘across-the-board’ elevation of prolactin that was observed in all drug-treated groups.
## Table 1-1: Chronic effects of atypical antipsychotics on body weight gain and fat mass in male and female rats.

<table>
<thead>
<tr>
<th>Study</th>
<th>Strain</th>
<th>Sex</th>
<th>Dose (mg/kg)</th>
<th>Study Length</th>
<th>Drug Delivery</th>
<th>Body Weight</th>
<th>Fat Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baptista et al, 1987</td>
<td>Wistar</td>
<td>F</td>
<td>10, 20</td>
<td>21 days</td>
<td>i.p.</td>
<td>↑↑</td>
<td>n/a</td>
</tr>
<tr>
<td>Baptista et al, 2002</td>
<td>Wistar</td>
<td>M</td>
<td>20</td>
<td>21 days</td>
<td>i.p.</td>
<td>NC</td>
<td>n/a</td>
</tr>
<tr>
<td>Baptista et al, 2002</td>
<td>Wistar</td>
<td>M</td>
<td>0.125-0.5</td>
<td>16 days</td>
<td>s.c.</td>
<td>NC</td>
<td>n/a</td>
</tr>
<tr>
<td>Ota et al, 2002</td>
<td>SD</td>
<td>M</td>
<td>0.005 b.i.d.</td>
<td>21 days</td>
<td>s.c.</td>
<td>↑↑</td>
<td>n/a</td>
</tr>
<tr>
<td>Baptista et al, 1993</td>
<td>Wistar</td>
<td>M</td>
<td>0.5-5</td>
<td>21 days</td>
<td>i.p.</td>
<td>NC</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>10-20</td>
<td>21 days</td>
<td>i.p.</td>
<td>NC</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.5-20</td>
<td>21 days</td>
<td>i.p.</td>
<td>NC</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Cooper et al, 2008</td>
<td>SD</td>
<td>F</td>
<td>6-12 b.i.d.</td>
<td>21 days</td>
<td>i.p.</td>
<td>↓</td>
<td>n/a</td>
</tr>
<tr>
<td>Clozapine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choi et al, 2008</td>
<td>SD</td>
<td>M</td>
<td>10</td>
<td>11 days</td>
<td>Infusion</td>
<td>NC</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>F</td>
<td>10</td>
<td>11 days</td>
<td>Infusion</td>
<td>NC</td>
<td>n/a</td>
</tr>
</tbody>
</table>

(Table 1-1 cont’d on next page)
1.3.2 Insulin Resistance and β-Cell Dysfunction

Since the atypical antipsychotics have been associated with a high incidence of type II diabetes, insulin resistance and β-cell dysfunction have been the focus of several recent studies. To date, numerous studies have suggested that atypical antipsychotics – both acute and chronic
administration – may not only decrease peripheral insulin sensitivity, but also impair glucose-stimulated insulin secretion from the β-cell. Currently, clamp studies that have examined insulin resistance and β-cell dysfunction directly have been limited to male animals, though the reasons for this are unclear. Studies in female animals are needed, especially given the marked differences in food intake and body weight gain responses. With chronic drug-treatment, though, it is not surprising that obesity (female rats) or increased adiposity (male rats) might lead to insulin resistance and β-cell dysfunction. Interestingly, in some cases acute effects on these parameters have been observed which are independent of changes in body weight or adiposity. The literature on these effects is reviewed here, however the underlying mechanisms have not been determined.

1.3.2.1 Sulpiride

Regardless of the mechanism of sulpiride-induced obesity in female rats, several interesting features of the obesity should be noted. In sulpiride-treated obese female rats, oral glucose tolerance is improved, evident from decreased AUC-glucose and/or AUC-insulin from a glucose tolerance test (76, 112, 113), suggesting that sulpiride increases rather than decreases insulin sensitivity. Moreover, hyperglycemia and/or hyperinsulinemia are not associated with sulpiride-induced obesity in female rats (63). These findings seem counterintuitive since these obese animals have significantly more body fat, which is strongly associated with insulin resistance. These effects on glucose tolerance were observed after 10 days of treatment, but appeared to be lost with longer sulpiride treatment (63). Of note, Ravussin has postulated that increased insulin sensitivity might be a causative factor in populations (e.g. Pima Indians) that are particularly susceptible to obesity and insulin resistance (114), which may partially explain the effect of sulpiride on body weight gain.
In contrast to the findings in females, chronic sulpiride (20 mg/kg) does not affect food intake or body weight gain in male rats, but does cause hyperglycemia, hyperinsulinemia and impairs oral glucose tolerance (77). Acute sulpiride does not elevate blood glucose in mice, suggesting that the effects observed in male rats are not immediate (115). Collectively, these findings imply increases and decreases in insulin sensitivity in female and male rats, respectively, are intriguing and warrant further investigation.

1.3.2.2 Risperidone

In contrast to sulpiride, chronic risperidone (0.5 mg/kg 16-21 days) does not appear to affect glycemia or glucose tolerance in male or female rats (81) or dogs (83). Unfortunately, the effects of chronic risperidone on either euglycemic-hyperinsulinemic or hyperglycemic clamp endpoints have not been determined in rats. Furthermore, euglycemic clamp studies in rats after acute treatment have yielded conflicting data with regard to potentially rapid effects of the drugs on insulin sensitivity. Houseknecht et al (116) administered a single dose of risperidone intravenously during the plateau, or ‘clamp phase’, of a euglycemic-hyperinsulinemic clamp, but no change in insulin sensitivity was observed; similar to a chronic (3 wk) clamp study in healthy volunteers (117) and chronic treatment in dogs (83). In contrast Remington’s group, using a seemingly identical protocol besides route of drug administration (subcutaneous instead of i.v.), reported that risperidone decreased insulin sensitivity within ~2h of drug injection (118). Consistent with the potentially rapid decrease in insulin sensitivity, another report showed that even a low dose of risperidone (2 mg/kg) produced hyperglycemia in mice within 30 minutes that persisted for up to 6h post-administration (115). The cause of these discrepancies are not known, thus further studies are needed to clarify these observations.
Even though risperidone may have acute effects on glycemia and potentially on insulin sensitivity, hyperglycemic clamps studies to examine glucose-stimulated insulin secretion have shown that acute or chronic risperidone treatment do not appear to affect insulin secretion in rats or dogs (83, 118); findings that are consistent with a short-term (3 wk) hyperglycemic clamp study in healthy volunteers (119).

1.3.2.3 Clozapine

Along with olanzapine, clozapine carries one of the biggest risks of weight gain and type II diabetes; however, few in vivo studies have examined its potential diabetogenic effects. This is likely because the effects of chronic clozapine in rats did not appear to model the common clinical endpoints of obesity and diabetes, as clozapine-treated rats lose weight from depressions in food intake (90). Clozapine, like risperidone, has been shown to acutely cause hyperglycemia (115), but this may be a short-lived effect since chronic studies have not demonstrated fasting hyperglycemia or hyperinsulinemia (87).

In contrast to its lack of effect on body weight gain, hyperinsulinemic-euglycemic clamp studies have reported that a single-dose of clozapine (1-10 mg/kg) can rapidly and dose-dependently decrease whole-body insulin sensitivity (116, 118), mostly due to hepatic insulin resistance. When given sub-chronically (10 mg/kg/day, 4 days), insulin resistance can be triggered by intravenous injection of the drugs during euglycemic clamp studies. The insulin resistance can be re-elicited by repeat drug injection during a euglycemic clamp for at least four days of dosing. The duration of this drug-induced insulin resistance is obviously less than 24h, since a repeat injection is required to elicit the insulin resistance. Nevertheless, these data showing acute development of insulin resistance in males may help explain the observation of weight gain-independent increased visceral adiposity in female rats (96). Whether or not these
same findings are present in clozapine-treated male rats, which also lack clozapine-induced hyperphagia and weight gain, is currently unknown. In addition to changes in insulin sensitivity, hyperglycemic clamp studies have also demonstrated that acute clozapine (10 mg/kg), also suppresses glucose-stimulated insulin secretion.

### 1.3.2.4 Olanzapine

Studies have recently begun examining the effects of chronic and acute olanzapine treatment on glycemia, insulin resistance and insulin secretion. Similar to sulpiride-treated female rats, blood glucose was not elevated following chronic olanzapine (2-8 mg/kg). In some studies, blood glucose has been reported to be lower in female olanzapine-treated animals (120). As with the other atypical antipsychotics, clamp studies after acute and chronic olanzapine treatment have been quite informative. Ader et al demonstrated that chronic olanzapine treatment (10 mg/kg, 6 wk) caused hepatic insulin resistance in dogs during a hyperinsulinemic-euglycemic clamp (83). Whole-body and hepatic insulin resistance was also present after chronic (4 week) olanzapine (2.5 or 7 mg/kg/day) in female rats (121). These effects may not necessarily be time-dependent, though, as a single-dose olanzapine (similar to clozapine and risperidone) also produced insulin resistance during euglycemic clamp studies in male rats (116, 122). Even though the findings of animal models suggest hepatic and/or peripheral insulin resistance following short-term (1-5 days) olanzapine treatment, clamp studies in healthy volunteers failed to show insulin resistance (117).

Findings from hyperglycemic clamp studies have not been consistent. Chronic treatment (4 week) in male rats with infused olanzapine (2.5 or 7 mg/kg) showed no changes in glucose-stimulated insulin secretion during a hyperglycemic clamp study (121), similar to findings in healthy volunteers (119). However, 6-week olanzapine treatment in dogs was shown to impair
insulin secretion as assessed by hyperglycemic clamp (83). Similar to euglycemic clamps, acute hyperglycemic clamps have been conducted (122) that show olanzapine appears to impair insulin secretion, which may be a short-lived effect compared with more chronic findings.

1.4 Summary

Determining the mechanism behind the obesity and diabetogenic side effects of the atypical antipsychotics warrants continued study, given the clinical and economic impact of this problem. The non-specific nature of these drugs has made it difficult to determine the targets of these metabolic side effects, although investigation of animal models gives the best chance at elucidating these effects, as they are likely centrally and/or hormonally mediated. From the literature reviewed there are several key comparisons between the atypical antipsychotics that suggest common effects that warrant further attention:

1) In the absence of weight gain, patients and healthy volunteers alike have experienced impaired glucose tolerance, insulin resistance and the development of type II diabetes. Given that risperidone, clozapine and olanzapine all appear to impair insulin resistance and/or acutely affect insulin secretion in animals suggests that similar mechanisms exist between animals and humans. Thus, identifying the molecular mediators of this rapid effect is necessary to understanding and identify short-term versus long-term drug effects.

2) The drug-induced hyperphagia and weight gain observed with sulpiride, risperidone, and olanzapine is apparently a sex-dependent effect. Whether or not such a sexual dimorphism exists clinically has not been systematically examined (123), thus the mechanistic basis for the sexually dimorphic hyperphagia in rats may contribute to antipsychotic-induced weight gain in humans. Further examination of this mechanism may lead to a better understanding the
biological basis for this sex-dependent phenomenon in rats, which could lead to better understanding of appetite and body weight regulation in humans.

3) Even though antipsychotics exhibit a sexually dimorphic effect on body weight gain, studies suggest that olanzapine and clozapine increase body fat in male and female rats. Thus, this drug effect is consistent between the sexes and whether the changes in body composition are applicable clinically should be further studied. Identifying the mechanism of these changes in body composition may underlie, at least in part, the biological basis for the human side effect.

4) Finally, one of the more interesting findings that is consistent across several studies, is that when female rats become obese after antipsychotic treatment (sulpiride, risperidone and olanzapine), they clearly do not respond with an expected increase in circulating leptin concentration. A similar effect may also present clinically (124). This could be due to biological variability in leptin concentration and/or insufficient sample size. However, an alternative explanation is that leptin may be important to the drug-induced hyperphagia observed in female rats. Given the importance of leptin in the long-term regulation of body weight, further studies should focus on direct and/or indirect effects on leptin and leptin-mediators.
Chapter 2

Materials and Methods

This chapter details the common materials and methods used in the completion of the dissertation research. Any deviation from these methods is detailed in the subsequent chapters.

2.1 Research Approvals

The research described within this dissertation utilized vertebrate animals as well as radioactive biological compounds. All animal protocols and housing facilities were reviewed and approved by the Institutional Animal Care and Use Committee of the Penn State College of Medicine prior to the conduction of the research. Experiments requiring the use of radioactive isotopes ($^3$H, $^{14}$C, $^{125}$I) were approved by the Milton S. Hershey Medical Center / Penn State Hershey College of Medicine Radiation Safety Committee prior to the conduction of the research.

2.2 Animal Subjects

Male (~200-225 g) and female (~220-225 g) Sprague-Dawley and Wistar rats were purchased from Charles River Laboratories (Cambridge, MA). Female C57Bl/6J and A/J mice (~6 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were maintained on a 12:12h light-dark cycle (Lights on from 0700 to 1900h) with food (Harlan-Teklad Rodent Chow, no. 2018 18% protein diet; Madison, WI) and water provided ad libitum. Animals were housed in groups of 4-5 unless 24h food intake was measured or in studies in which drug was administered in a food treat (described below). Prior to the start of individual
experiments, animals were routinely acclimated (~1 week) to the experimental conditions by
daily gavage and handling to reduce potential stress effects during individual experiments.
Experimental groups were matched for body weight in all experiments.

2.3 Surgical Procedures for Catheter Implantation

In some experiments, vascular access was needed for arterial blood sampling and/or
delivery of metabolic tracers for whole-body metabolic and tissue metabolite uptake studies.
Prior to these studies, animals were anesthetized using volatile isofluorane (4% induction, 1.5%
maintenance). Using aseptic surgical technique, sterile surgery was performed and a single
arterial polyethylene catheter (PE-50) for blood sampling was implanted in the left carotid artery
such that the tip lay in the aortic arch. A venous catheter (PE-50) with a silicon tip (silicon
tubing; ID: 0.51 mm, OD: 0.94 mm; Braintree Scientific) was inserted in the right jugular vein for
infusion of non-radioactive glucose and insulin. In some studies a second ‘hybrid’ venous
catheter (PE-50/PE-10) was inserted for uninterrupted infusion of 3-[3H]-glucose. At the
conclusion of the operation, catheters were filled with a heparinized saline solution (200 U/ml) to
prevent lumen thrombus formation and sealed with metal plugs. During postoperative recovery,
rats were given subcutaneous, 37°C saline (16 ml/kg) and intramuscular ceftriaxone (100 mg/kg).
At least 4 days recovery was allowed before conducting clamp experiments. At this time body
weight was greater than or within 5% of the pre-operative value. Animals not meeting this
criterion were not used in clamp experiments.
2.4 Drug Preparation and Administration

Olanzapine was provided as a generous gift from Neuland Laboratories Ltd (Ameerpet/Hyderabad, Andhra Pradesh, India) and Dr. Reddy’s Laboratories Ltd (Ameerpet/Hyderabad, Andhra Pradesh, India). Clozapine was purchased from Novartis Pharmaceuticals (East Hanover, NJ). Topiramate was purchased from Johnson & Johnson (Raritan, NJ) or obtained as a gift from Solvay Pharmaceuticals (Marietta, GA). Risperidone was purchased from Janssen-Cilag (Raritan, NJ), sulpiride from Sigma-Aldrich (St. Louis, MO), ziprasidone from Pfizer (New York, NY), aripiprazole from Bristol-Myers Squibb (New York, NY), and quetiapine from AstraZeneca (Wilmington, DE).

Chronic Drug Preparation and Administration. To reduce animal stress and the likelihood of adverse events associated with chronic drug administration via intraperitoneal injection or oral gavage, drug was chronically self-administered in a small quantity of chocolate chip cookie dough to animals trained to eat drug-free dough. Individual batches of dough were prepared separately from a pre-prepared dry mix. To prepare control dough, approximately 496g of Betty Crocker® Chocolate Chip Cookie Mix (General Mills, Inc., Minneapolis, MN) was mixed with 113g Imperial® Margarine (Unilever, Canada) and 61g Egg Beaters® egg substitute (ConAgra Foods Inc, Omaha, NE) per manufacturer’s directions. A 325-W Kitchen Aid mixer (Artisan) was used to complete all mixing. After the addition of wet ingredients, all batches of dough were prepared to a similar consistency by mixing batter on setting 2 until the chips broke up into 2-3 mm pieces (approximately 15-20 minutes). For drug-containing dough, drug(s) was added to the dry mix prior to the addition of wet ingredients and mixed thoroughly to ensure equal distribution. For rat studies, the initial drug concentration for olanzapine or clozapine was 0.2 mg/gram of dough; dough was provided at an amount of 2 g dough per 100 g of the previous day’s body weight. For mice, initial drug concentration was 0.1 mg/gram of dough; dough was
provided at a rate of 4 g per 100 g of the previous day’s body weight. Control animals received an equal quantity of drug-free dough. The drug-free (control) and drug-containing doughs were stored in airtight containers at 4°C for the duration of the experiment.

For chronic studies, cookie dough portions were provided in 1-oz. or ¾ oz “American” portion control cups (waxed paper) from Hoffmaster (Oshkosh, WI, stock nos. 67-100 & 67-075). Dough was administered between 16:00 and 18:00h daily, at which time body weight and 24-hour food intake were also measured. Rats and mice were first trained to eat the drug-free dough from the wax paper cups for 3-6 days prior to the introduction of the drug. During this time, all of the cookie dough was generally consumed within 30 minutes. After this training period, drug could be added to the dough without affecting its extent of consumption. Parts of the waxed paper cups were frequently destroyed by chewing or partially consumed.

The dose of olanzapine was determined from preliminary studies in which 4 mg/kg of olanzapine resulted in a general increase in body weight gain and hyperphagia in almost all drug-treated animals. Therefore, this dose of drug was used initially in chronic dosing experiments. A dosing regimen was designed from pilot studies to insure continued weight gain. Thus, olanzapine was self-administered at 4 mg/kg/day during days zero through six. On the seventh day of treatment, the dose was increased to 8 mg/kg/day. In longer experiments, the dosage was again increased on day 21 to 12 mg/kg/day, and then to 20 mg/kg/day on day 29 through the end of the study.

In chronic clozapine studies, drug was provided at 4 mg/kg for the first eight days of treatment. On the ninth day of administration, the dose was increased to 8 mg/kg. Beginning on day 13, animals received clozapine-containing dough (8 mg/kg) supplemented with olanzapine (4 mg/kg). In experiments where animals received olanzapine and topiramate concurrently, dough contained both drugs. The dosage of topiramate used was 100 mg/kg body weight.
**Acute Drug Preparation and Administration – Female Rats.** For acute experiments, clozapine or olanzapine solution was prepared for intraperitoneal (i.p.) injection or oral administration by gavage. Both drugs were first solubilized in 0.1N HCl. After adjusting pH to 5.5 with 0.01N NaOH, saline was added to reach the desired concentration. For control animals, a comparable pH-adjusted saline was prepared. In experiments with topiramate, which had to be prepared as an oral suspension, olanzapine (solution) and topiramate (suspension) were administered in a vehicle solution (2% polyethylene glycol in 1% carboxymethylcellulose, pH 5.5). Before undergoing the euglycemic clamp experiment, animals received two doses of olanzapine by oral gavage. For dosing via oral gavage, drug was solubilized in 0.1N HCl and pH was adjusted to 5.5 with 0.01N NaOH. For control animals, a comparable pH-adjusted solution was prepared. The first dose of olanzapine (4 mg/kg body weight) was given at approximately 1700h the day before the clamp experiment. The second dose (4 mg/kg body weight) was given at approximately 0800h on the morning of the experiment, two hours before the beginning of the euglycemic-hyperinsulinemic clamp.

In acute studies, animals received four doses of drug over a 28h period (i.p. or p.o.) with food and water provided ad libitum for the first 24 hours. The total daily doses were; olanzapine, 4 mg/kg/day; clozapine, 4 mg/kg/day; olanzapine plus topiramate, 4 mg/kg/day and 100 mg/kg/day, respectively. One-third of the total daily dose was administered on the first day between 08:00 and 09:00h. The other two-thirds dose was provided between 16:00 and 17:00h (food and water still provided ad libitum). The following day, one-third of the total daily dose was administered between 08:00 and 09:00h. At this point, animals were food deprived. Four hours later, the remaining two-thirds of total daily dose was administered. An oral glucose tolerance test was performed one hour after this final dosing (details provided below).

**Acute Drug Administration – Male Rats.** For acute drug administration, animals received two doses of drug over a two-day period by oral gavage (2 ml/kg). The first dose of olanzapine
or vehicle was administered before the dark cycle at approximately 1600h. Food and water were provided \textit{ad libitum} or animals were food restricted as indicated per figure. The following morning a second dose of drug was administered typically between 0700 and 0800h. The final drug or vehicle gavage was always given approximately two hours prior to the beginning of any experiment or blood/tissue sampling. The dose of olanzapine given in all acute experiments was 10 mg/kg unless otherwise specified, which was a dose of olanzapine that showed consistent elevations in blood glucose in pilot experiments.

\subsection*{2.5 Oral Glucose Tolerance Test (OGTT)}

Animals were food restricted for either 5h (Acute) or 14h (Chronic). Acutely exposed animals received drug according to the acute dosing regimen (see above) while chronically exposed animals received half the daily dose of drug (6 mg/kg) one hour prior to the start of the OGTT. During the OGTT, basal blood samples and glucose measurements were obtained and then glucose was given via oral gavage. In female studies, chronic and acutely treated animals received 2.5 g glucose/kg via oral gavage. In male studies, acutely treated animals received 2.5 g glucose/kg, while chronically treated animals received 1.5 g glucose/kg. The different amounts of glucose used in acute vs. chronic OGTTs were based on pilot experiments that showed these amounts adequately raised blood glucose concentration in age-matched male and female rats. Blood samples were collected in Microvette® 500 Potassium-EDTA coated or Microvette® 300 lithium-heparin coated sample tubes (Sarstedt Inc, Newton, NC). Samples were then centrifuged at 1,800x g for 10 min at 4°C; plasma was separated and frozen at -80°C for further analysis.
2.6 Insulin Tolerance Test (ITT)

Animals were food restricted for either 5h (Acute) or 14h (Chronic). Olanzapine or vehicle solution was given according to the acute or chronic dosing schemes. Two hours after the final drug/vehicle gavage, baseline blood samples were collected via tail vein and animals received an intraperitoneal (i.p.) injection of insulin (0.75 U/kg, Humulin-R, Lilly; Indianapolis, IN). Blood glucose was measured every 30 minutes for two hours and data expressed as percent change from the baseline (Time 0) glucose concentration for each animal. The inverse area-under-the-curve (ITT-AUC) was calculated as the absolute value of the negative area under the curve, as the blood glucose is decreased following insulin injection. Thus, greater insulin sensitivity is observed as a larger change from baseline glucose and higher inverse area under the curve.

2.7 Oral Lipid Tolerance Test (OLTT)

Weight-matched animals were food-restricted for 14h prior to the start of the OLTT. After collection of baseline blood samples, olive oil (Giant®; Carlisle, PA) was administered by oral gavage (6 ml/kg). Blood samples were collected via a tail snip using a sterile scalpel. A bandage covered the snipped tail between blood samplings at 1, 2, 4 and 6 hours post-gavage. Samples were centrifuged at 1,800 x g for 10 min at 4°C; plasma was collected and frozen at -80°C for further analysis.

2.8 Body Composition Analysis

Longitudinal changes in body composition were tracked non-invasively in conscious animals using a ¹H-NMR analyzer (Bruker LF90 proton-NMR Minispec; Bruker Optics; The
Woodlands, TX) for rapid determination of total body lean, adipose tissue, and paracellular fluid masses and then returned to their respective cages. Initially, groups were matched for total body adiposity and lean body mass (week 0) and then subsequent measurements were made weekly on days 7, 14, 21, 28 and 35.

2.9 Locomotor Activity and Energy Expenditure

Locomotor activity was measured using infrared technology (OPT-M3, Columbus Instruments). Activity in all three dimensions was measured by counting of three-dimensional infrared beam breaks (X total, X ambulatory or ‘y’, and Z) were measured for 24h. Energy expenditure was measured concurrently using indirect calorimetry (Oxymax, Columbus Instruments). Constant airflow (~8 ml/g/min) was drawn through the chamber and monitored by a mass-sensitive flow meter. O₂ and CO₂ concentrations were measured at the inlet and outlet of sealed chambers to calculate O₂ consumption and CO₂ expiration. Each chamber was measured for 1 minute at 15-minute intervals. Animals were acclimated to the specialized calorimetry cages and had *ad libitum* access to food and water throughout the 24h period.

2.10 Hyperinsulinemic-Euglycemic Clamp

All hyperinsulinemic-euglycemic clamp experiments were performed at the same time in the morning on conscious, unrestrained animals after 14h of food restriction to ensure animals were in the post-absorptive state. On the evening prior to clamp experiments, animals received either vehicle or olanzapine (10 mg/kg) via oral gavage between 1700 and 1800h and then food-restricted. The following morning animals received another gavage of vehicle or olanzapine (10 mg/kg) and then placed in small, dark cages for the clamp experiment. During the basal period (t
= -120 to 0 min), saline was infused through the venous catheter and a tracer amount of tritiated glucose (3-[\textsuperscript{3}H]-glucose; Perkin-Elmer, Waltham, MA) was infused as a primed-continuous infusion (10 µCi bolus, 0.2 µCi/min) through the hybrid venous catheter for measurement of basal hepatic glucose output. At time zero (t = 0), a primed-continuous insulin infusion (75 mU/kg bolus, 1 mU/kg/min, 0.3% BSA) was started and glucose (20% dextrose) was co-infused to maintain euglycemia (~100 mg/dl). Blood glucose concentration was monitored every 10-15 minutes. Additionally, the rate of tritiated glucose infusion was increased to 0.4 µCi/min to maintain a constant specific activity for the measurement of insulin-stimulated glucose kinetics. Blood samples (< 250 µl) were collected at -20, 0, 60, 120, 160 and 180 minutes for measurement of insulin and specific activity of plasma glucose.

2.11 Synthesis of \textsuperscript{125}I-BMIPP for FFA Uptake Studies

Radioisotopic beta-methyl-iodo-phenyl-pentadecanoic acid ([\textsuperscript{125}I]-BMIPP) was prepared by heating non-radioactive BMIPP (Molecular Insight, Cambridge, MA) and sodium [\textsuperscript{125}I] iodide in propionic acid at 180ºC in the presence of a copper catalyst to produce an exchange of radioactive \textsuperscript{125}I for stable iodine on the BMIPP molecule. Upon completion of the exchange reaction, the resulting product was reconstituted in ethanol/water and passed through a C18 Sep Pak cartridge to separate copper, propionic acid, and free \textsuperscript{125}I from the resulting [\textsuperscript{125}I]-BMIPP. The cartridge was washed with 20 ml of water, followed by incremental washings of 60% and 70% ethanol. The active product was eluted with 100% ethanol. The results demonstrated an average radiochemical purity of 97% (n = 2) and a radiochemical yield of 35%. The HPLC analysis, using a C18 reverse phase column, demonstrated no degradation of the radiochemical product for up to 5 days at room temperature.
2.12 FFA and Glucose Uptake

Tissue specific FFA uptake was measured using the non-metabolizable fatty acid analog, [\(^{125}\)I]-BMIPP, as previously described and validated (125). Glucose uptake was measured in parallel using [\(^3\)H]-2-deoxyglucose. Four days after catheter implantation animals were dosed with olanzapine or vehicle according to the acute dosing protocol (see above). At approximately 0700h, tracer amounts of [\(^{125}\)I]-BMIPP (10 µCi) and [\(^3\)H]-2-deoxyglucose (5 µCi) were given as an intravenous (i.v.) bolus (t = 0 min). Serial blood samples (~250 µl) were collected (t = 2, 5, 10, 20, 30, 40) to determine the AUC for plasma [\(^{125}\)I]-BMIPP and [\(^3\)H]-2-deoxyglucose during the 40 minute \textit{in vivo} labeling period. After final blood samples were collected (t = 40 minutes), animals were euthanized and tissues (i.e. gastrocnemius, soleus, heart, liver, kidney, proximal duodenum, terminal ileum, skin, adipose, brain) collected and freeze-clamped in liquid nitrogen. Total [\(^{125}\)I]-BMIPP activity was measured in plasma (25 µl) and whole-tissue (~100 mg) for calculation of FFA metabolic rate (R_f). Tissue and plasma [\(^{125}\)I] radioactivity was counted using a gamma counter (Beckman Coulter; Fullerton, CA). For calculation of glucose metabolic rate (R_g), frozen tissue was pulverized and homogenized in ice-cold 0.5 N perchloric acid (0.4 ml/100 mg tissue) and centrifuged at 3,000 x g for 15 minutes. The supernatant was collected and neutralized with an equal molar amount of potassium hydroxide and assayed for total [\(^3\)H] radioactivity using liquid scintillation (Beckman Coulter; Fullerton, CA). The [\(^3\)H] radioactivity in these samples represents the total counts from both the 2-deoxyglucose and the phosphorylated 2-deoxyglucose present in the tissue sample. An aliquot of the neutralized extract was subjected to Somogyi extraction to remove the non-phosphorylated 2-deoxyglucose and then counted. Thus, total counts of phosphorylated 2-deoxyglucose were calculated as the total counts minus the counts remaining after Somogyi extraction.
2.13 Tissue Glucose Uptake

A tracer amount of 1-[^14]C]-2-deoxyglucose (8 µCi; MP Biochemicals, Irvine, CA) was given as an intravenous bolus during the steady-state phase (t = 140 min) of the hyperinsulinemic-euglycemic clamp for the determination of the in vivo rate of glucose uptake, or tracer was alternatively given as a bolus in 14h food-restricted or ad libitum fed animals. Serial blood samples (< 250 µl) were collected (t = 142, 145, 150, 160, 170, 180) to determine the area under the curve (AUC) of plasma 2-deoxyglucose activity during the final 40 minutes of the experiment, or similar in vivo labeling periods in food-restricted or fed animals. At the end of the clamp (t = 180 min), animals were euthanized and tissues quickly excised and immediately freeze-clamped in liquid nitrogen. Frozen tissues and plasma samples were processed and counted for radioactivity as described above.

2.14 Heart Rate, Blood Pressure, and Echocardiography

Heart rate and mean arterial blood pressure were measured approximately two hours after olanzapine gavage (10 mg/kg) in conscious, unrestrained animals by way of a pressure transducer (Grass Analyzer, Model 79E; Quincy, MA) within an arterial catheter. The catheter was implanted in the left carotid artery four days prior to the study and inserted so that the tip rested in the aortic arch.

Following measurement of heart rate and blood pressure, heart function was assessed by echocardiography using a Sequoia C256 Echocardiography System (Siemens Medical Solutions, Mountain View, CA) equipped with a 7.5-MHz transducer, as previously described (126, 127). Rats were lightly anesthetized by intraperitoneal injection of ketamine (40 mg/kg) + acepromazine (1 mg/kg), and body temperature was maintained during the procedure by
placement of rats on a heating pad. The transducer was placed on the thorax, and M-mode recordings were performed by direction of the ultrasound beam at the midpapillary muscle level. Endpoints were obtained after well-defined, continuous interfaces of the septal and posterior walls were visualized. The operator was blinded to the experimental condition (olanzapine or control), and measurements from three to four consecutive cardiac cycles were averaged for all animals.

2.15 Calculations

During euglycemic clamp studies the glucose rates of appearance (Ra) and disappearance (Rd) were calculated using the isotope dilution method (128). Briefly, glucose turnover rate was calculated as the ratio of the rate of [3H]-glucose infusion (dpm/min) to the glucose specific activity (dpm/mg). Hepatic glucose output (HGO) in the basal state was defined as the tracer determined rate of glucose turnover. During the steady-state, insulin-stimulated portion of the clamp experiment (t = 120 to 180 min) the rates of glucose appearance and disappearance are equal and thus the residual hepatic glucose output during the clamp equals the total glucose turnover minus the exogenous glucose infusion rate (GIR).

The glucose metabolic rate, Rg (µmol/g tissue/min) and FFA metabolic rate, Rf (nmol/g tissue/min) for selected tissues was calculated using the general equation for flux through a metabolic pathway (129-131). The glucose metabolic rate, Rg, was calculated by taking the total counts of phosphorylated [14C]-2-deoxyglucose and dividing by the AUC of [14C]-2-deoxyglucose during the 40 minute in vivo labeling period and multiplying by the mean plasma glucose concentration (132, 133). Similarly, the FFA metabolic rate, Rf, was calculated by taking the total tissue counts of [125I]-BMIPP and dividing by the AUC of [125I]-BMIPP during the 40 minute in vivo labeling period and multiplying by the mean plasma FFA concentration (125).
Under non-euglycemic clamp conditions, tissue metabolic clearance rate for glucose was calculated by dividing the calculated $R_g$ by the plasma glucose concentration.

In some chronic feeding studies ‘Food-Conversion Efficiency’ was calculated as the ‘change in body weight’ divided by the ‘cumulative food intake’ (134). For insulin tolerance tests, the inverse area-under-the-curve (ITT-AUC) was calculated as the absolute value of the negative area under the curve from baseline glucose, as blood glucose normally decreases following insulin injection.

In studies examining cardiac structure and function, cardiac minute work (dyne-cm/min) was calculated as the product of pulse (beats/minute), stroke volume ($cm^3$), and arterial pressure (mmHg) using the conversion factor of 1 mmHg = 1332 dyne/cm². An index of cardiac energy expenditure, the ‘double-product’, was calculated as the product of pulse (beats/min) and arterial pressure (mmHg).

2.16 Isoproterenol Challenge Test

To gauge adipose tissue lipolytic capacity an isoproterenol challenge test was performed. Isoproterenol (Isuprel®; 20%) was purchased from Abbott Laboratories (Abbott Park, Illinois) and diluted in sterile normal saline immediately before use. Animals were given ad libitum access to food and water and received the final vehicle or olanzapine gavage 2h prior to the start of the challenge test. After collection of basal blood samples, an i.p. injection (2 ml/kg) of isoproterenol (0.05 mg/kg) was given to vehicle- and olanzapine-treated animals. Serial blood samples were collected at 30-minute intervals for two hours to measure blood glucose and to prepare plasma by centrifugation for FFA and glycerol analysis.
2.17 Hepatic VLDL-Triglyceride Secretion

To measure the rate of hepatic very low density lipoprotein (VLDL)-triglyceride secretion, tyloxapol (600 mg/kg) was given intravenously in 14h food-restricted animals as a 20% (w/v) solution. This dose of tyloxapol inhibits lipoprotein lipase for at least two hours (135). With inhibition of lipoprotein lipase, triglycerides cannot be cleared from the circulation and the increase in plasma triglyceride concentration over time is solely due to hepatic secretion in animals in the post-absorptive state. Baseline blood samples were collected for measurement of triglycerides and subsequent blood samples collected at t = 15, 30, 60, and 90 minutes post-injection to measure plasma triglycerides. Rates of hepatic triglyceride secretion were calculated based on the slope of the linear increase in plasma triglyceride concentration between 30 and 90 minutes, assuming a plasma volume of 3.5% (136, 137).

2.18 Human Acetyl-CoA Carboxylase (ACC) Assays

*In vitro* IC$_{50}$ values for the human ACC1 and ACC2 isoforms (hACC1 and hACC2) were determined using recombinant enzyme expressed in a baculovirus system. Full-length versions of the two cDNAs were first cloned into pET30a (Novagen, Madison, WI). The cDNAs along with the in-frame 3’-his tag sequences from the pET vector were then PCR amplified and TOPO-cloned into the Gateway® entry vector pENTR/D-TOPO. To remove mitochondrial targeting sequences, the ACC2 primers were designed to delete amino acids 1-148 and add Met-Gly in front of Lys149 in the final product. The resulting entry clones were transferred to BaculoDirect™ linear DNA (Invitrogen corp.) using the Gateway® LR recombination reaction. The recombinant baculovirus DNA was transfected into insect cells and viral amplification was performed according to the manufacturer’s protocols. For protein expression sf9 cells were
infected with a P3 viral stock in the presence of 50 µM biotin. The cells were harvested after 48-72 hr, lysed by sonication, and the resulting extract was clarified by centrifugation. ACC in the crude extract was concentrated by ammonium sulfate precipitation (40% w/v) and purified by Ni²⁺-NTA chromatography (Novagen) followed by anion exchange chromatography on a UNO-Q column (BioRad).

Overall ACC activity in the hACC preparations was measured by following incorporation of [¹⁴C]-bicarbonate into acetyl-CoA essentially as described (138). Briefly, the reaction mixture (50 µl) contained 50 mM HEPES (pH 7.4), 20 mM K-Citrate, 20 mM Mg-Acetate 1.5 mM MgSO₄, 2 mM DTT, 2 mM acetyl-CoA, 12 mM NaHCO₃, 0.2 mM NaH¹⁴CO₃ (~50 mCi/mmol), 0.01% (v/v) Triton X-100, 0.75 mg/ml BSA, 1% (v/v) DMSO (with or without inhibitor), and 0.3 µg (hACC1) or 0.15 µg (hACC2) enzyme. The known ACC inhibitors Soraphen A and CP-640186 were used as positive controls in all assays. Reactions were initiated by the addition of ATP to a final concentration 2 mM. After 10 minutes at 37°C, the reaction was stopped by the addition of 50 µl 2N HCl. The samples were evaporated in a hood overnight, resuspended in 200 µl H₂O, and incorporated label was quantified by scintillation counting in 3 ml scintillation cocktail. Inhibition data were fit to dose-response curves and IC₅₀ values are reported (i.e. the concentration of inhibitor that gives 50% inhibition).

2.19 Western Blotting

Briefly, frozen powdered tissues were prepared in a 1:4 ratio of ice-cold homogenization buffer (20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM NaF, 100 mM KCl, 0.2 mM EDTA, 50 mM β-glycerophosphate, 1 mM DTT, 0.1 mM PMSF, 1 mM benzamidine, and 0.5 mM sodium vanadate, 0.4% 3-([3-cholamidopropyl] dimethylammonio)-1-propanesulfonate (CHAPS), 1 M microcystin LR and 1% Triton X-100) with a Polytron homogenizer and centrifuged at 10,000
rpm for 10 min. A Bio-Rad protein assay was performed to determine the protein concentration of the samples. The samples were normalized in homogenization buffer. The supernatant was aliquoted into microcentrifuge tubes, and 2x sample buffer (2 ml of 1M Tris, pH 6.8, 4 ml of glycerol, 4 ml of 10% SDS, 0.4 ml of β-mercaptoethanol, 0.32 g of bromophenol blue, and 5.6 ml of water to a final volume of 16 ml) was added in a 1:1 ratio. The samples were boiled for 5 min and cooled before being used for Western blot analysis. Samples were then subjected to electrophoresis on 10% polyacrylamide gel and then proteins were electrophoretically transferred to PVDF membranes. The resulting blots were blocked with 5% nonfat dry milk for 1 hour. Blots were incubated with one of the following antibodies: total AMPK (no. 2532), phospho-specific AMPKα antibody (Thr 172; no. 2531), total AKT (no. 4685), phospho-specific AKT (Ser 473; no. 9271, or Thr 308; no. 9275), total ACC (no. 3662) all obtained from Cell Signaling Technology (Beverly, MA), phospho-specific ACC (Ser 79; no. 07-303, Upstate), phospho-specific PDHe1-alpha (Ser 293; no. NB110-93479 from Novus Biologicals), or total PDHe1-alpha (no. 459400 from Invitrogen). All blots were washed with Tris-buffered saline (TBS)-T (1x TBS including 0.1% Tween-20) and incubated with secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit) at room temperature for 1h. The blots were developed with enhanced chemiluminescence (ECL) Western blotting reagents as per the manufacturer’s (Amersham) instructions. Blots were exposed to X-ray film in a cassette equipped with a DuPont Lightning Plus intensifying screen. After development, the film was scanned (Microtek ScanMaker IV) and analyzed using NIH Image 1.6 software.

2.20 Clinical Chemistry, Metabolite and Hormone Measurements

Glucose concentrations from whole blood were measured using One Touch Ultra® glucometers (Lifescan Technologies, Milpitas, CA) or Ascensia® Contour Blood glucometers
(Bayer Healthcare LLC; Mishawaka, IN). These meters were chosen because of the small sample size required (~0.6 µl) and because they correct for differences in hematocrit which may confound data analysis. Glucose specific activity was determined on plasma samples deproteinized with equal molar volumes of barium hydroxide and zinc sulfate (Somogyi Extraction).

Alanine aminotransferase, aspartate aminotransferase, albumin, very low-density lipoprotein cholesterol, total cholesterol, triglycerides, and lactate were determined using the DT60 II and DTSc II modules of the Vitros™ Chemistry System (Ortho-Clinical Diagnostics, Rochester, NY). The following plasma metabolite concentrations were made using colorimetric assays: plasma glucose (Thermo Scientific, Waltham, MA), FFAs (Wako Diagnostics, Richmond, VA), glycerol (Cayman Chemical, Ann Arbor, MI) and beta-hydroxybutyrate (Stanbio Laboratory, Boerne, TX). Branched-chain amino acid and plasma alanine concentrations were measured using enzyme-linked reactions (139). A commercially available RIA was used to measure plasma concentrations of leptin and active ghrelin (Linco Research, St. Charles, MO). Plasma prolactin concentration was measured by the National Hormone and Peptide Program (Harbor–UCLA Medical Center, Torrance, CA).

2.21 Assessment of Central Dopamine Receptors and Liver Histology

At the conclusion of most chronic studies, peripheral tissues were removed for morphometry and histology, whereas brains were saved for radioligand binding studies. After decapitation, trunk blood was collected, peripheral tissues were weighed and, in some cases, formalin fixed for subsequent histological analysis by standard hematoxylin and eosin staining. Also at this time, brains were immediately removed and immersed in -40°C isopentane (2-methylbutane) and stored at -80°C. Brains were sectioned on a cryostat in the coronal plane at 20
µm and thaw-mounted on poly-lysine coated slides. In this particular experiment, the brain regions examined were from the dorsal and ventral striatum (1.7 to 1.1 mm from Bregma; inclusive of the NAcc and dorsal striatum) and midbrain region (-5.6 to -6.1 mm from Bregma; inclusive of the ventral tegmental area, VTA rostral and caudal, and the substantia nigra pars compacta, SNpc). Four sections from each brain region were serially mounted on a slide. Approximately 8 slides were taken from each brain region (representing an approximate distance of 160 µm between sections). One slide from each brain region was subject to cresyl violet staining to accurately determine anterior-posterior anatomical coordinates for proper analytical comparisons.

2.22 Autoradiography of D2 Receptors

Slide-mounted matched sections were removed from -80°C freezer storage and brain sections were allowed to thaw for at least 3 minutes at room temperature (RT). Following thawing, sections were air dried for 3 minutes, pre-incubated for 15 minutes in 50 mM Tris HCl (pH 7.4), 120 mM NaCl at RT followed by an incubation for 30 minutes at RT in 50 mM Tris HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 2 mM CaCl\(_2\), 1 mM MgCl\(_2\) and a 0.6 nM concentration of \([^{125}\text{I}]-\)iodosulpride (Amersham Biosciences/ GE Healthcare, Piscataway, NJ; 2000 Ci/mmol). This radioligand has a K\(_d\) of 0.6 nM and 1.2 nM for rat D2 and D3 receptors, respectively (140). The concentration of iodosulpride was determined previously by running a series of test sections at stepped concentrations bracketing a suggested concentration of 0.3 nM. Slides were rinsed 2 times for 5 minutes, each time in ice-cold 50 mM Tris-HCl (pH 7.4) buffer and placed before a stream of air at RT to dry for several hours. Non-specific binding was determined on a set of 'test' sections incubated in a parallel manner as described above with the addition of a 50 µM concentration of (+)-apomorphine to the \([^{125}\text{I}]-\)iodosulpride incubation solution. Subsequently, the
slides were placed in a cassette with \[^{125}\text{I} \] microscale standards (Amersham, Arlington Heights, IL) to expose Kodak X-OMAT AR film (Eastman Kodak, Rochester, NY) for 18h (141). Using the developed film, receptor density was determined semi-quantitatively by a densitometry procedure using the microscales to generate a standard curve via computer software provided with an Analytical Imaging Station (Imaging Research Inc. St. Catherines, Ontario). Binding was assessed for each target region unilaterally in all tissue. Both background density (as sampled from the corpus callosum) and nonspecific binding density were subtracted from all assayed tissue.

### 2.23 Statistical Analysis

For all results, data are expressed as the mean ± standard error of the mean. To calculate statistical significance (P<0.05), a two-tailed Student’s t-test or one-way analysis of variation (ANOVA) with or without repeated measures and Bonferroni’s multiple comparison post-test was used where appropriate. Asterisks indicate a particular level of statistical significance (***P<0.001, **P<0.01, *P<0.05). Sample sizes for each experiment are included in the respective figure legends. All statistical analyses and data manipulations were made using GraphPad Prism or InStat computer software (GraphPad Software, San Diego, CA), or Statistica 6.0 software (Statistica, Tulsa, OK).

For D2 receptor autoradiography studies, absolute \[^{125}\text{I} \]-iodosulpride binding was expressed as fmol/mg of tissue equivalent ± S.E. Binding for the Olanzapine group was also expressed as percentage of the binding of the Saline group ± S.E., percent binding. The assessed brain regions were the following: the core and the shell of the nucleus accumbens (NAcc core, NAcc shell) representing the terminal regions of the mesoaccumbens dopamine projections, the dorsolateral aspect of the striatum (dorsolateral striatum or DLS) that receives exclusive
projections from the nigrostriatal dopaminergic neurons, and the somatodendritic regions of both systems respectively: the rostral and caudal ventral tegmental area of the brain stem (rostral and ventral VTA) and the SNpc. Results for absolute specific binding and percent specific binding were analyzed by separate two-way analyses of variance (ANOVA; treatment versus structure) and post hoc LSD tests were made where applicable. Statistical significance regarded at P<0.05.
Chapter 3

Lowered plasma glucose and leptin precede hyperphagia in a model of atypical antipsychotic-induced obesity

3.1 INTRODUCTION

A number of drugs exhibit unexpected effects related to body weight changes in humans. Determining the mechanism of these side effects might lead to new targets for the treatment of obesity and overweight. In addition, such research may reveal ways to design new drugs with reduced side effects. Well-known examples of these side effects include the unexpected changes in body weight experienced by patients taking the anti-seizure drug topiramate (weight loss), or atypical antipsychotics olanzapine and clozapine (weight gain).

Atypical antipsychotic drugs are potent antagonists with a higher affinity for 5HT2A than D2 receptors (142), they also inhibit the G-protein coupled receptors for other several biogenic amines including cholinergic, adrenergic and histaminergic as well as other dopaminergic receptors (for reviews see 143, 144, 145). Typical antipsychotic drugs on the other hand, like haloperidol, act as high affinity antagonists for dopamine D2-like receptors (D2, D3 and D4 receptors) with a consequence of extrapyramidal side effects, whereas atypical antipsychotics, like olanzapine and clozapine, generally have lower incidences. Both typical and atypical antipsychotics are principally used to treat schizophrenia and other psychoses (40, 146). The atypical antipsychotics tend to cause weight gain as a side effect in some but not all individuals, suggesting a genetic predisposition (for reviews see 147, 148, 149). Even those patients who do not become obese frequently report increases in appetite and meal-size (150, 151). Thus,

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1 This chapter has been modified and reprinted with permission. Original publication: Hormonal and metabolic effects of olanzapine and clozapine related to body weight in rodents. Albaugh et al 2006 Obesity (Silver Spring) 14:36-51.
hyperphagia is associated with this side effect in humans. Accordingly, it has been demonstrated that olanzapine induced weight-gain is caused by an increase in caloric intake, rather than alterations in basal energy expenditure (51). It is believed that olanzapine stimulates appetite through serotonergic or histaminergic mechanisms in the lateral hypothalamus (38). However, the exact mechanism of olanzapine-induced appetite stimulation is unknown. Patients with olanzapine-induced obesity have elevated plasma concentrations of the adiposity signaling hormone, leptin (e.g. 124, 152). This is not surprising, though, given their increased adipose mass. On the other hand, inconsistent changes have been reported in the serum concentrations of the appetite stimulating hormone ghrelin in olanzapine treated patients (151, 153).

In the following chapter, we describe the characterization of a new animal model of atypical antipsychotic-induced obesity where the drug is self-administered to help reduce handling stress and potential adverse events associated with chronic drug administration.

In addition to characterizing peripheral changes in the model, we have investigated central dopaminergic pathways for three reasons. First, dopamine has been considered as a neural substrate for reward. D2 receptors serve as both presynaptic and postsynaptic receptors. Thus, alteration in D2 receptor availability is a sensitive indicator of altered DA function in general. Secondly, there is accumulating evidence in support of the notion (53, 54) that decreased concentrations of D2 dopamine receptors predispose subjects to search for reinforcers; specifically in the case of drug-addicted subjects for drug and in the case of obese subjects for food as a means to temporarily compensate for decreased sensitivity of DA D2 regulated reward circuits. Third, atypical antipsychotics are D2 receptor antagonists, which in turn may alter functioning of the reward circuit. For example, natural rewards including palatable food, such as sugars, increase dopamine in the NAcc (154). Importantly, this effect can be augmented by food restriction (155) indicating integration between peripheral physiological factors (i.e., metabolic and hormonal signals) and dopaminergic mechanism within the brain. To control for this effect,
it is important to assess D2 receptor binding within the reward-related areas (e.g., nucleus accumbens). To investigate the role of these dopamine receptors in the olanzapine-induced obesity, we measured D2 (D2/D3) receptor density in the dorsal and ventral striatum (representative terminal fields of the motor and motivational system, respectively) in parallel with development of olanzapine-induced weight gain in the rat.

As mentioned earlier, topiramate is a popular anti-seizure agent that exhibits an unexpected weight loss side effect, particularly in obese and overweight patients (e.g. 156, 157-160). Its mechanism for anti-seizure activity has never been firmly established, as is the case for its weight loss side effect. Weight loss from topiramate appears to be most rapid/effective in the forms of obesity caused by olanzapine and clozapine (161-166). Thus, animal models reproducing this effect are potentially valuable in elucidating the mechanisms of both weight loss and weight gain. Therefore we have also examined the effects of topiramate on our rat model of olanzapine-induced obesity. The effects of topiramate, together with other aspects of the obesity (e.g. hyperphagia) observed in the animal model, have parallels in the human side effect. These findings suggest this model may not only be relevant for study of the human effect, but the model may also be a valuable, rapid and inexpensive approach for screening potential anti-obesity drugs.

Lastly, to address the mechanism of this side effect, we also examined the acute metabolic effects of olanzapine. We posited that changes in endocrine or metabolic factors might precede the onset of hyperphagia and thereby help explain it. Our results show that olanzapine brings about early changes in the plasma concentrations of the satiety hormone leptin and other factors recognized to impact appetite, including plasma glucose and insulin concentrations as well as the response to a glucose challenge. These effects readily explain the hyperphagia in this model that in turn causes weight gain.
3.2 RESULTS

3.2.1 Effects of Chronic Olanzapine or Clozapine on Body Weight Gain and Food Intake

We sought to develop and characterize an animal model of the obesity side effect of atypical antipsychotic drugs frequently observed in humans. Different strains of rats and mice were initially tested with olanzapine. C57Bl/6J or A/J mice were chosen for two reasons. First, they exhibit a different susceptibility to diet-induced obesity. Second, consomic strains of these animals are available that can be used to address weight gain mechanisms using genetic approaches. Olanzapine, though, did not increase body weight in either strain (Fig 3-1). In contrast, A/J mice displayed a non-significant trend of weight loss (Fig 3-1A), along with a non-significant trend for decreased food intake (data not shown).

Figure 3-1: Chronic, self-administration of olanzapine in female mice. (A) A/J and (B) C57Bl/6J mice were provided olanzapine (4 mg/kg) daily in a small amount of cookie dough (Olanzapine) or cookie dough alone (Control), as indicated. On day 10, the dosage was increased to 8 mg/kg. Body weight was monitored daily for the duration of the experiment (n = 10). Data are represented as the mean body weight ± S.E.
Similarly, no effect was observed after chronic administration of either clozapine or olanzapine on body weight in male rats (data not shown). In contrast, self-administered olanzapine increased body weight gain in female Wistar (data not shown) and Sprague-Dawley rats (Fig 3-2A). These findings are in agreement with several previous studies showing effects of olanzapine in female but not male rats (97, 101, 167, 168). The increase in body weight was coupled with an increase in average daily food intake (Fig 3-2B). The food intake became significant only after the first 24h. In the experiment shown in Figure 3-2, there was an apparent trend for an increase in food intake in the first 24h, but this was not always observed and was not statistically significant in any experiment. To compensate for a tendency of drug effects to level off after 7-10 days, a dosing paradigm was designed and used wherein the dose was increased as

**Figure 3-2:** Effect of chronic olanzapine exposure on body weight and food intake in female rats. Sprague-Dawley rats were matched for body weight and then trained to eat cookie dough as indicated in Chapter 2, “Materials and Methods” (Day 0 body weights not significantly different: Control, 246 ± 3; Olanzapine, 246 ± 3). Cookie dough ± olanzapine (4 mg/kg) was then self-administered daily starting at Day 0. The dose of olanzapine was increased at days 7, 21, and 29 to 8, 12, and 20 mg/kg, respectively. (A) Body weight and (B) food intake were monitored daily. Data represent the mean ± S.E. (n = 12). Change in body weight significantly different (*P<0.05) between days 3 and 29.
described in the methods. When drug concentrations were increased, increased food intake was generally maintained but not increased (Fig 3-2B). A sedating effect was evident after the dose was increased to 20 mg/kg on day 29; this sedation may explain the decreased daily food intake in the olanzapine group observed after that dose was administered (Fig 3-2B). Accordingly, animals receiving this amount of olanzapine were noticeably less active and unresponsive which is presumed to have led to the decreases in daily food intake at this dosage. Since the weight gain effect of olanzapine did eventually plateau, even with ramped dosing, other experiments were terminated earlier than 20 days as indicated.

**Figure 3-3:** Clozapine lacks the body weight gain side effect in rats. Female, Sprague-Dawley rats were matched for body weight and then trained to eat cookie dough. Day 0 body weights were not significantly different (Con: 256 ± 4 vs. Clz/Olz: 259 ± 4 g). Animals began self-administration of clozapine-containing dough (4 mg/kg) or cookie dough alone, as indicated. The dose of clozapine was increased to 8 mg/kg on Day 8. Starting with Day 13 (indicated by arrow), animals receiving clozapine-containing dough were given dough supplemented with olanzapine (4 mg/kg). Body weights were monitored daily, and the change in weight from day zero is expressed as the mean ± S.E. (n = 12). Change in body weight differed significantly between groups from Day 17 through the end of the study (P<0.05).

When female animals were provided with clozapine-containing dough, there was no statistically significant effect on body weight (Fig 3-3, days 0-12). Interestingly, when clozapine-containing dough was replaced with dough containing both olanzapine and clozapine, weight gain was observed (Fig 3-3, days 13-27).
3.2.2 Effect of Chronic Olanzapine on OGTT

Several oral glucose tolerance tests were performed after chronic olanzapine administration (i.e. 13 days). Olanzapine treated animals displayed a trend for lower, rather than higher, blood glucose values compared to control animals at all time points (Fig 3-4A). While these trends were not significantly different, they were nevertheless unexpected given the adiposity (see below) and increased food intake (Fig 3-2B) in the olanzapine group; i.e., elevated glucoses were expected. In spite of this observation, the plasma insulin concentrations in the olanzapine group were significantly higher at several time points reflective of increased insulin resistance (Fig 3-4B). Accordingly, the olanzapine group displayed a significantly lower

Figure 3-4: Glucose tolerance after chronic olanzapine administration. Female rats were food-restricted for 14h and then challenged with an OGTT. A fasting blood glucose measurement was taken at time zero, and glucose was administered by oral gavage immediately afterward. (A) Blood glucose measurements were taken at 30-min intervals for 2h post-glucose gavage. (B) Corresponding insulin concentrations were measured for each time point. Data represent the mean ± S.E. (n = 24). Asterisks indicate significant differences from time-matched controls (*P<0.05).
quantitative insulin sensitivity check index [QUICKI (169); Con: 0.31 ± 0.01 vs. Olz: 0.29 ± 0.01 (n = 21-23); P<0.05] consistent with increased insulin resistance.

3.2.3 Other Peripheral Effects of Chronic Olanzapine

After receiving olanzapine for 19 days via oral gavage, animals were deprived of food overnight and then euthanized to obtain tissue and blood samples on day 20. Figure 3-5 shows that gastrocnemius muscle weights were not significantly different between the control and drug groups (P = 0.85); however, weights of the parametrial fat pads were greater in the olanzapine treated group (P<0.01). Such changes were also observed in studies terminated at 13 or 14 days and were not significantly different in the two rat strains tested (data not shown).

The increase in adipose tissue mass with chronic administration of olanzapine was associated with an increase in plasma leptin (Table 3-1). Chronic administration had no significant effect on

![Figure 3-5: Effect of chronic olanzapine exposure on tissue weights in female rats. Animals received olanzapine (4 mg/kg) by oral gavage (Olz) or saline (Con) for 20 days. Tissues were collected and weighed after overnight period of food-restriction on day 20. Data represent the mean ± SE (n = 8). Gastroc = Gastrocnemius. An asterisk indicates a significant difference from time-matched, control tissue weight (*P<0.05).]
two other obesity related hormones: adiponectin and the ‘active’ form of ghrelin (Table 3-1).

Also, there were no significant changes observed in plasma triglycerides, VLDL cholesterol, or free fatty acids. Since no changes in the lipid profiles were observed (Table 3-1), this suggests that even though these animals are obese they have not yet developed the metabolic syndrome.

A small, but statistically significant, increase in liver wet weight was routinely observed (P=0.02), irrespective of strain or route of drug administration (Fig 3-5). While no gross morphological abnormalities were noted in the livers, there was concern that the drug might be eliciting some hepatic toxicity or steatosis. This possibility was investigated in part by measuring liver enzymes and albumin (a measure of visceral protein synthesis from liver). As shown in Table 3-1, we observed no significant changes in albumin, alanine aminotransferase (a.k.a., ALT, serum glutamic pyruvate transaminase, SGPT) or aspartate aminotransferase (a.k.a., AST, serum glutamic oxaloacetic transaminase, SGOT). Microscopic analysis of fixed and hematoxylin and eosin-stained as well as oil red O stained liver slices indicated no microscopic pathology or hepatic steatosis that could readily explained the increase in liver wet weight with olanzapine.
Table 3-1: Plasma chemistries following chronic olanzapine treatment in female rats.

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Strain</th>
<th>Condition</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>Wistar</td>
<td>Control</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine + Topiramate</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Sprague-Dawley</td>
<td>Control</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>1.6 ± 0.4*</td>
</tr>
<tr>
<td>Ghrelin (active) (pg/ml)</td>
<td>Wistar</td>
<td>Control</td>
<td>198.1 ± 38.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>223.3 ± 26.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine + Topiramate</td>
<td>209.8 ± 23.0</td>
</tr>
<tr>
<td>Alanine Aminotransferase (U/L)</td>
<td>Wistar</td>
<td>Control</td>
<td>56 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>Aspartate Aminotransferase (U/L)</td>
<td>Wistar</td>
<td>Control</td>
<td>245 ± 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>244 ± 34</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>Wistar</td>
<td>Control</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>Sprague-Dawley</td>
<td>Control</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>6.0 ± 0.4</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>Sprague-Dawley</td>
<td>Control</td>
<td>50 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>52 ± 2</td>
</tr>
<tr>
<td>VLDL Cholesterol (mg/dl)</td>
<td>Sprague-Dawley</td>
<td>Control</td>
<td>10.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>10.5 ± 0.5</td>
</tr>
<tr>
<td>Free Fatty Acids (mM)</td>
<td>Sprague-Dawley</td>
<td>Control</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>0.8 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± S.E; n = 8-23 rats per group. An asterisk indicates significantly different (*P<0.05) compared to measurements from time-matched, control animals.
3.2.4 Effect of Chronic Olanzapine Treatment on Central Dopamine Pathways

Dopaminergic pathways in the CNS are involved in motivational aspects of eating and taste preference. Since olanzapine blocks dopamine receptors it is conceivable that modulating those pathways may result in hyperphagia. To test this possibility, CNS dopamine receptors were quantified by autoradiography. $[^{125}\text{I}]$-iodosulpride D2-labeled binding was analyzed in the dorsal and ventral striatum and in the ventral medial and ventral lateral midbrain. Absolute D2 binding for each brain region and group is shown in Table 2 and the detailed method of statistical analysis is described in Bello et al (141). Absolute binding was significant for treatment, brain region and their interaction ($P<0.05$). Post hoc tests revealed that absolute binding in the dorsolateral striatum of the olanzapine group ($30.77 \pm 1.56 \text{ fmol/mg tissue equivalent}$) was significantly higher than that of the control group ($25.67 \pm 1.26 \text{ fmol/mg tissue equivalent}; P<0.001$). Not surprisingly, D2 binding in the dorsolateral striatum (olanzapine group) was higher than any other brain region ($P<0.05$). When this binding was taken as a percentage of the saline controls, the percent binding was still higher in the olanzapine group compared with the control group ($P<0.05$). The olanzapine group’s percent binding was higher in all brain regions, except in the SNpc ($97.90 \pm 4.57\%$). Post hoc tests again revealed a significant difference in the percent binding in the dorsolateral striatum for the olanzapine group ($119.88 \pm 6.01\%$) compared with saline control ($P<0.05$). This increase in D2 binding most likely resulted from the direct pharmacological effect of olanzapine. No significant difference was observed in percent binding between brain regions or their interaction. Except for the increase in the dorsolateral striatum, which was not studied previously, these findings are essentially in agreement with Wurtman’s group (97) and suggest that the observed overeating is not a
consequence of changes in the dopamine system that might be expected to influence motivational aspects of appetite.

| **Table 3-2:** Absolute binding of $[^{125}]$iodosulpride in the striatum and midbrain regions of chronic olanzapine- and vehicle-treated female rats. |
|---|---|---|---|
| **Brain Regions** | **Treatment** | **Olanzapine (n = 12)** | **Saline (n = 12)** |
| | | $\pm$ S.E. | $\pm$ S.E. |
| NAcc Shell | | 15.00 ± 0.58 | 13.65 ± 0.87 |
| NAcc Core | | 15.90 ± 0.74 | 14.10 ± 0.66 |
| VTA (r) | | 5.08 ± 0.26 | 4.87 ± 0.46 |
| VTA (c) | | 7.66 ± 0.24 | 7.04 ± 0.26 |
| DLS | | 30.77 ± 1.56* | 25.67 ± 1.26 |
| SNpc | | 7.56 ± 0.35 | 7.72 ± 0.36 |

Binding is expressed in femtomoles per milligram of tissue equivalent ± S.E. NAcc shell, nucleus accumbens shell region; VTA (r), ventral tegmental area, rostral expanse; VTA (c), ventral tegmental, caudal expanse; DLS, dorsolateral striatum; SNpc, substantia nigra pars compacta. An asterisk indicates a significant difference from saline (Control) group (*P<0.001).

3.2.5 **Effect of Topiramate on Olanzapine-induced Weight Gain**

A characteristic of atypical antipsychotic drug-induced obesity in humans is that it is rapidly attenuated by topiramate (156, 161, 162, 165). Therefore, the effect of topiramate was investigated on olanzapine-induced weight gain in rats. Animals were provided with cookie dough containing olanzapine (4 mg/kg/day) with or without topiramate (100 mg/kg/day) or cookie dough alone (control). Each form of the dough was completely consumed (data not shown). As can be seen in Figure 3-6A, topiramate co-administration attenuated olanzapine-induced weight gain. Figure 3-6B shows that olanzapine caused a significant increase in adipose
tissue content relative to control, in agreement with the results shown in Figure 3-5. Animals receiving olanzapine and topiramate had a significantly lower amount of fat tissue compared to animals receiving olanzapine alone (Fig 3-6B). Thus, topiramate was effective at reducing adiposity caused by olanzapine in the rat.

![Figure 3-6: Topiramate blunts the olanzapine-induced weight gain in female rats. Animals received olanzapine (Olz), olanzapine + topiramate (Olz-Top), or cookie dough alone (Con), as indicated. (A) Change in body weight after 5 days of treatment was determined for each condition. Data represent mean ± S.E. (n = 8), Asterisks indicate significantly different from other values (**P<0.01). (B) Effects of drugs on tissue weights. Data represent mean ± S.E. (n = 8). ‘a’ indicates significantly different from the control condition in the same tissue. ‘b’ indicates significantly different from animals receiving only olanzapine. Gastroc, gastrocnemius.]

3.2.6 Effects of Acute Olanzapine Administration

Olanzapine did not exhibit a statistically significant increase in food intake during the first 24h of drug administration in any of our studies. An increase was usually detectable on the second day that generally reached a maximum by the third day. We posited that metabolic
changes might be apparent in the animals which preceded the hyperphagia that might possibly explain it. To examine this possibility, animals were treated acutely with drug or vehicle and food deprived according to the schedule described in Chapter 2 “Materials and Methods.” In the first 24h food was provided to both groups of animals. During that first 24h, the amount of food consumed was not significantly different [Con: 16.1 ± 0.9 (n = 23) vs. Olz: 18.2 ± 0.7 g (n = 23)]. On the second day of treatment, animals continued on drug but were food deprived for five hours before an OGTT was administered (Fig 3-7). The basal and peak glucose was significantly lower in the olanzapine group compared to the control (Fig 3-7A). The rise in insulin in response to a glucose challenge was also blunted. The QUICKI index indicated that groups did not differ in insulin sensitivity [Con: 0.29 ± 0.01 (n = 22) vs. Olz: 0.31 ± 0.01 (n = 19)]. Total areas under the glucose tolerance curves were also not significantly different between olanzapine and control groups (data not shown).

**Figure 3-7:** Glucose tolerance after acute olanzapine administration. Female rats received four doses of olanzapine divided over a 29h period. An OGTT was performed after a 5h period of food-restriction. (A) Blood glucose and (B) plasma insulin measurements are shown. Data are the mean ± S.E. (n = 19-22). Asterisks indicate a difference from time-matched, control animals (*P<0.05).
Plasma from the OGTT was used to measure other endpoints – namely leptin and active ghrelin. In the olanzapine group, the mean concentration of the satiety hormone leptin was significantly depressed compared to the control animals (Fig 3-8). With regard to ghrelin, increases in active ghrelin have been reported to stimulate appetite; however, active ghrelin was actually decreased in animals receiving olanzapine. Active ghrelin in plasma from control animals (n = 23) contained 51.9 ± 3.0 pg/ml while plasma from olanzapine treated animals (n = 23) contained 43.2 ± 2.2 pg/ml (P<0.05). In contrast, plasma albumin concentrations did not differ significantly (P=0.755) between vehicle- and olanzapine-treated animals [3.50 ± 0.07 vs. 3.46 ± 0.10 g/dl (n = 23)]. Leptin was also measured at the ninety-minute time point of the OGTT (Fig 3-8). Plasma leptin in the olanzapine group increased at the ninety-minute time point, however, this elevated leptin was still significantly lower than the basal leptin measurement from control animals.

**Figure 3-8:** Plasma leptin before and 90 minutes after an intraperitoneal glucose injection. Female rats received olanzapine (4 mg/kg) acutely. Plasma leptin was measured before and at 90 minutes post-glucose injection in animals receiving either olanzapine or saline (Control) through oral gavage, as indicated. Data represent the mean ± S.E. (n = 21-23). ‘a’ indicates significantly different with control animals at time zero. ‘b’ indicates significantly different compared with olanzapine-treated animals at time zero.

To address the specificity of the acute lowering of leptin and glucose, we acutely treated animals with either olanzapine (which does increases body weight) or the same dose of the chemically and pharmacologically related compound, clozapine (which did not increase body
weight gain in the rat). Clozapine failed to lower the plasma glucose (not shown) or lower leptin, while olanzapine did (Fig 3-9B). Topiramate added together with olanzapine did not affect the glucose or leptin significantly (Fig 3-9A). Thus, its attenuation of food intake and body weight gain is unrelated to this early effect of olanzapine.

![Graph](image.png)

**Figure 3-9:** Plasma leptin concentrations after acute olanzapine, olanzapine and topiramate, or clozapine treatment. (A) Female rats received vehicle (Control), olanzapine (Olz), or olanzapine and topiramate (Olz + Top) by oral gavage, or (B) clozapine by oral gavage after a 5h period of food-restriction (n = 10-18). Data represent the mean ± S.E. An asterisk indicates significantly different from the control group (P<0.05).

3.3 DISCUSSION

In this chapter we have characterized a novel animal model of atypical antipsychotic drug-induced obesity in which the drug is provided by self-administration in a food treat. The obesity was verified by increased adipose tissue mass. Several aspects of the olanzapine-induced obesity in the chronically studied rat model are in accordance with the weight gain side effect in humans; these are hyperphagia (150, 151, 170), hyperleptinemia (e.g. 124, 152), hyperinsulinemia/insulin resistance (171) and antagonism by topiramate (161-165). The
olanzapine-induced hyperphagia observed here is in agreement with other reports where the drug was injected (e.g. 97, 168) and from many studies in humans. Thus, this model appears to be relevant in several respects to the obesity observed in humans taking atypical antipsychotics. Two to three weeks of olanzapine treatment in rats did not lead to diabetes or plasma lipid abnormalities associated with the metabolic syndrome. Therefore this model represents a less complicated form of obesity compared to others. We not only characterized potential changes in the animals after weight gain, changes which have not yet been reported, but also determined acute metabolic alterations that might explain the overeating observed in the olanzapine-treated animals. Thus another new finding is that olanzapine has three, possibly related, acute metabolic effects preceding hyperphagia. These three acute effects include hypoleptinemia with food deprivation, blunted response of insulin and leptin during a glucose challenge, and acute hypoglycemia with food deprivation. It is posited that these three observations could readily explain the drug-induced overeating and consequent overweight/obesity observed in the rats.

Because olanzapine-induced obesity eventually results in an apparent hyperleptinemia and insulin resistance similar to humans, our model for how olanzapine works acutely may validly suggest the mechanism by which the drug effects appear to become saturable with time. That is, eventually, the excess of fat from overeating compensates for the acute drug-induced decreases in glucose, insulin and leptin by contributing to an eventual increase in the plasma concentrations of these satiety factors secondary to insulin resistance.

This is the first report of olanzapine-induced acute hypoleptinemia in animals following brief food deprivation, despite the fact that a number of laboratories have reported weight gain effects of olanzapine in animals (97, 101, 172) and humans (e.g. 124, 152). The acute hypoleptinemia undoubtedly contributes to the hyperphagia because rises in plasma leptin inhibit feeding in rats (e.g. 173, 174). Active-ghrelin was not increased, as might have been expected if it were involved in the hyperphagic mechanism. Accordingly, active ghrelin measured in chronic
studies showed no significant differences. Indeed, reports of olanzapine effects on active ghrelin in humans have been inconsistent (151, 153). The potential involvement of leptin in the weight gain side effect of olanzapine may also warrant a valid explanation of why the drug effect plateaus with time at the same dose. The rise in leptin caused by the increase in fat mass may offset the drug’s leptin lowering effect. In other words, the effect of olanzapine to lower leptin after chronic treatment may become masked by the leptin elevating effects of the increased adipose tissue mass that develops over time with hyperphagia. It would be interesting to see if olanzapine acutely lowers leptin in humans or, for that matter, if humans taking olanzapine have appropriate plasma leptin concentrations for their given fat mass. In at least one human study where this was addressed, leptin concentrations were determined to be lower in the olanzapine treated group than was expected based on body mass index (124).

The response of leptin and insulin to a glucose challenge was blunted in the acute experiments. Because of this attenuation, it is expected that more food would need to have been consumed by the drug-treated animals in order to provide the same concentration of leptin, a known satiety factor. This could reasonably be a second contributing factor explaining the increased food intake that is observed in this model. The generalized decrease in leptin, as well as the decrease in response to glucose challenge, may be secondary in part to a generalized lowering of the basal glucose and insulin concentrations, which are other known regulators of leptin secretion.

Lastly, some metabolic partitioning of glucose is apparent in the olanzapine-treated animals. The drug caused a greater lowering of plasma glucose after food deprivation and this led to a blunting in the rise of glucose in response to a challenge. No acute changes in glucose tolerance were observed, though, evident by equal areas under the OGTT curves. Since plasma glucose concentration is a known direct and indirect regulator of satiety (e.g. 175, 176), it is tempting to speculate that the lowering of plasma glucose upon food restriction and glucose
challenge could be a third contributor to the apparent decrease in satiety and increased food intake we observed. This acute effect of olanzapine to lower plasma glucose following food deprivation (Fig 3-8) appears even in the chronic setting as a non-statistically significant trend (Fig 3-4), a time point where we would have expected frank elevation of glucose given the increase in fat mass of the olanzapine treated animals.

3.3.1 Olanzapine-Induced Obesity in Rats as a Model for the Human Side Effect

It is currently unclear whether this model is an appropriate system for studying atypical antipsychotic-induced obesity in humans. Several findings have been supportive of the idea (97, 177, 178); however, the ability of haloperidol to also cause obesity in rats but not in humans has been claimed as non-supporting (168). The sexual dimorphism of the olanzapine-induced obesity in rats also seems to argue against human relevance. Our data, and that from other groups, indicate that olanzapine stimulates weight gain in female, but not male rats. Another surprise was that the drug did not work in the female mouse strains examined. We chose to study the A/J and C57Bl/6J mouse strains because they exhibit different susceptibilities to diet induced obesity. We hoped for a differential effect on antipsychotic-induced obesity in order to take advantage of existing consomic strains of these animals. Unfortunately, two different doses of olanzapine that worked in female rats did not cause weight gain in either strain of female mice. In contrast to our rat model, olanzapine causes weight gain in male and female humans. The reasons for this sexual dimorphism in rats is presently unclear, but may relate to one or more of the following: (a) responses to prolactin (90, 167), (b) the fact that humans and female rats are more weight stable than male rats (i.e., weight changes easier to discern in females), and/or (c) the sexual dimorphism in leptin which may be different in humans and rats depending on age or stage in the estrous cycle.
We did not observe an effect of clozapine on weight gain in rats. This was surprising because clozapine is both structurally-related to olanzapine and has a reported weight gain side effect in humans. While our findings are in agreement with the lack of effect of clozapine in female rats reported by another group (90), this is admittedly in contrast to the situation in humans. Although, it should be noted that the therapeutic dose of clozapine in humans is typically ten times higher than olanzapine. This would mean using 40-50 mg/kg of clozapine per day in rats. However, even doses half as much as that produced such severe sedation that the animals could not eat and lost rather than gained weight in preliminary studies (not shown). Consequently it may not be the obesity side effect that is different, but rather the sedation side effect. In this regard, it should be recognized that clozapine and olanzapine are unselective or so-called “dirty drugs.” Both antagonize receptors for several biogenic amines in mammals (179). The potency at these receptors varies for each compound and might be different between human and rat depending on the receptor involved. For example, whereas clozapine and olanzapine are approximately equipotent at D1 and D4 dopamine receptors, clozapine is more potent at blocking H1-histamine, α2-adrenergic, muscarinic and serotonin (5-HT2) receptors (147). Olanzapine is more potent blocking D2 dopamine and α1-adrenergic receptors (147).

Several observations were consistent with the atypical antipsychotic-induced obesity in humans. The first was the previously reported hyperphagia that has been reported (170). Chronic hyperleptinemia and hyperinsulinemia were observed in our chronically treated animals, which have also been reported in humans taking atypical antipsychotic drugs (e.g. 124, 152). Another interesting characteristic of the side effect in humans is the rapid attenuation by topiramate (156, 161, 162, 165), which we observed in our model. This is in contrast to diet-induced obesity models in which topiramate is not particularly effective (180).
3.3.2 Other Potential Effects of Olanzapine Investigated

In this study, olanzapine-induced weight gain and increased D2 receptor binding in dorsolateral striatum but not in the ventral striatum (i.e. shell and core of the nucleus accumbens) or in the somatodendritic domains of both nigrostriatal and mesoaccumbens dopamine projections (i.e. in the SN or the VTA, respectively). This increase in D2 binding most likely resulted from the direct pharmacological effect of olanzapine. For example, in patients treated with olanzapine (>14 days) it has been demonstrated using single photon emission computed tomography (SPECT) and positron emission tomography (PET) that olanzapine causes a significant amount of striatal D2 receptor occupancy, e.g. 46-82%; (181, 182). In addition, chronic (28 days) subcutaneous delivery of olanzapine via an osmotic pump in rats resulted in an increase in D2 but not D3 binding in the dorsolateral striatum and the NAcc. Although they account for small percentage of total D2 binding in the striatum (~15%), D4 receptor binding was also increased in the dorsolateral striatum and NAcc in another study (183). Together, these studies suggest that chronic treatment of olanzapine increases occupancy and up-regulates D2 receptors. It is conceivable that this enhanced D2 binding and subsequent activity may also lead to an increased food intake with a corresponding increase in weight gain.

In contrast, using PET, Wang and colleagues (54) reported that striatal D2 receptor availability is lower in morbidly obese individuals compared with normal weight controls and body mass was negatively correlated with D2 receptor availability. This finding does not necessarily contradict our observation that acute treatment of olanzapine increased D2 binding. First, in individuals with a long history and potential inherited factors (see the second point below), an adaptation to the dopamine system may occur. In fact, a decreased D2 receptor availability could result from dietary constraints and feeding patterns, rather than directly from weight gain. In a study by the Hoebel laboratory it was shown that restrictive feeding in rats with
a 12 hour daily access to glucose and chow for 30 days resulted in a decrease in D2 binding in the ventral and dorsal striatum (184). We also demonstrated that daily limited access (i.e. 20 min) to sucrose over a 7 day feeding regimen decreased D2 dopamine receptors binding in the NAcc shell and dorsolateral striatum compared with rats that had limited access to just food (141). The second potential difference between development of chronic and acute (e.g. drug or diet-induced) obesity is that whereas the latter does not assume pre-existing genetic conditions, inheriting variants of dopamine may be causally related to some forms of obesity. For example, it has been suggested that the Taq IA and Ser311Cys polymorphisms in the D2 receptor gene are associated with obesity (185, 186). Future studies should focus on the early affects of olanzapine preceding the hyperphagia.

Other results from the present studies indicate that the obesity in the olanzapine-treated animals is uncomplicated (at least in a few weeks) by significant metabolic syndrome or diabetes. In support of this, we did not see any changes in adiponectin or the lipid profile. Thus, this model, at least over the time course we studied, would seem to be an uncomplicated form of obesity.

Overall our results indicate that an acute lowering of plasma leptin and glucose/insulin concentrations may cause olanzapine-induced overeating and obesity in female rats. These changes, revealed by brief food deprivation, would be expected to increase hunger and decrease satiety in the rat. A blunted rise in glucose, insulin and leptin upon glucose challenge was also observed, and is posited to contribute to the hyperphagia that results in obesity in this model. These effects were not observed with the same dose of the structurally related compound clozapine, which in contrast to olanzapine did not cause weight gain in weight-stable female rats. Several aspects of the obesity seen in female rats are similar to the obesity observed in humans taking olanzapine. Future studies should focus on the acute effects of the drug in humans, the
mechanism of regulation (e.g. transcriptional or translational), and whether or not it is a direct effect of olanzapine on adipose tissue or secondary to effects on a leptin regulator.
Chapter 4

Olanzapine promotes fat accumulation in male rats by decreasing physical activity, repartitioning energy and impairing lipolysis

4.1 INTRODUCTION

Atypical antipsychotic drugs are a mainstay of psychiatric pharmacotherapy and are used to treat a variety of psychiatric illnesses, most notably schizophrenia. These second generation drugs lack the extrapyramidal and other debilitating movement side effects that complicated treatment with first generation compounds. The popularity of the atypical antipsychotics, especially olanzapine, led to the observation that these drugs have distinct metabolic side effects that were not detected during preclinical testing; including, insulin resistance, diabetes, and obesity (35, 171, 187-190). The mechanisms underlying these metabolic side effects in humans are not known. However, case reports and other observational studies have suggested that effects of atypical antipsychotics on glucose homeostasis and insulin sensitivity probably precede significant weight gain in humans (25, 29, 31). Regardless, clinically significant weight gain is observed in almost one-third of patients treated with olanzapine, predisposing these patients to the metabolic syndrome and additional comorbid conditions (Reviewed in 35, 191). Olanzapine, one of the most popular antipsychotics, confers the greatest potential for weight gain and is the focus of our current effort.

Numerous animal studies have examined the potential metabolic side effects of olanzapine, though many questions remain unanswered. A common finding is that, unlike the clinical situation, olanzapine-associated weight gain in rats is sex-dependent with female, but not male rats, displaying a robust increase in body weight gain (97, 101-103, 168). Body weight gain
in female rats is strongly associated with hyperphagia, while effects of olanzapine in male rats appear to be limited to increased meal size without altering 24h food intake (78, 84, 172, 178). However, recent reports (107, 108) have demonstrated that olanzapine does indeed have metabolic effects in male rats. When treated chronically (>3 weeks), olanzapine increased adiposity without altering food intake or body weight gain, similar to chronic (6 week) administration in dogs (83). The mechanism of the sexual dimorphism in rats and the increased adiposity in male rats and dogs is currently unknown.

In the following chapter, we confirm that chronic olanzapine administration leads to increased deposition of fat (adiposity) without a change in total body weight in male rats and demonstrate that these increases can be detected as early as the first treatment week. After acute olanzapine treatment animals eat more food for their amount of physical activity, which declines. In addition, acute metabolic effects that precede changes in body composition appear to repartition the excess nutrients toward adipose tissue with subsequent trapping of those stored nutrients in adipose tissue by impairing lipolysis in vivo.

4.2 RESULTS

4.2.1 Effects of Chronic Olanzapine on Male Rats

Previous work has demonstrated that olanzapine increases adiposity over an extended time course (~3 weeks), though the mechanism of this effect is not clear (107, 108). Similar to previous reports, neither body weight gain (Fig 4-1A) nor 24h food-intake (Fig 4-1B) differed between vehicle- and olanzapine-treated rats throughout the study. Moreover, food-conversion efficiency was not different during the first week of treatment [23.1 ± 1.4 vs. 21.8 ± 1.8 %, for
control and olanzapine treated groups, respectively] or after three weeks of treatment [17.0 ± 0.8 vs. 15.1 ± 1.4 %].

![Figure 4-1: Effect of chronic olanzapine on body weight and food intake in male rats. Animals were trained to eat drug-free cookie dough and then allocated to experimental groups for chronic olanzapine treatment (see Chapter 2 for details). (A) Body weight and (B) food intake were measured daily and data represent the mean ± S.E. (n = 10).](image)

In contrast to weight gain and food intake, olanzapine increased percent of total body fat by the seventh day of treatment (Fig 4-2). The increase in total body fat relative to control animals was maintained for the duration of the study but reached a plateau at three weeks. Percentages of adipose, lean and fluid masses over the course of the study are shown in Table 1. Accordingly, as olanzapine did not change body weight gain during chronic administration, the observed increase in fat mass was balanced by a decrease in total body lean mass (Table 1).
Table 4-1: Minispec $^1$H-NMR body composition data during chronic olanzapine treatment (4-12 mg/kg) in male rats.

<table>
<thead>
<tr>
<th>Time</th>
<th>Weight Component</th>
<th>Control (%)</th>
<th>Olanzapine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>Adipose</td>
<td>20.7 ± 0.5</td>
<td>20.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Lean</td>
<td>65.1 ± 0.7</td>
<td>65.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Fluid</td>
<td>6.3 ± 0.2</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>Week 1</td>
<td>Adipose</td>
<td>20.7 ± 0.8</td>
<td>22.6 ± 0.4*</td>
</tr>
<tr>
<td></td>
<td>Lean</td>
<td>59.2 ± 1.4</td>
<td>58.3 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Fluid</td>
<td>5.3 ± 0.2</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>Week 2</td>
<td>Adipose</td>
<td>23.3 ± 0.7</td>
<td>25.3 ± 0.5*</td>
</tr>
<tr>
<td></td>
<td>Lean</td>
<td>63.5 ± 0.8</td>
<td>62.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Fluid</td>
<td>5.7 ± 0.3</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Week 3</td>
<td>Adipose</td>
<td>25.2 ± 0.5</td>
<td>27.5 ± 0.4**</td>
</tr>
<tr>
<td></td>
<td>Lean</td>
<td>63.4 ± 0.3</td>
<td>62.1 ± 0.4*</td>
</tr>
<tr>
<td></td>
<td>Fluid</td>
<td>5.7 ± 0.1</td>
<td>5.6 ± 0.1</td>
</tr>
<tr>
<td>Week 4</td>
<td>Adipose</td>
<td>24.7 ± 0.6</td>
<td>27.4 ± 0.4**</td>
</tr>
<tr>
<td></td>
<td>Lean</td>
<td>61.3 ± 0.9</td>
<td>60.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Fluid</td>
<td>5.5 ± 0.3</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>Week 5</td>
<td>Adipose</td>
<td>25.7 ± 0.7</td>
<td>27.6 ± 0.5*</td>
</tr>
<tr>
<td></td>
<td>Lean</td>
<td>62.8 ± 0.6</td>
<td>61.3 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>Fluid</td>
<td>6.2 ± 0.1</td>
<td>6.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± S.E. (n = 10 rats per group). Data represent the average component mass in percent of total body weight. Asterisks indicate significantly different (*P<0.05, **P<0.01) compared to measurements from time-matched control animals.
Physical activity was measured during the third week of olanzapine or vehicle treatment, as decreased activity has been implicated in olanzapine-induced obesity in female rats (97, 192). However, locomotor activity did not differ between experimental groups during the third treatment week [‘X dimension 25,820 ± 1,121 vs. 22,445 ± 1,616; ‘Y dimension’ 14, 589 ± 820 vs. 12, 201 ± 1,095; ‘Z dimension’ 2,431 ± 285 vs. 1,899 ± 278 (n = 10), all values ‘Control vs. Olanzapine’, respectively].

Increased adiposity is strongly associated with insulin resistance, and thus we challenged animals with an oral glucose tolerance test after 4 weeks of olanzapine treatment. Consistent with their increased adiposity, the olanzapine-treated animals displayed hyperglycemia (Fig 4-3A) and hyperinsulinemia (Fig 4-3B) after 14h of food-restriction. Glucose and insulin concentrations remained significantly elevated compared to vehicle-treated controls throughout the OGTT. Moreover, the AUC_{Glucose} was significantly elevated by olanzapine [194 ± 78 vs. 401 ± 134 (mM-min), P<0.01], suggesting decreased whole-body insulin action. To confirm such changes, an insulin tolerance test was performed on day 42 of drug treatment. Once again, drug-treated
animals showed elevated plasma glucose following 14h food-restriction [5.0 ± 0.2 vs. 6.1 ± 0.2 mM, P<0.001]. Olanzapine blunted the fall in blood glucose following insulin injection (Fig 4-3C), with glucose concentrations remaining elevated compared to control levels at 60, 90 and 120 minutes post-injection. The ITT-AUC was lower in the olanzapine group compared to control animals [6,226 ± 564 vs. 3,797 ± 569 (min-% Baseline), P<0.01], which further suggests decreased insulin sensitivity.

Figure 4-3: Effect of chronic olanzapine on oral glucose tolerance and insulin tolerance in male rats. On day 28 of olanzapine or vehicle treatment an OGTT was performed. Animals were food-restricted for 14h and administered a half-dose of olanzapine (6 mg/kg) or vehicle 1h prior to beginning the OGTT. Baseline blood samples were collected 1h after the final gavage and then glucose administered orally. Blood samples were collected at 30-min intervals for 2h following glucose gavage for measurement of (A) blood glucose and (B) plasma insulin. (C) On day 42 of treatment, an ITT was performed. Animals were food restricted for 14h and given a half dose of olanzapine (6 mg/kg) or vehicle 1h prior to the start of the ITT. The response to injected insulin was measured as the % change in baseline blood glucose. Data represent the mean ± S.E. (n = 6-10). *P<0.05, **P<0.01, ***P<0.001, compared to time-matched control values.
4.2.2 Effects of Acute Olanzapine on Locomotor Activity and Energy Expenditure

Because primary disturbances in energy expenditure might increase adiposity and weight gain over a similar time period, we examined potential drug effects on these parameters. Locomotor activity (Fig 4-4) and energy expenditure were measured following acute oral olanzapine administration (Fig 4-5A-B, indicated by arrows) at the beginning of the dark cycle (shaded area) and then again at the beginning of the light cycle on the following morning. As seen in Figure 4-4, olanzapine significantly decreased locomotor activity in all dimensions (x, y, z) during the dark and light cycles, but had no effect on 24h food intake (data not shown); thus relative food intake was more than expected for the amount of physical activity.

**Figure 4-4:** Locomotor activity following acute olanzapine administration. Male rats were placed in specialized chambers to measure locomotor activity. Following acclimation, olanzapine (10 mg/kg) or vehicle solution was administered by oral gavage. Locomotor activity was significantly different from control in all three dimensions (x, y, z) during both the dark cycle (shaded area) and light cycle (P<0.001). Data represent the mean ± S.E. (n = 12).

Leptin, an adipocyte-derived hormone and known satiety factor, has been posited as contributing to the hyperphagia observed in female rats following olanzapine treatment (102). Consistent with those observations, plasma leptin concentration was also decreased following acute olanzapine-treated male rats [5h Fast: Control = 5.29 ± 0.55, Olz = 3.16 ± 0.44 ng/ml (n = 9-10), P<0.01]. Thus, a decreased plasma leptin may also contribute to the relative increase in food intake.
O2 consumption (Fig 4-5A) and CO2 production (Fig 4-5B) were measured throughout a complete dark and light cycle. As the olanzapine-treated group had less physical activity, they unexpectedly had similar VO2 (Fig 4-5C) and VCO2 (Fig 4-5D) relative to vehicle-treated controls during the dark cycle, while these parameters were significantly elevated during the light cycle.

Rectal temperature was also measured in separate groups of animals before and 2h after olanzapine gavage, though no change from baseline body temperature was detectable (data not shown). Thus, energy expenditure is not decreased following olanzapine administration, even though drug-treated animals have less locomotor activity. In fact, the drug-treated animals maintain similar oxygen consumption to vehicle-treated animals during the dark cycle and elevated oxygen consumption during the light cycle. These results demonstrate that olanzapine-treated rats use some of the excess caloric intake to support a process other than locomotor activity.
Figure 4-5: Energy expenditure following acute olanzapine administration. Male rats were placed in specialized chambers to measure energy expenditure using indirect calorimetry. Following acclimation, olanzapine (10 mg/kg) or vehicle solution was administered by oral gavage (indicated by arrows). Animals retained ad libitum access to food and water for the duration of the experiment. (A) VO$_2$ and (B) CO$_2$ were measured at 15-min intervals for 24h. Average (C) VO$_2$ and (D) VCO$_2$ for the dark and light cycles were calculated. All data represent the mean ± S.E. (n = 12); asterisks indicate significant differences (**P<0.01, ***P<0.001) compared to time-matched control values.
4.2.3 Effects of Acute Olanzapine on Glucose Tolerance and Insulin Action

The worsening glucose and insulin tolerance observed after chronic treatment with olanzapine might be secondary to increased adiposity. To test this hypothesis, OGTTs and ITTs were conducted in separate cohorts of rats on the second treatment day before significant accumulation of adipose tissue. As seen in Figure 4-6, acute olanzapine-treated rats displayed mild hyperglycemia (Fig 4-6A) and hyperinsulinemia (Fig 4-6B) after 5h of food-restriction following a low dose of olanzapine (4 mg/kg/day), suggesting insulin resistance. During the OGTT, glucose concentration tended to be higher relative to control values, with baseline and 120 minute blood glucose measurements being significantly elevated in the olanzapine group. Plasma insulin was similar between experimental groups throughout the OGTT, even though increased circulating insulin was expected given the mild elevations in blood glucose. To better assess insulin sensitivity, an insulin tolerance test (Fig 4-6C) was conducted in vehicle-treated rats or in rats injected with one of two doses of olanzapine (4 or 10 mg/kg/day). Olanzapine administration led to an apparent dose-dependent decrease in ITT-AUC (Fig 4-6C). The 4 mg/kg/day dose [6,054 ± 344 vs. 4,436 ± 415 (min ⋅ % Change), P<0.01] decreased and the 10 mg/kg/day dose further decreased the ITT-AUC [4,436 ± 415 vs. 1,514 ± 296 (min ⋅ % Change), P<0.001]. Together these data demonstrate that insulin resistance following olanzapine administration precedes and is therefore independent of the change in body composition observed in the chronically treated animals.
Figure 4-6: Effects of acute olanzapine on oral glucose and insulin tolerance in male rats. An OGTT or ITT was conducted on the second treatment day after acute olanzapine (4 or 10 mg/kg) gavage (see Chapter 2 for dosing protocol). Animals were food-restricted for 5h and received a final olanzapine or vehicle gavage 1h prior to the start of the tolerance tests. (A-B) Baseline blood samples were collected at 1h following the drug or vehicle gavage and then glucose (2.5 g/kg) administered orally. Blood samples were collected at 30-min intervals for 2h post-gavage for measurement of (A) blood glucose and (B) plasma insulin concentrations (n = 12). (C) For the ITT, animals received high dose olanzapine (10 mg/kg), low dose olanzapine (4 mg/kg) or vehicle by gavage 1h prior to beginning the ITT. Blood glucose was measured for 120 min as an indicator of insulin sensitivity. All Data represent the mean ± S.E. (n = 18-20). Asterisks represent significant differences (***P<0.001, **P<0.01, *P<0.05), compared to time-matched control values.

Since drug-induced insulin resistance precedes the changes in body composition, it is tempting to speculate that it might play a role in the development of the olanzapine-induced adiposity. For example, Caro et al (193) proposed a mechanism of obesity wherein tissue-specific insulin resistance, in which muscle but not fat was affected, would lead to shunting of
substrates away from muscle and towards fat. To test this hypothesis, hyperinsulinemic-euglycemic clamp studies using tracer methodology were conducted on the second treatment day.

Plasma insulin concentration was maintained at a mild, hyperinsulinemic level to examine drug effects on peripheral tissues as well as hepatic insulin sensitivity [Plasma insulin: 200 ± 28 vs. 205 ± 25 pM in control vs. olanzapine treated animals, respectively]. During the basal state (14h food-restricted), the tracer-determined rate of glucose turnover, which is equivalent to hepatic glucose output (Fig 4-7A), did not differ between experimental groups. Moreover, insulin-mediated suppression of hepatic glucose output, a measure of hepatic insulin sensitivity, was similar in the control and olanzapine groups. Consistent with two previous studies (116, 118), the tracer-calculated rate of whole-body glucose disposal during the clamp was significantly

Figure 4-7: Basal and insulin-stimulated glucose kinetics following acute olanzapine administration in male rats. Hyperinsulinemic-euglycemic clamp studies were conducted after olanzapine administration. (A) Hepatic glucose output was measured during basal (14h food-restricted) and clamp conditions. (B) Whole-body glucose disposal, a measure of whole-body insulin action, was measured during hyperinsulinemic-euglycemic conditions and compared to basal glucose turnover. Data represent the mean ± S.E. (n = 10-14). Asterisks indicate significant differences compared to control group under clamp conditions (**P<0.001).
decreased in the olanzapine group (Fig 4-7B). As a result of these changes, whole-body insulin-mediated glucose disposal was increased by ~85% in control animals, while olanzapine-treated animals showed no increase in whole-body glucose disposal, confirming the insulin resistance suggested by the glucose and insulin tolerance tests.

To determine the tissue(s) responsible for the insulin resistance, tissue glucose metabolic rates (Rg) were calculated for muscle and adipose tissue during the steady-state portion of the euglycemic clamp (Table 4-2). Consistent with the decrease in insulin-stimulated whole-body glucose disposal, Rg was decreased by 31% in gastrocnemius and 40% in soleus, which represent both fast- and slow-twitch skeletal muscle fibers, respectively. Unlike skeletal muscle Rg, a 53% increase in subcutaneous adipose tissue glucose uptake was observed relative to control animals, while epididymal and pararenal adipose tissue Rg remained unchanged. Thus, olanzapine acutely perturbs insulin sensitivity in a tissue-specific manner, decreasing insulin sensitivity in muscle tissue while either increasing or not affecting sensitivity in adipose depots.
Table 4-2: Tissue metabolic clearance rates and insulin-stimulated glucose metabolic rates for individual tissues during either basal or euglycemic-hyperinsulinemic clamp conditions in male rats on the second day of acute olanzapine treatment (10 mg/kg).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Basal Conditions</th>
<th>% Change From Control</th>
<th>Clamp Conditions</th>
<th>% Change From Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metabolic Clearance Rate</td>
<td></td>
<td>Glucose Metabolic Rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(ml/g tissue/min)</td>
<td></td>
<td>(nmol/g tissue/min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Olanzapine</td>
<td>Control</td>
<td>Olanzapine</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>1.28 ± 0.10</td>
<td>1.62 ± 0.24</td>
<td>ns</td>
<td>198 ± 20</td>
</tr>
<tr>
<td>Soleus</td>
<td>2.36 ± 0.28</td>
<td>3.20 ± 0.46</td>
<td>ns</td>
<td>381 ± 25</td>
</tr>
<tr>
<td>Epididymal Adipose</td>
<td>0.67 ± 0.09</td>
<td>0.59 ± 0.10</td>
<td>ns</td>
<td>51 ± 6</td>
</tr>
<tr>
<td>Pararenal Adipose</td>
<td>0.67 ± 0.12</td>
<td>0.74 ± 0.09</td>
<td>ns</td>
<td>65 ± 13</td>
</tr>
<tr>
<td>Subcutaneous Adipose</td>
<td>1.62 ± 0.25</td>
<td>2.68 ± 0.37</td>
<td>+65%*</td>
<td>89 ± 8</td>
</tr>
<tr>
<td>Liver</td>
<td>10.71 ± 1.46</td>
<td>11.04 ± 2.05</td>
<td>ns</td>
<td>195 ± 23</td>
</tr>
</tbody>
</table>

Values are means ± S.E. (n = 6-14 per tissue); asterisks indicate significant differences (*P<0.05, **P<0.01) compared to control. ns = not significantly different from control.
Tissue-specific glucose uptake was also measured in 14h food-restricted animals, under similar conditions as the basal period of the clamp studies. Tissue metabolic clearance rate instead of tissue Rg is shown in Table 4-2, as olanzapine elevated glucose and insulin concentrations under these conditions. Despite the insulin resistance, tissue clearance of glucose by skeletal muscle and two adipose depots was not affected, presumably because the plasma glucose and/or insulin were elevated. However, clearance by subcutaneous adipose tissue was increased. Glucose uptake into adipose tissue depots was also measured in fed animals (Fig 4-8). In these animals blood glucose was elevated in the olanzapine group, however, insulin concentration was not significantly different (data not shown). In this condition, there was a trend (P=0.10) for increased uptake into epididymal adipose tissue of olanzapine-treated animals, but not other depots, which only showed a non-significant tendency to be increased.

Figure 4-8: Adipose tissue glucose uptake in ad libitum fed male rats after acute olanzapine. Glucose uptake was measured in epididymal (EPI), pararenal (Renal), and subcutaneous (SC) adipose tissue depots after acute olanzapine administration (10 mg/kg) using the 2-deoxyglucose technique. Data represent the mean ± S.E. (n = 8).
4.2.4 Effects of Olanzapine on Adipose Tissue FFA Flux

In 14h food-restricted animals, acute olanzapine treatment decreased plasma FFA (Fig 4-9A), as well as glycerol [17.5 ± 1.3 vs. 9.6 ± 1.1, P<0.001]. This was surprising as insulin resistance is normally associated with elevated FFA. To examine the mechanism underlying this effect, we measured the uptake of FFA into adipose tissue and their release following isoproterenol-stimulated lipolysis in vivo. Lowered plasma FFA might reflect increased plasma FFA clearance from the circulation. To test this hypothesis, adipose tissue FFA metabolic rates were measured using the non-metabolizable FFA analog $^{125}$I-BMIPP in ad libitum fed animals (Fig 4-9B) in which FFA levels were not different (data not shown). Olanzapine increased FFA metabolic rate 2-fold in epididymal, pararenal, and subcutaneous adipose depots.

![Figure 4-9: Acute effects of olanzapine on circulating FFAs and adipose tissue FFA uptake in male rats. (A) Plasma FFAs were measured in 14h food-restricted animals following olanzapine (10 mg/kg) gavage. (B) In a separate cohort of animals, adipose tissue FFA uptake was measured on the second day of olanzapine treatment using a non-metabolizable FFA analog ($^{125}$I-BMIPP) in animals that had ad libitum access to food and water. Two hours after the final dose of olanzapine (10 mg/kg), an intravenous bolus of the FFA tracer was given and blood samples were collected during a 40-min in vivo labeling period. Fat depots were excised and measured for tracer uptake, an index of tissue FFA flux. Data represent the mean ± S.E. (n = 8-14). Asterisks indicate significant differences compared to control (***P<0.001, *P<0.05).](image-url)
Adipocytes and hepatocytes mobilize, respectively, stored fatty acids and glucose fuels in the post-absorptive state, during longer periods of food-deprivation, or in times of stress when catecholamines are elevated. Lipolytic impairment could serve as an additional pathway leading to the chronic changes in body composition by trapping stored fuels, and thus we tested lipolytic capacity in vivo with an isoproterenol challenge test in fed animals in which the FFA are not different.

**Figure 4-10:** Effects of olanzapine on isoproterenol-stimulated lipolysis. An isoproterenol challenge test was performed with ad libitum male rats on the second treatment day after olanzapine (10 mg/kg) gavage. Serial blood samples were collected following isoproterenol injection to measure the lipolytic response, as measured by (A) FFA and (B) glycerol, as well as the (C) hepatic glycolytic response (n = 8-10). Data represent the mean ± S.E. ***P<0.001, **P<0.01, *P<0.05, compared to time-matched control values.

Following injection of a low dose of isoproterenol, rises of plasma free fatty acids (Fig 4-10A) and glycerol (Fig 4-10B) were blunted by olanzapine. Even though lipolysis was attenuated, isoproterenol-stimulated hepatic glucose output remained intact (Fig 4-10C). Thus, the effects of olanzapine on lipolysis probably do not involve the early steps in the beta-adrenergic signaling
pathway shared by the lipolytic and hepatic glucose output response to isoproterenol. Overall, through the above actions, olanzapine seems to promote trapping of energy stores in adipose tissue.

4.3 DISCUSSION

In this chapter we confirmed that chronic olanzapine administration in male rats increases total body adiposity independent of a change in either body weight gain or food intake. The increased fat mass was detectable by $^1$H-NMR within the first week of drug administration and is associated with impaired oral glucose tolerance and insulin resistance. The present study extends these previous findings by demonstrating that olanzapine has at least three acute metabolic effects that may act in concert to favor adipose tissue deposition and development of insulin resistance. First, olanzapine decreases physical activity without a compensatory reduction in food intake, resulting in a relative over-nutrition in drug-treated animals. This was associated with a decline in the satiety hormone leptin. Second, olanzapine causes tissue-specific changes in insulin sensitivity that support a tendency for increased glucose uptake into adipose tissue. Importantly, FFA uptake into adipose tissue was also increased. Lastly, olanzapine impaired in vivo release of FFAs during an isoproterenol challenge, which suggests that adipose tissue is functionally trapping metabolic fuels. These acute effects of olanzapine are present on the second treatment day and are associated with detectable increases in adipose tissue by the seventh treatment day. The accumulation of adipose tissue is self-limiting, reaching a plateau after approximately three weeks of treatment, but most likely further worsens the drug-induced insulin resistance and glucose intolerance observed before body composition is affected.
4.3.1 Decreased Physical Activity Without Lowering Food Intake

A prolonged decrease in physical activity without reduction of food intake favors the accumulation of adipose tissue by excess nutrient storage. In contrast to humans, olanzapine-induced weight gain and hyperphagia in rats is sexually dimorphic. Studies have shown that female rats, but not males, are susceptible to drug-induced weight gain (e.g. 102, 192), and our results are consistent with those findings (107, 108). However, while female rats show decreased activity and increased food intake (97, 194), male rats show decreased activity without a change in food intake. Decreased locomotor activity was not observed at three weeks, suggesting this effect diminishes with time. Interestingly, percent body fat also reached plateau over a similar time course. Thus, in the short term, male rats have a relative over-nutrition for their given level of activity. It is tempting to speculate this mismatch is due to the drug-induced reduction in plasma leptin concentrations. The reason for this lack of weight gain appears to be due to maintained energy expenditure. The energy-consuming process is currently unknown, though it might represent activation of a futile cycle or thermogenesis that dissipates heat energy. We did not observe differences in body temperature before or 2h after olanzapine or vehicle gavage (data not shown). However, both of these processes would be thought to decrease body weight, which is not consistent with deposition of adipose tissue and loss of lean mass that occurs with chronic olanzapine treatment. Although speculative, a more likely alternative is that male rats are expending energy for de novo lipogenesis, which is supported by the observed increase in body fat. In individuals consuming a mixed macronutrient diet (carbohydrates, fat, protein) de novo lipogenesis usually accounts for a small fraction of total energy expenditure (<5%), as most stored lipid is recycled from dietary sources (195). However, de novo lipogenesis from carbohydrate precursors can account for upwards of ~20-30% of energy expenditure (196, 197). The standard rat chow used in this study is ~6% crude fat and, therefore, increased adiposity
would be thought to come mostly from carbohydrate precursors. It is noteworthy that adipose tissue did not exhibit the insulin resistance observed in skeletal muscle and therefore was under different conditions of either increased glucose uptake or a trend for increased glucose uptake under physiological hyperinsulinemic conditions. Regardless, further studies are needed to identify the process maintaining energy expenditure.

4.3.2 Increased Energy Uptake Into Fat

Tissue-specific insulin resistance has been posited to underlie the pathogenesis of obesity in humans and monogenetic obesities in rodents (193). We noted such tissue-specific insulin resistance after acute olanzapine. Under some conditions, a tendency or significant increase in glucose uptake into fat was observed. Additionally, there was a large increase in FFA uptake into adipose tissue in fed animals. In a recent study we showed that other peripheral tissues were similarly affected, and this may explain the sparing of glucose/hyperglycemia and apparent muscle insulin resistance after acute olanzapine (102). Increased glucose and FFA uptake appears to be a second mechanism through which olanzapine contributes to increased adiposity in response to chronic treatment.

It should be emphasized that these effects are acute and therefore not secondary to increased adiposity, but may instead promote the accumulation of fat seen with chronic treatment. Previous studies have documented weight gain-independent insulin resistance during hyperinsulinemic-euglycemic clamp studies (116, 118). Consistent with those reports, we demonstrate a similar decrease in insulin-stimulated whole-body glucose disposal (Fig 3E). Furthermore, this decreased glucose disposal is due to skeletal muscle insulin resistance (Table 2), a conclusion suggested from previous studies but most likely undetected due to a small sample size (116). In contrast, insulin-stimulated glucose uptake in epididymal and pararenal adipose
tissue was unchanged, while subcutaneous adipose tissue showed increased glucose flux. The mechanism by which skeletal muscle becomes insulin resistant and not adipose tissue is currently unknown. Moreover, the differential effects on glucose flux in visceral and subcutaneous adipose depots is not known, but may be due to intrinsic differences between these distinct tissue subtypes (198-200). Because olanzapine elevated plasma glucose and insulin in the fed and food-restricted states, excess glucose should be repartition to adipose tissue with the prevailing differences in tissue insulin sensitivity.

Increased adipose tissue glucose uptake relative to skeletal muscle would be expected to increase adipocyte triglyceride storage, which is further potentiated by the nearly 2-fold increase in FFA flux present in visceral and subcutaneous fat depots. The increased FFA flux likely contributes to triglyceride storage by providing FFAs for esterification to phosphoglycerol moieties from glycolysis. These changes in glucose and FFA flux are consistent with increased adiposity and adipocyte hypertrophy previously reported (105). Because tissue FFA uptake is both passive and regulated by insulin-sensitive fatty acid transporters (201), the mechanism of the increased tissue FFA uptake may involve either increased fatty acid oxidation, stimulation of fatty acid transporters by a direct drug effect, or by drug-induced increases in plasma insulin affecting lipogenic pathways. Increased VO₂ during the light cycle supports the theory of increased lipogenesis.

4.3.3 Impaired Fuel Mobilization From Adipose Tissue

A third factor contributing to increased adiposity during olanzapine administration is impaired mobilization of stored fuels. Normally, stored adipose triglyceride is mobilized via lipolysis during the post-absorptive state to conserve glucose fuel for the brain. Alternatively, FFA are mobilized in times of stress by elevated plasma catecholamines. Olanzapine blunted
increases in plasma FFA and glycerol in 14h food-restrict rats, suggesting lipolytic impairment that was confirmed by decreased FFA release after injected isoproterenol in fed animals when FFAs were not significantly different between the experimental groups. This suggests that in vivo lipolysis is impaired following olanzapine administration. As adipocyte lipolysis is a dynamic process (reviewed in 202), whether these effects on net FFA release reflect increased re-esterification or decreased lipase activity are currently unknown. Consistent with these findings, decreased plasma FFA have also been reported in patients following acute (2-3 weeks) olanzapine treatment (203). Moreover, catecholamine-stimulated lipolysis is attenuated in

![Model for altered nutrient partitioning in olanzapine-treated male rats.](image)

**Figure 4-11:** Model for altered nutrient partitioning in olanzapine-treated male rats. (Upper panel) In the post-prandial state nutrients (e.g. glucose) are taken up by insulin sensitive tissues (predominately by skeletal muscle, but also by adipose tissue for fuel storage. During the post-absorptive state, stored adipose tissue fuel reserves are liberated in the form of FFAs that are oxidized by other tissues (i.e. skeletal muscle, liver) for fuel. (Lower Panel) Olanzapine-induced skeletal muscle insulin resistance decreases glucose uptake by the muscle tissue and increases adipose tissue glucose disposal in the post-prandial state. Moreover, fasting-induced lipolysis (i.e. FFA release) is blunted by olanzapine and effectively ‘traps’ stored fuel. These changes may underlie the decreased muscle mass and increased adipose mass with chronic drug treatment.
adipose tissue harvested from chronically treated rats (105), suggesting a primary defect in the adipocyte. The mechanism of the decreased lipolysis is currently unknown, though several hypotheses may be considered. Decreased sympathetic tone will reduce lipolysis (204), however, this is likely not responsible because the response to exogenous catecholamines remains intact. Previous radioligand binding studies have not detected binding of olanzapine to β2-adrenergic receptors (205) and hepatic glucose output, which is regulated by β2-adrenergic receptors in response to isoproterenol, was not affected. It is possible that olanzapine inhibits β3-adrenergic receptors that are found only on adipose tissue. As many of the early steps in β-adrenergic signaling are shared between β2- and β3-adrenergic receptors in fat and β2-adrenergic receptors in liver, it seems unlikely that these are the primary targets of the impairment.

In summary, olanzapine increases the accumulation of fat mass in male rats independent of a change in body weight gain and food intake. Current findings demonstrate that olanzapine has at least four effects that collectively predispose male rats to increased adiposity, namely decreased physical activity, tissue-specific insulin resistance leading to repartitioning of glucose fuel, increased uptake of FFA into fat, and impaired lipolysis which traps excess fuel taken up. Further studies are needed to identify the molecular mechanisms leading to these early drug effects and determine their relevance to antipsychotic-induced weight gain and insulin resistance seen clinically.
Chapter 5

Olanzapine rapidly shifts peripheral fuel selection and increases whole-body lipid fuel oxidation

5.1 INTRODUCTION

Second generation antipsychotic drugs, including olanzapine, are a chemically diverse class of medications prescribed for a variety of mental illnesses and lack the sedative and movement side effects that plagued first generation compounds (206, 207). While their clinical utility continues to rise (208-210), atypical antipsychotics have their own unique metabolic side effects; including, body weight gain (e.g. 21, 81, 211, 212), type 2 diabetes (e.g. 213, 214, 215), dyslipidemia (e.g. 216, 217) and hypertension (e.g. 33, 191, 218). In general, the mechanisms leading to these metabolic side effects remain unknown, though animal studies have suggested that insulin resistance is an early drug effect and is independent of increased body weight, hyperphagia or adiposity (77, 116, 121). Insulin resistance is a key component of the metabolic syndrome in humans, as it plays a major role in diabetic, obesigenic, and atherosclerotic propagation.

Metabolic flexibility, which is strongly correlated with insulin sensitivity, is the ability to switch between carbohydrate and fatty acid fuels in the well-fed and post-absorptive states, respectively. The ability to switch between glucose and fatty acid fuels is reciprocally regulated, a phenomenon known as the Randle Cycle (219). Randle and colleagues (220) demonstrated that the end products of fatty acid oxidation (ATP and acetyl-CoA) allosterically inhibit key glycolytic enzymes, namely the pyruvate dehydrogenase complex and phosphofructokinase to limit glucose oxidation via glycolysis when fatty acid oxidation was increased. Years later, it was
shown that another level of cellular regulation exists, involving the glucose-derived metabolite malonyl-CoA. In contrast to the Randle Cycle, when glucose and the insulin:glucagon ratio are high, malonyl-CoA concentration is increased within the cell. Malonyl-CoA effectively shuts down entry of long-chain fatty acyl-CoA entry into the mitochondria via allosteric inhibition of Carnitine Palmitoyl Transferase-1 (CPT-1), thus limiting beta-oxidation (221). Thus, cellular malonyl-CoA concentration is an important determinant of cellular energy production; increased malonyl-CoA rises with feeding and inhibits fatty acid oxidation, which causes the cell to rely on glucose metabolism for ATP-generation. However, inhibition of fatty acid oxidation may increase the build-up of long-chain acyl-CoA intermediates, which might have negative effects on insulin signaling (222). The Randle Cycle and/or malonyl-CoA/CPT-1 axis appear to be perturbed in states of metabolic inflexibility, namely insulin resistant, type II diabetics, who paradoxically oxidize a higher percentage of glucose than fatty acids in the post-absorptive state (223-225). As the atypical antipsychotics have recently been shown to affect insulin sensitivity and whole-body glucose disposal (116, 122), the potential role of metabolic fuel selection or ‘flexibility’ in these rapidly developing effects are unknown.

We report here the effects of chronic olanzapine administration on plasma FFA, glycerol and whole body insulin tolerance. To help understand the mechanism underlying the lower FFA after chronic treatment, the acute effects of olanzapine on whole-body and tissue-specific fuel uptake and utilization were examined. We demonstrate that olanzapine can switch fuel utilization in peripheral tissues from carbohydrate to oxidation of fatty fuels with amazing rapidity, thereby increasing whole-body lipid utilization while at the same time blunting lipolysis. Potential mechanisms of this effect are discussed along with their potential involvement in drug-induced insulin resistance.
5.2 RESULTS

5.2.1 Effect of Olanzapine on Plasma FFA and Insulin Sensitivity

As previously reported by others (108), chronic olanzapine treatment increased adiposity (not shown) and impaired insulin tolerance as evidenced by a reduction in the Insulin Tolerance Test-AUC (Table 5-1). Chronic olanzapine treatment also lowered plasma FFA and glycerol, despite increased adiposity.

To investigate the mechanism underlying these effects, studies were performed in rats acutely treated with olanzapine. Comparable changes in the ITT-AUC were also observed after acute olanzapine treatment, ruling out increased adiposity as a potential causative factor. Further, acute olanzapine decreased glucose infusion rate (whole-body glucose uptake, $R_g$) during a euglycemic-hyperinsulinemic clamp (Table 5-1; in agreement with 116, 118) also suggesting an effect on whole-body insulin action. Despite the apparent insulin resistance, FFA concentrations were ~40% lower in olanzapine-treated rats (Table 1). As mentioned already, the lower FFA concentrations were unexpected, given that insulin resistance is usually associated with increased not decreased plasma fatty acids (226).
<table>
<thead>
<tr>
<th>Condition</th>
<th>Treatment</th>
<th>Dose mg/kg/day</th>
<th>Food Restriction</th>
<th>Endpoint</th>
<th>Control</th>
<th>Olanzapine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>Ramp, 4-12³</td>
<td>14h</td>
<td>FFA</td>
<td>0.91 ± 0.05</td>
<td>0.58 ± 0.04***</td>
</tr>
<tr>
<td>(Chronic)</td>
<td></td>
<td></td>
<td></td>
<td>Glycerol</td>
<td>1.79 ± 0.13</td>
<td>1.34 ± 0.12*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ITT-AUC</td>
<td>6,226 ± 564</td>
<td>3,797 ± 569**</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>5h</td>
<td>FFA</td>
<td>0.63 ± 0.05</td>
<td>0.38 ± 0.02***</td>
</tr>
<tr>
<td>(Acute)</td>
<td></td>
<td></td>
<td></td>
<td>Glycerol</td>
<td>1.95 ± 0.08</td>
<td>1.61 ± 0.05**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ITT-AUC, GIR</td>
<td>6,054 ± 344</td>
<td>4,436 ± 415**</td>
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<td></td>
<td></td>
<td></td>
<td>81.4 ± 5.3</td>
<td>43.2 ± 5.5***</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.E. (n = 10-12); asterisks indicate significant differences from time-matched controls (*P<0.05, **P<0.01, ***P<0.001). Parameters reported in SI units: FFA, mM; glycerol, mM; (GIR) Glucose Infusion Rate, μmol/kg/min, ITT-AUC (Time · % Change in Baseline Glucose). ³Ramped dosing protocol for chronic studies detailed in ‘Materials and Methods’.

Female rats were also treated with olanzapine acutely and similar decreases were observed in FFA (0.63 ± 0.04 vs. 0.51 ± 0.04 mM, P<0.05), glycerol (1.89 ± 0.15 vs. 1.38 ± 0.10 mM, P<0.01) and ITT-AUC (6,598 ± 188 vs. 4,639 ± 311, P<0.001). The magnitude of the FFA lowering effect appeared greater in male than female rats (40% vs. 20%, respectively).

A lower FFA concentration in the postabsorptive state could be due to impaired lipolysis and/or increased fat oxidation. Plasma glycerol concentrations were also depressed by olanzapine (Table 5-1) in both chronic and acute studies suggesting olanzapine impairs lipolysis in vivo, in agreement with previous in vitro studies (105). These findings indicate that both acute and chronic administration of olanzapine may impair lipolysis, which could explain the increased adiposity after chronic administration that we observed (data not shown) and as previously
reported (105). However, this conclusion does not exclude the possibility that chronic olanzapine treatment might also increase fat oxidation. Consistent with this idea, the olanzapine-induced reduction of FFA and glycerol appeared greater in rats treated chronically with olanzapine (FFA decreased 37% vs. 25% for glycerol) than acutely (FFA decreased 40 vs. 18% for glycerol).

5.2.2 Plasma Metabolites in Fed and Food-Deprived Rats

Nutrient and hormone concentrations change depending on the nutritional state. To provide additional mechanistic insight for the lowering of the FFAs, we examined the effect of acute olanzapine on a panel of nutrients during the transition from fed to fasting (Figs 5-1 and 5-2). However, the design of this study was complicated by olanzapine’s relatively short half-life in rats [$t_{1/2} \sim 2h$ (109), compared to humans, $t_{1/2} \sim 21-54h$]. We opted for a design in which the plasma concentration of olanzapine was near its peak at every time point and this required the use of different cohorts of animals for each time point. Continuous infusion via mini-pumps was not used since olanzapine is unstable in solution and this results in unstable plasma levels (111). As detailed in Experimental Procedures, plasma samples were drawn in the morning, 2h after the second dose of olanzapine from either fed rats or different cohorts of rats that were food-deprived for the indicated time prior to the morning blood draw.

As seen in Figure 5-1A, blood glucose concentration declined as expected with increasing food-restriction. Olanzapine acutely raised the blood glucose about 20%, but did not affect its rate of decline following food-restriction. Despite the elevated blood glucose, plasma insulin concentrations were not different in the fed state (Fig 5-1B). As expected, insulin concentration dropped sharply with food-restriction in control, but not as drastically in olanzapine-treated animals, consistent with whole-body insulin resistance indicated by the ITT and clamp studies (Table 5-1). In the olanzapine group, insulin remained at fed levels during the
first 5h of food deprivation; it was elevated by 40% and 140%, respectively, after 5h and 14h of food-restriction compared to the time-matched controls.

**Figure 5-1:** Circulating glucose, insulin, lactate, and alanine concentrations in fed and food-deprived male rats. Blood samples were collected in different cohorts of animals in the fed state (time 0), or after 5h or 14h of food-restriction. All animals received either olanzapine (10 mg/kg) or vehicle via oral gavage two hours prior to blood sampling, as described in the ‘methods’. (A) Glucose was measured from whole blood, while (B) insulin, (C) lactate, and (D) alanine were measured in plasma. All data represent the mean ± S.E. (n = 10-20); asterisks indicate significant differences (**P<0.01, *P<0.05) compared to time-matched controls.

The gluconeogenic substrate lactate (Fig 5-1C) did not differ between fed or 5h food-restricted animals, though a significant decrease was observed after 14h of food-restriction. The
major glucogenic amino acid alanine (Fig 5-1D) did not differ between fed or 5h food-restricted animals, though a small but significant increase in plasma alanine was observed after 14h of food-restriction. Circulating concentrations of the branched-chain amino acids did not differ between groups in any nutritional state (not shown). A normal (900%) increase in plasma beta-hydroxybutyrate concentration was observed with prolonged fasting (14h), but no differences were observed between the experimental groups (Fig 5-2B).

In contrast to branched-chained amino acids and glucose, the effects of olanzapine on the plasma concentrations of lipid fuels (i.e. triglycerides and FFAs) differed depending on the metabolic state. Plasma triglycerides in the fed state tended to be lower after olanzapine, with the olanzapine group being significantly lower than control in some but not all of three separate experiments (data not shown). Triglycerides (Fig 5-2A) were consistently lower after five hours of food restriction, with olanzapine-treated animals reaching ‘fasting’ triglyceride levels (14h) more rapidly than control animals, suggesting that triglycerides were being used more rapidly during food-deprivation by the olanzapine group. FFA (Fig 5-2C) and glycerol (Fig 5-2D) were not significantly affected by olanzapine in the fed state. However, expected rises in these metabolites following food restriction were blunted by olanzapine, in agreement with data from a separate cohort of rats presented in Table 5-1. Both FFA and glycerol were significantly lower in the olanzapine group after 5h and 14h of food-restriction. Collectively, olanzapine lowers plasma concentrations of triglycerides, FFA and glycerol in the fed and/or food-restricted states, while glucose remains elevated in the presence of persistent hyperinsulinemia.
Figure 5-2: Circulating triglycerides, β-hydroxybutyrate (BHBA), FFAs and glycerol in fed and food-restricted male rats. Blood samples were collected in different cohorts of animals in the fed state (time 0), or after 5h or 14h of food-restriction. All animals received either olanzapine (10 mg/kg) or vehicle via oral gavage two hours prior to blood sampling, as described in Chapter 2, 'Materials and Methods'. (A) Triglycerides, (B) β-hydroxybutyrate (C) FFA, and (D) glycerol were measured in plasma. All data represent the mean ± S.E. (n = 10-20); asterisks indicate significant differences (***P<0.001, *P<0.05) compared to time-matched controls.
5.2.3 Effects of Olanzapine on Metabolic Fuel Preference

One explanation for the above results could be that olanzapine shifts whole-body fuel metabolism toward utilization of FFA from plasma FFA and triglycerides. Such “fuel switching” might explain the concurrent decrease in lipid fuels (FFAs, triglycerides) and accumulation of plasma glucose following acute olanzapine administration. This hypothesis was tested by examining the respiratory exchange ratio (RER), the ratio of CO2 produced to O2 consumed, which provides information on the fuel mixture being utilized for whole-body energy production. RER fluctuates in response to feeding and fasting, approaching a value of 1.0 when carbohydrate is oxidized and a value of 0.7 indicating exclusive lipid oxidation.

Indirect calorimetry was used to examine the effects of olanzapine on RER in three separate experiments. The first examined the effects of olanzapine in fed rats (Fig 5-3A), the second examined the fed to fasted transition (Fig 5-3B) and the third measured RER in fasted and refed rats (Fig 5-3C and D). In fed rats the RER increased during the dark cycle (Fig 5-3A), which is normal and considered a result of food intake. However, olanzapine administration (indicated by arrows) elicited a rapid decrease in RER that was absent in vehicle controls [RER significantly different between 0.75-3.75 hours]. The olanzapine-mediated decline in the RER slowly returned to control levels over the course of the dark cycle with a time course that was consistent with the half-life of olanzapine in rats (109). When olanzapine was re-administered shortly after the beginning of the light cycle, it again decreased the RER, which again returned to control values over the same time course [RER significantly different between 14.5-15.5 hours]. The 24h food intake did not differ between control and olanzapine-treated animals [27.2 ± 1.0 vs. 26.3 ± 0.9 g] during this 24h period (Fig 5-3A). Experiments were continued for 36h in a subset of the animals to determine if the robust RER decrease was a short-lived phenomenon, but, as seen in
Figure 5-3: Effects of olanzapine on respiratory exchange ratio (RER) in ad libitum fed, food-restricted, and “fasting-refed” states. (A) Male rats were placed in metabolic chambers and subsequently received olanzapine via gavage while retaining ad libitum access to food and water. RER (A) was calculated based on expired CO$_2$ and O$_2$ consumed (n = 12). A subset of animals remained in the cages for up to 36h for additional measurements. (B) In another set of experiments, ad libitum fed animals were placed in metabolic chambers and then food-restricted. In a third experiment (C-D), animals were 14h food-restricted and gavaged with olanzapine (10 mg/kg) 45 minutes prior to the start of the dark cycle. Re-feeding began approximately 30 minutes after drug dosing. (C) RER and (D) food intake was measured during the experiment (n = 6). All data represent the mean ± S.E. Background shading or lack thereof indicates the dark and light cycles, respectively. Arrows indicate the time of drug gavage.
Fig 5-3A, a third dose of olanzapine at 25 hours was again able to elicit a decrease in RER comparable to that observed after the first dose. On average, RER in both the dark and light cycles was significantly lower in olanzapine animals compared to control due to the robust decreases following olanzapine. Consistent with the changes in plasma lipids, drug-treated animals appear to shift their whole-body substrate metabolism from a mixture of carbohydrate and fat to one in which energy expenditure is derived predominately from fat oxidation.

The effect of olanzapine on RER was also examined after food-restriction (Fig 5-3B). Ad libitum fed animals were given olanzapine or vehicle and re-placed in calorimetric cages without food. RER declined after food deprivation in control animals as expected. However, in the olanzapine group RER was already significantly lower by the time of the first measurement and remained lower than control values for 2.5h. Thereafter, there was no difference between the groups. By the end of the dark cycle both experimental groups had been food-restricted for ~12 hours and the RER in each group was ~0.7 indicating that animals were predominately using fat for fuel. Upon repeat gavage at the beginning of the light cycle and continued food restriction, there was no further decline in RER in the olanzapine treated group (Fig 5-3B).

In a third experiment, the effect of olanzapine was determined on refed rats after food-deprivation, often referred to as “fasted-refed”. Animals were food-restricted for 14h and re-fed approximately 30 minutes after oral olanzapine or vehicle administration. RER in the 14h food-deprived (a.k.a. fasted) rats (Fig 5-3C) was approximately 0.7. Olanzapine significantly blunted the rate of increase in RER with re-feeding (Fig 5-3C), even though food intake in the first 2h was the same between groups (Fig 5-3D) [RER significantly different from 3.0 to 7.25h]. Consistent with previous studies, 12h food intake, like 24h food intake, did not differ between control and drug-treated animals (Fig 5-3D). Overall, acute, orally administered olanzapine causes a rapid and robust decrease in RER that reflects a ‘switch’ from the oxidation of mixed but
mostly carbohydrate fuels (RER = ~0.9, consistent with their 6% fat diet) to oxidation of lipids (RER = ~0.7).

5.2.4 Triglyceride Clearance and Hepatic Secretion after Olanzapine

To confirm our observation that olanzapine preferentially switches utilization to lipid fuels from carbohydrates, the effect of olanzapine on oral lipid tolerance was examined. The lipid challenge test is usually performed using olive oil in food-restricted animals. Therefore, animals were food-restricted overnight (14h) and provided an oral gavage of olive oil. Baseline triglyceride concentrations (t = 0) were similar to early experiments (Fig 5-3A) and did not differ between groups after 14h of food restriction. Following lipid gavage, plasma triglycerides increased and reached their peak concentration at approximately 2h in both control and drug-treated groups (Fig 5-4A). Peak concentrations were lower after olanzapine treatment. Overall, the AUC for plasma triglycerides during the lipid tolerance test was significantly decreased by olanzapine treatment (Fig 5-4B), which suggests that olanzapine increases the clearance of orally administered triglycerides.

As plasma triglycerides are also derived from hepatic secretion, the effect of olanzapine on the hepatic secretion of VLDL-triglyceride particles was measured. Using intravenously administered tyloxapol to inhibit lipoprotein lipase, the increase in plasma triglycerides can be measured and used to estimate the hepatic rate of VLDL-triglyceride secretion. As seen in Figure 5-4C, the increased triglyceride concentration following tyloxapol injection is near linear and was not different between control and olanzapine-treated groups; calculated VLDL-secretion rates did not differ (Fig 5-4D). Collectively, our data suggest that the observed decrease in plasma triglyceride concentrations observed in fed and food-restricted rats following olanzapine is most likely due to increased plasma clearance rather than decreased hepatic secretion.
Figure 5-4: Effects of olanzapine on triglyceride metabolism. (A-B) Oral lipid tolerance tests were conducted in narrow-weight range male rats after 14h of food restriction. An oral gavage of olive oil (6 ml/rat) was administered as a single bolus on the second day of olanzapine treatment (10 mg/kg). (A) Plasma triglycerides at times before and following the olive oil challenge. (B) Area under the curve for plasma triglycerides (n = 20). (C) Hepatic triglyceride secretion in 14h food-restricted male rats. Baseline blood samples were collected and Tyloxapol (600 mg/kg) was then administered intravenously to inhibit lipoprotein lipase and thus triglyceride clearance. Serial blood samples were collected at 15, 30, 60 and 90 minutes post-injection to measure the rise in plasma triglycerides and calculate the (D) rate of hepatic VLDL-triglyceride secretion. Data represent the mean ± S.E. (n = 6-7), asterisks indicate significant differences (***P<0.001).
5.2.5 Tissue-specific FFA and Glucose Uptake

The above data indicate that olanzapine increases whole-body lipid fuel utilization, but it is unclear whether this is a tissue-specific phenomenon or generalized effect on all or most peripheral tissues. This was addressed by measuring tissue fatty acid uptake in vivo using the radiolabeled, non-metabolizable fatty acid analog [$^{125}$I]-BMIPP (Fig 5-5, panels A, C, E) as previously described (125). Glucose uptake was simultaneously measured using the [$^3$H]-2-deoxyglucose technique (Fig 5-5, panels B, D, F). [$^{125}$I]-BMIPP and [$^3$H]-2-deoxyglucose were given as a bolus i.v. injection in the morning shortly after the beginning of the light cycle to animals that had ad libitum access to food. Fed rats were used because under fed conditions FFA concentrations were the same in vehicle- and olanzapine-treated groups, as opposed to after overnight food-restriction when there are large differences between the FFA concentration in control and drug-treated animals (Fig 5-3). Here, differences in FFA uptake cannot be attributed to differences in the prevailing plasma FFA concentration between groups. As seen in Figure 5-5A, olanzapine increased FFA uptake by 120% in gastrocnemius and soleus, which are representative fast- and slow-twitch muscle. Two tissues with normally high rates of fatty acid oxidation, namely the liver and kidney, showed similar 100-120% increases in FFA uptake (Fig 5-5C). Interestingly, another tissue with a high rate of fatty acid oxidation, the heart, showed no significant difference in FFA uptake (Fig 5-5A). FFA uptake was also increased 80-120% in the proximal duodenum and terminal ileum as well as skin (Fig 5-5D). As expected, brain FFA uptake was too small to determine any affect of olanzapine reliably (<0.1 nmol/g tissue/min). Thus, FFA uptake was increased in all peripheral tissues, with the exception of the heart, irrespective of whether the tissue was dependent or independent of insulin for glucose transport.

Glucose uptake in the fed state was not different in the two skeletal muscles (Fig 5-5B). Because of the insulin resistance produced by olanzapine, one might have expected muscle $R_g$ to
Figure 5-5: Tissue uptake of FFA and glucose following olanzapine administration. The non-metabolizable fatty acid and glucose analogs, $[^{125}I]$-BMIPP and $[^3H]$-2-deoxyglucose, respectively, were used to measure fatty acid and glucose uptake in *ad libitum* fed male rats. At least 4 days prior to experiments, arterial and venous catheters were implanted in male rats. On the second day of olanzapine (10 mg/kg) or vehicle treatment, a bolus i.v. injection of fatty acid and glucose tracers was administered and blood samples drawn for calculation of tracer specific activity during a 40-min labeling period. Tissues samples were counted for radioactivity and calculation of FFA uptake (FFA Metabolic Rate = nmol/g tissue/min) and glucose uptake ($\mu$mol/g tissue/min). Tissues sampled included (A) gastrocnemius, soleus and cardiac muscle, (B) liver and kidney, and (C) proximal duodenum, terminal ileum, and skin. Data represent the mean ± S.E. (n = 8), asterisks indicate significant differences (*P<0.05, **P<0.01), compared to time-matched control values.
be reduced. However, it should be remembered that while insulin was similar in both groups in the fed state, the plasma glucose is elevated in the olanzapine group, which would increase $R_g$ by mass action. In contrast to skeletal muscle, glucose uptake by the heart was greatly depressed in the fed state by olanzapine (Fig 5-5C); AKT activity (phosphorylation state, Ser$^{473}$) in the heart and gastrocnemius tissues was similar between olanzapine and control (data not shown). The decreased cardiac glucose uptake suggests that the degree of insulin resistance is greater in the heart than skeletal muscle after olanzapine. The liver and kidney, where glucose transport is mostly insulin-independent, showed an increased glucose uptake following drug treatment (Fig 5-5D), presumably due to elevated plasma glucose (i.e. mass action effect). The intestine and skin are also insulin independent, but were assessed because they are responsible for a significant fraction of whole-body glucose utilization in normal rats, ~16-40% depending on metabolic state (133). Glucose uptake did not differ in either the gut or the skin (Fig 5-5F), although there was a trend for increased glucose uptake in skin ($P = 0.09$). Brain glucose uptake did not differ between groups (not shown).

5.2.6 Olanzapine and Acetyl-CoA Carboxylase

Our data thus far collectively demonstrate that olanzapine induces a generalized effect on most tissues to rapidly switch the metabolic ‘fuel’ that is being oxidized for fuel from mostly carbohydrates to almost entirely fats. It is noteworthy that these robust effects are observable within minutes after olanzapine gavage, suggesting that olanzapine, and not a metabolite is responsible for this phenomenon. One type of compound that can elicit a similar rapid decreases in RER are inhibitors of acetyl-CoA carboxylase (ACC), the enzyme which catalyzes the irreversible carboxylation of acetyl-CoA to form malonyl-CoA (227). We hypothesized that olanzapine directly inhibits ACC, given the similarities between the effects on RER of olanzapine
compared with ACC inhibitors. To test this hypothesis, \textit{in vitro} assays were conducted with human ACC1 and ACC2, which share >85% homology with rodent ACC isoforms (228-231), to measure the incorporation of radiolabeled bicarbonate into malonyl-CoA (Fig 5-6). The known ACC inhibitors Soraphen A (232) and CP-640186 (227) were used as positive controls and demonstrated marked inhibition of both hACC1 and hACC2. The IC$_{50}$ values of Soraphen A for ACC1 and ACC2 were 3.1 and 7.3 nM, respectively. CP-640184 was less potent than Soraphen A, but still produced frank inhibition of ACC1 and ACC2, with IC$_{50}$ values of 234 and 38 nM, respectfully. Olanzapine showed no inhibition of either enzyme over a wide range of drug concentrations.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5-6.png}
\caption{\textit{In vitro} inhibition assay of human acetyl-CoA carboxylase isoforms hACC1 and hACC2. Overall ACC activity in the hACC preparations was measured by following incorporation of $^{14}$C-bicarbonate into malonyl-CoA. Potential inhibition of hACC1 and hACC2 by olanzapine, with concentrations of olanzapine up to 135 \textmu M, and inhibition by the known ACC inhibitors Soraphen A and CP-640186 was measured.}
\end{figure}
5.3 DISCUSSION

The present study demonstrates for the first time that olanzapine is associated with rapid decreases in the plasma concentrations of fatty fuels, independent of an acutely developing insulin resistance. Both non-esterified (FFA) and esterified (triglycerides) fatty acids were affected. Olanzapine appears to bring about these effects by increasing whole-body lipid utilization, while at the same time impairing in vivo lipolysis. Compensatory increases in hepatic triglyceride secretion, to balance the increased utilization of triglyceride were not apparent in the drug-treated animals. Stimulation of whole-body lipid utilization by olanzapine is demonstrated by a rapid and robust lowering of the RER after olanzapine along with decreases in plasma FFA and triglycerides following food restriction, increased uptake of FFA into most peripheral tissues, and improved oral triglyceride tolerance. The ability to mobilize plasma glycerol and FFA after food deprivation was also impaired. In contrast to other peripheral tissues, olanzapine did not increase FFA uptake into the heart and appeared to produce cardiac insulin resistance. The insulin resistance appears to be caused at some point distal to the early steps in insulin signaling, as AKT phosphorylation was not affected.

5.3.1 Effects of Olanzapine on FFA and Lipolysis

Previous studies examining blood lipids or RER after a few days or shorter periods of olanzapine treatment in humans or rats are limited. However, reductions in plasma FFA and glycerol concentrations were observed in a metabolomics study on plasma of patients treated with olanzapine for 2-3 weeks (203), which is in agreement with our findings. Consistent with our conclusion that the reduction in plasma FFAs is partially due to impaired in vivo lipolysis, olanzapine has been reported to block lipolysis in isolated rat adipocytes in vitro (105).
In addition to the effects on lipolysis, olanzapine lowered fasting plasma FFA and triglyceride levels by increasing whole-body lipid utilization. It seems likely that the transient nature of this effect results from olanzapine’s short half-life in rats. Further studies, in which olanzapine is given continuously will be needed to verify this observation. While we only examined whole-body calorimetry for 36h, the magnitude of the change in RER was not attenuated after the second and third doses of olanzapine. Again, further studies are needed to determine whether this effect persists with chronic administration.

5.3.2 Olanzapine and Metabolic Fuel Selection

The present study clearly demonstrates that olanzapine affects whole-body metabolic flexibility, the normal ability to adjust fuel oxidation to match fuel availability (233); for example, as observed during the switch from fasted to fed states. In the food-restricted state, healthy individuals mostly oxidize FFAs for fuel and preserve glucose, which is reflected by low RER values (~0.7). Upon feeding or insulin-stimulation (e.g. during hyperinsulinemic-euglycemic clamp), these values robustly swing towards 1.0, reflecting increased glucose oxidation and decreased fat oxidation. In contrast to healthy individuals, those with insulin resistance typically have a restricted ability to make these shifts; this is referred to as metabolic inflexibility. In such individuals, fasting RER is more elevated with fasting compared to healthy individuals and RER does not increase as much with feeding. Olanzapine caused insulin resistance but also a unique kind of metabolic inflexibility in most of the peripheral tissues. Rather than shifting the animals into midrange RERs, olanzapine blocked expected elevations in RER with feeding. ACC inhibition is associated with enhancement of fat oxidation, which is considered beneficial (234, 235), and therefore has been implicated as a potential therapeutic target for diabetes and obesity. However, enhanced fat oxidation by olanzapine is strongly
associated with insulin resistance. Regardless, we are not aware of other drugs or conditions that possess this rapid and unusual effect on fat utilization and/or simultaneous impairment of lipolysis.

The mechanism by which olanzapine switched whole-body fuel oxidation from mainly carbohydrate to mostly lipid is not yet known; however, observations from this study may provide some insight. For example, it could be hypothesized that the ability of olanzapine to acutely produce insulin resistance and thereby reduce glucose uptake, forces peripheral tissues to rely more heavily on long-chain fatty acids as metabolic fuels. While this is not consistent with the lack of effect on the heart, the heart may have already achieved maximal FFA uptake (236). Two observations argue against a causative role of insulin resistance in the switch to lipid fuel. First, olanzapine increased FFA uptake into tissues that are not dependent on insulin for glucose uptake – namely kidney, liver, gut, and skin. Second, during a time course study, the time to achieve a 50% reduction in insulin-stimulated glucose uptake (i.e. a measure of insulin resistance) following intravenous olanzapine was approximately 90 minutes with a maximal effect appearing at 2h (116); another study showed similar results (118). In contrast, the time to half-maximal effect on RER was ~30 min following the same dose of olanzapine, with a maximal effect on RER observed after ~60 min. We provided olanzapine orally but, assuming the target of olanzapine is the peripheral tissues, oral administration should increase not decrease these effect times compared to intravenous administration. Collectively, these findings suggest the switch to lipid oxidation is independent of the drug-induced insulin resistance.

Notably, with the exception of ACC inhibitors, there are few examples of drugs or conditions in which whole-body metabolism switches so rapidly in the well-fed state and we can only speculate on how olanzapine mediates these effects. Olanzapine did not exhibit any inhibitory effects on human ACC isoforms, thus it is likely that the highly homologous rat isoforms (>85%) would not be inhibited. The rapidity of this effect argues against a metabolite of
olanzapine mediating the changes in fuel oxidation, however this possibility still exists. Moreover, it is also unlikely that this effect is mediated by the olanzapine-induced increase in synthesis of a new protein. Besides this possibility, few other mechanisms would be thought to lead to such rapid changes. On the cellular level, the ability to switch between carbohydrate and fat fuel oxidation (i.e. metabolic flexibility) may involve pyruvate dehydrogenase (PDH) and/or AMP-activated protein kinase (AMPK) (233, 237). Inhibition of PDH can affect substrate utilization by restricting tissues to oxidation of fatty acids, effectively preserving glucose and other three carbon metabolites for gluconeogenesis (238). Thus, a direct effect of olanzapine on PDH or a PDH regulator (e.g., one or more of the PDH kinases or phosphatases) could explain these effects. An effect on a PDH regulator seems more plausible, given that a global inhibition of PDH including the brain would most likely lead to CNS effects (e.g. seizures) related to the inability of neurons to oxidize glucose. Alternatively, fuel switching might occur if olanzapine lowered cellular concentrations of the carnitine-palmitoyl transferase-1 (CPT-1) inhibitor, malonyl-CoA, by accelerating its metabolism or decreasing its synthesis. Olanzapine activation of AMPK or an AMPK regulator (239) might also affect malonyl-CoA concentrations. However, evidence of differential AMPK activation or phosphorylation of its downstream target, ACC, was not observed in heart or skeletal muscle in the fed or 14h food-restricted conditions (data not shown).

5.3.3 Increased Fatty Fuel Utilization and Insulin Resistance

As the effect of olanzapine on RER may precede the effects on insulin resistance, it is temping to speculate that the increased fatty acid uptake into skeletal muscle underlies the whole-body insulin resistance. There are several potential mechanisms through which increased fatty acid uptake might lead to the development of insulin resistance; including the Randle Cycle
('Glucose-Fatty Acid Cycle') (220), protein kinase C activation via increased intracellular fatty acids and fatty acyl-CoAs (240-242), as well as increased incomplete fatty acid oxidation due to mitochondrial overload (243). A metabolic cause is appealing, as AKT phosphorylation was unaffected in either muscle or heart. Further studies are needed to explore the tissue metabolomic effects of olanzapine to better understand the potential role of one or more of these mechanisms in the development of the insulin resistance.

Collectively, the present study has demonstrated a new metabolic effect of olanzapine. That is, olanzapine causes a rapid shift in the major fuel being oxidized by peripheral tissues from mostly carbohydrate to mostly fat while insidiously preventing the mobilization of that fuel. After food-deprivation, and perhaps by extension between meals, these actions of olanzapine more rapidly deplete lipid fuel than would otherwise occur. Olanzapine also caused whole-body insulin resistance and this preferentially affected the heart more than skeletal muscle. The shift in fuel utilization appears to precede the development of insulin resistance and therefore has the potential to explain its development. The enhanced oxidation of fatty fuels appears to precede the development of insulin resistance and, therefore, may underlie its development. In contrast, because higher peripheral fat oxidation is generally associated with lean body composition and good health, the fat burning effects of olanzapine could be leveraged if the mechanism(s) for insulin resistance, diabetes and anti-lipolysis can be separated from that responsible for the increased fat oxidation.
Chapter 6

Olanzapine may increase cardiac risk by depleting fatty fuels, cardiac insulin resistance, anti-lipolysis and tachycardia

6.1 INTRODUCTION

Olanzapine and other atypical antipsychotics are widely known to have numerous unexpected metabolic side effects related to insulin resistance and obesity. The mechanisms of these side effects have been difficult to identify, as the atypical drugs are known to antagonize numerous G-protein coupled receptors expressed both peripherally and centrally (46, 244). These side effects are likely mediated through multiple types of receptors, and this promiscuity has made determining which receptor mediates each of these metabolic side effects even more challenging.

Recently a new side effect of these drugs has emerged; they increase the risk of ventricular arrhythmias and sudden cardiac death (245). Because the particular type of arrhythmia induced involves prolongation of the QT interval, it has been speculated that these drugs may affect K+-channels. Indeed, first generation antipsychotics are known to affect re-polarizing currents and are also associated with an increased risk of sudden cardiac death. However, QT interval prolongation can also occur during malnourishment or other situations that interfere with the production of ATP and 2,3-diphosphoglycerate, vital for many cellular processes.

We became interested in the cardiac side effect during efforts to characterize the chronic metabolic effects of olanzapine, in which decreased plasma concentrations of the heart’s preferred fuel, FFA, were noted in several cohorts of animals. This observation was surprising
given the strong association of olanzapine and insulin resistance, a state in which FFAs are typically elevated. However, a similar lowering of FFAs has recently been observed in olanzapine-treated patients (203). Subsequently, we demonstrated that olanzapine rapidly increases lipid fuel utilization, which decreases availability of fatty fuels in the plasma with brief food restriction. Free fatty acids are the preferred fuel of the heart, and whether decreasing the availability of the preferred cardiac fuel has functional consequences is unknown.

Metabolically, the heart is a complex, insulin-sensitive tissue that utilizes glucose and FFAs for fuel. In the adult heart as much as 90% of ATP generated can be derived from fatty acid oxidation, though in the normal physiologic state this value typically ranges between 60-80% (246). Regardless of the condition, glucose remains an important substrate for generation of ATP not met by lipid oxidation, as well as under pathological conditions that limit aerobic metabolism (e.g. ischemia). The reciprocal relationship between glucose and fatty acid metabolism in the heart is unique because, unlike other muscles, changes in work (i.e. contractility, cardiac output, wall tension) must be quickly compensated or energy deficits will ensue and lead to failure, end-organ damage (inadequate perfusion), and possibly death. In addition to maintaining contractile function, ATP production must remain relatively constant to support the electrophysiological processes intrinsic to the heart, or risk the development of arrhythmias. Accordingly, impaired myocardial metabolism is associated with several cardiomyopathies, including diabetic cardiomyopathy, heart failure and ventricular hypertrophy. Evidence suggests that metabolic perturbations lead to functional defects, though metabolic changes also appear to be compensatory to pathologic stresses (reviewed in 247, 248, 249).

Overall, in the following chapter we show that in addition to depleting the heart’s preferred fuel olanzapine simultaneously increases cardiac work. The increase in whole-body lipid utilization that decreases the plasma availability of fatty fuels is also associated with severe cardiac insulin resistance, which appears to affect the heart more so than other tissues, making it
more difficult to obtain glucose. The metabolic impact of decreasing fuel availability while increasing fuel requirements for an insulin resistant heart is unknown at this time. Thus we propose that, collectively, these metabolic effects might help explain the increased risk of sudden cardiac death observed in patients taking olanzapine, as well as other atypical antipsychotics.

### 6.2 RESULTS

#### 6.2.1 Olanzapine and Cardiac Function

As olanzapine seemed to affect nutrient uptake in the heart differently compared to other tissues in previous studies (Chapter 5, Fig 5-5), it was not clear if these differences might affect cardiac function or metabolism during the fed to fasting transition. In particular, we questioned whether our previous observations (Chapter 5) of reduced uptake of glucose in the fed state or limited availability of fatty acid fuels during the fed to fasting transition had functional consequences. To address this, heart rate and blood pressure were measured in conscious, unrestrained animals via a centrally placed arterial catheter after 14h of food-restriction, a time when fatty fuels are limited. Compared with control animals, acute olanzapine administration increased heart rate by ~80 beats per minute, without a change in mean arterial blood pressure (Fig 6-1). Estimated cardiac energy expenditure calculated as the ‘double-product’ (heart rate multiplied by blood pressure), a clinically reliable index that strongly correlates with cardiac energy expenditure, was increased following olanzapine treatment [Con: 4.04 ± 0.34 vs. Olz: 5.30 ± 0.31 (x 10³ pulse-mmHg, P<0.05], (250, 251). Echocardiography was performed immediately following heart rate and blood pressure measurement to examine functional (i.e. stroke volume) and structural parameters. As expected, no structural differences were detected in hearts (Table 6-1) following acute drug treatment (e.g. posterior wall thickness, intraventricular septal
thickness, left ventricular mass). Stroke volume did not differ between olanzapine and control groups. However, cardiac minute work was increased by olanzapine \([1.64 \pm 0.18 \text{ vs. } 2.62 \pm 0.35 \times 10^7 \text{dyne}\cdot\text{cm/min}], P<0.05\) \((251)\).

**Figure 6-1:** Effects of olanzapine on heart rate, blood pressure, and cardiac function in male rats. (A) Heart rate and (B) mean arterial blood pressure were measured in unrestrained, conscious animals acutely treated with olanzapine, 2h after their last dose, using a pressure transducer attached to an intra-arterial catheter. Echocardiography was used to examine functional and structural parameters in the same animals. Representative M-mode recordings in (C) control and (D) olanzapine animals are shown. An asterisk indicates \((*P<0.05)\) significant differences compared to time-matched control values. Data represent the mean ± S.E. \((n = 6-7)\).

Based on glucose uptake measurements in the fed state, our previous work suggested that insulin resistance observed in the heart was more severe than that seen in skeletal muscle. To more thoroughly examine this possibility, we measured cardiac glucose uptake during a hyperinsulinemic-euglycemic clamp. Insulin-stimulated cardiac glucose uptake was decreased
42% by olanzapine (Fig 6-2A). Since inability to take up glucose by cardiac myocytes secondary to insulin resistance may compromise myocardial energy balance when fatty fuels become limited due to increased peripheral utilization. Thus, we sought to determine how cardiac energy balance was maintained in the food-deprived state (14h) when lipid fuels were limited and cardiac work was increased.

Figure 6-2: Effects of olanzapine on basal and insulin-stimulated cardiac glucose uptake. A 3-hour, hyperinsulinemic-euglycemic clamp was performed in acutely treated, 14h food-deprived male rats, beginning 120 min after their second dose of vehicle or olanzapine. All clamp studies were conducted at the same time of day. (A) Glucose infusion rate during hyperinsulinemia-euglycemic clamp studies. (B) Heart glucose uptake under ‘Clamp’ euglycemic conditions was measured in animals using a bolus injection of H-2-deoxyglucose tracer (n = 10-12). Heart glucose uptake during ‘Basal’ conditions after 14h of food-restriction was measured in a separate group of animals. Data represent the mean ± S.E. (n = 8), asterisks indicate significant differences (***P<0.001), compared with control values.

As seen in Figure 6-2, cardiac glucose uptake by the olanzapine group was markedly higher in the basal condition than in control animals, in contrast to the fed state. No changes in either AKT Ser\(^{473}\) phosphorylation nor in AMPK Thr\(^{172}\) phosphorylation were observed in the hearts or in the gastrocnemius, both for fed (Figure 6-3) and 14h food-deprived states (data not shown). No changes in AKT Thr\(^{308}\) phosphorylation state were detected in the fed and food-restricted states (data not shown). These findings suggest that olanzapine increases cardiac work
and produces myocardial insulin resistance. Whereas glucose uptake by the heart was impaired during the fed state, in the food-deprived state when the heart’s preferred lipid fuels were limited there was an increased rate of glucose metabolism, which is presumably insulin-independent and obligatory to maintain the increased work.

Figure 6-3: Effect of olanzapine on AKT and AMPK activity in skeletal muscle of *ad libitum* fed male rats. Gastrocnemius tissues were excised an immediately freeze-clamped approximately 2h from vehicle- or olanzapine-treated animals. Tissue homogenates were subjected to western blotting and quantified using chemiluminescence to measure the activity (ratio of phosphorylated isoform to total protein isoform) of (A) AKT (Ser473) (B) AMPK (Thr172). Representative images from actual blots are shown along with relative quantification representing the mean intensity ± S.E. (n = 8).
### Table 6-1: Echocardiographic parameters of vehicle- or olanzapine-treated male rats on the second day of acute olanzapine treatment (10 mg/kg).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Olanzapine</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV systolic dimension, mm</td>
<td>0.85 ± 0.37</td>
<td>1.21 ± 0.41</td>
<td>0.52</td>
</tr>
<tr>
<td>LV diastolic dimension, mm</td>
<td>5.28 ± 0.23</td>
<td>5.56 ± 0.33</td>
<td>0.53</td>
</tr>
<tr>
<td>LVPW thickness, mm</td>
<td>2.53 ± 0.24</td>
<td>2.33 ± 0.18</td>
<td>0.51</td>
</tr>
<tr>
<td>IVS dimension, mm</td>
<td>1.78 ± 0.10</td>
<td>1.69 ± 0.10</td>
<td>0.49</td>
</tr>
<tr>
<td>IVS/LVPW</td>
<td>0.74 ± 0.09</td>
<td>0.75 ± 0.06</td>
<td>0.87</td>
</tr>
<tr>
<td>LV stroke volume, ml</td>
<td>0.35 ± 0.04</td>
<td>0.41 ± 0.07</td>
<td>0.47</td>
</tr>
<tr>
<td>Left Ventricular Mass, g</td>
<td>1.23 ± 0.08</td>
<td>1.20 ± 0.05</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E. (n = 6-7). LV = left ventricle; LVPW = LV posterior wall; IVS = Intraventricular septum.

### 6.3 DISCUSSION

In animals and man, approximately 60-80% of the cardiac ATP production is due to fatty acid oxidation (252). Therefore, although fatty acids are the preferred cardiac fuel, in response to various conditions glucose may also be extensively metabolized, demonstrating the ability of this tissue to “substrate switch”. In some cases this shift is associated with cardiac pathology (reviewed in 253). As FFA and triglycerides became limited with food-restriction, an obligatory AMPK-independent increase in glucose uptake was observed, presumably required to maintain increased cardiac work, which was also stimulated by olanzapine. These changes in fuel
metabolism and cardiac work are reported in the context of a recent study demonstrating that olanzapine increases the risk of sudden cardiac death (245). Our findings may be relevant to that report since increased cardiac morbidity is frequently associated with any of the following: switching of cardiac substrates from lipid to carbohydrate (as we observed following food deprivation), tachycardia and whole-body insulin resistance, all of which were observed here in response to olanzapine (253).

Importantly, the changes in fuel utilization decreased the availability of the heart’s preferred fuel (i.e. FFA and triglycerides) and were observed in the setting of increased heart work and cardiac insulin resistance. The increased heart rate could potentially be explained as a compensatory response to the increased tissue oxygen requirements due to elevated FFA oxidation. However, in the 14h food-restricted state, RER was already low in the absence or presence of olanzapine (~0.7), and nevertheless heart rate remained elevated. Thus, a more likely mechanism for the tachycardia, a commonly observed effect in patients taking olanzapine, is olanzapine’s known anti-cholinergic action (205). It is unlikely, though, that the fuel switching or anti-lipolysis is an anti-cholinergic effect, as infusion of atropine does not affect plasma FFAs (254).
Figure 6-4: Physiologic fatty fuel partitioning in the ad libitum fed rat. In the prandial and post-prandial states the heart relies predominately on the oxidation of fatty fuels (i.e. free fatty acids and triglycerides) for the majority of cellular ATP production (~60-80%), unlike most other peripheral tissues (i.e. liver, skeletal muscle and adipose) which rely mainly on glucose in the well-fed state. Cardiac glucose uptake remains an important energy substrate in maintaining energy balance in the constantly working cardiac myocytes. As seen above, dietary fats enter the circulation as plasma fatty fuels and are then available to the peripheral tissues. The heart extracts a high fraction of plasma fatty fuels under these conditions relative to other peripheral tissues.

As FFAs became limited during food-restriction due to drug-induced increases in fatty acid oxidation, obligatory increases in glucose uptake were observed. The mechanism behind this apparent non-insulin dependent increase in glucose uptake is unknown and may reflect an adaptation to the metabolic stress; similar effects of olanzapine on glucose uptake have been suggested from in vitro studies (255). As mentioned earlier we did not observe evidence of
AMPK activation based on its phosphorylation pattern (Fig 6-3 and unpublished data).

Moreover, as the heart can also use lactate as an energy substrate (256, 257), this may help explain the observed decreases in plasma lactate (Fig 5-2C) with food-restriction in drug-treated animals.

**Figure 6-4:** Model for potential cardiac fuel starvation following olanzapine treatment. In the *ad libitum* fed state, olanzapine causes increased fatty acid uptake in peripheral tissues, including skeletal muscle, liver, and adipose tissue. This increased flux of fatty fuels (FFA and triglycerides) into the peripheral tissues causes a smaller fraction of circulating plasma fatty fuels to be available to the heart for energy production. During the transition to the post-absorptive state plasma fatty fuels become depleted secondary to increased peripheral usage and an already insulin resistant heart must increasingly rely on glucose oxidation to maintain cardiac energy balance.

Overall, it is tempting to speculate on the clinical relevance of the cardiometabolic effects we observed. In clinical practice, olanzapine and other atypical antipsychotics are associated with torsades de pointes and other potentially-fatal, ventricular arrhythmias (258, 259), which
result from prolongation of ventricular repolarization (i.e. QT interval) (260). Interestingly, torsades de points is also observed in severe diarrhea, chronic alcoholism and starvation, other situations that decrease RER. Electrolyte disturbances are thought to be the causal agent in these other situations. However, further studies are needed to determine whether the effects of olanzapine we observed contribute to the development of sudden cardiac death. Loss of its preferred fuel along with the severe cardiac insulin resistance caused by olanzapine may increase the risk for heart failure, especially in stress situations or during the sleep cycle when circulating fuels are reduced.
Chapter 7

Conclusions and Future Directions

Overall this project has described the modeling and characterization of the chronic and acute metabolic effects of olanzapine in rodents. Within this chapter, key findings are summarized, compared with potentially similar metabolic effects of other atypical antipsychotic drugs, and future studies are proposed.

7.1 Olanzapine-Induced Changes in Body Weight and Adiposity

Initially, we aimed to model the weight gain side effect of olanzapine in rats to examine the potential mechanisms of drug-induced hyperphagia and obesity. As described in Chapter 3, hyperphagia and increased body weight gain were evident after 48h of olanzapine treatment in female rats. This hyperphagia and subsequent weight gain continued for approximately 21 days even with a ‘ramped’ dosing protocol designed to maintain body weight gain. The weight gain secondary to olanzapine-induced hyperphagia was associated with increased visceral adiposity and mild increases in plasma leptin. There were no inter-strain differences (Sprague-Dawley vs. Wistar). With chronic drug administration, oral glucose tolerance was not impaired and slightly elevated insulin concentrations were detectable in response to oral glucose, indicative of only a mild insulin resistance. Given that the degree of insulin resistance was much less severe than expected, we re-examined body weight and food intake data from three separate cohorts of animals and realized that drug effects on food intake did not arise until the second dark cycle during drug administration. A consistent lag-time of at least 24h existed between the initiation of drug treatment and hyperphagia, thus we posited that changes might be detectable in female rats
prior to the onset of hyperphagia that might help explain this effect. Acute dosing experiments were conducted in the afternoon prior to the dark cycle during which the animals would begin to overeat. At this time point, olanzapine-treated female rats displayed enhanced oral glucose tolerance as well as lower circulating glucose and insulin concentrations in the basal state.

Plasma leptin, a known satiety signal, was also present at lower concentrations, which, along with glucose and insulin, may trigger the hyperphagic response in the female rat. Overall, olanzapine-induced weight gain appears to be due to at least three acute drug effects: 1) increased food intake, 2) decreased physical activity, and 3) decreases in circulating concentrations of the satiety-promoting factors glucose, insulin and leptin.

Like many other reports, we did not observe hyperphagia and/or body weight gain in male rats treated with olanzapine, as examined in Chapter 4. The reason for this lack of effect on food intake in male rats remains unknown. Perhaps more interesting, though, is that despite lacking an effect on food intake, olanzapine-treated male rats displayed increased adiposity as early as after one week of drug treatment. The increase of adiposity was maintained for three weeks in the male rats, after which time the total body adiposity reached a plateau. No change in adiposity was detectable over the last two weeks of drug treatment, but the increased adiposity of olanzapine-treated animals persisted relative to time-matched controls. In near identical studies to those performed in females, male rats displayed impaired oral glucose tolerance and fasting hyperglycemia and hyperinsulinemia. Acute studies in males identified four potential factors that may play a role in the accumulation of body fat in the male rat. These factors include: 1) decreasing physical activity without commensurate decreases in food intake, 2) repartitioning of nutrients away from muscle and towards fat due to a muscle-specific insulin resistance with either an increase or no change in insulin action in adipose tissue, 3) impairment of lipolysis, and 4) decreased plasma concentrations of leptin detectable after brief food-deprivation.
At first the metabolic effects of olanzapine in male and female rats may appear very different, though upon closer examination several similarities are present. Interestingly, both sexes respond to drug-treatment with increased adiposity, even though females also exhibit increased body weight gain. Since hyperphagia is necessary for olanzapine-induced obesity (100), it would be interesting to see if pair-fed, female rats responded to olanzapine treatment with increased adiposity without changes in body weight like males. Still, the depression of plasma glucose and insulin in female rats precedes the hyperphagia, with these decreases opposing the increased concentrations of glucose and insulin observed in males.

Figure 7-1: Effect of acute olanzapine on plasma leptin in male and female rats. Narrow weight range male and female rats received olanzapine (4 mg/kg/day) by oral gavage according to the acute dosing scheme described in ‘methods’. All animals were food-restricted for 5h prior to blood sampling. Data represent the mean ± S.E. (n = 9-12). Asterisks indicate significant differences from control (**P<0.01, *P<0.05).

A second similarity of chronic olanzapine treatment is that both rat sexes exhibit a type of hyperphagia. With the onset of drug treatment, female animals reliably increase 24h food consumption intake compared to vehicle-treated animals, an effect that has also been replicated with other atypical drugs. On the other hand, male rats do not change their 24h food consumption, but do exhibit a ‘relative hyperphagia’ because they consume more calories for their degree of physical activity. During brief food deprivation, plasma leptin was decreased on the second day of drug treatment in both sexes. This likely contributes to the hyperphagia in the
female rats, as leptin is a widely accepted satiety factor, and may also contribute to the relative overeating observed in males. In terms of plasma leptin, male and female rats responded similarly (Fig 7-1), though the differences in behavioral response are unknown; however, they may be related to differential sensitivity of the hypothalamus or other brain regions to leptin and insulin (261, 262).

The differences that are particularly difficult to understand between male and female rats are the differential changes in glycemia and oral glucose tolerance – enhancing glucose tolerance in females, while impairing it in males. Even though this effect is puzzling it is not without support from other investigations (263), including clinical case reports of hypoglycemic effects of olanzapine in some patients (264). Moreover, Baptista et al also reported similar findings in male and female rats with sulpiride treatment (63, 76, 77, 113). Unlike the present studies, Baptista et al observed these changes in glucose tolerance and/or glycemia early on (<10 days) after beginning chronic sulpiride administration. At this point animals were generally heavier than time-matched controls, but had not yet reached a weight gain plateau. At later time points the early improvements in female glucose tolerance were not observed, though this makes sense because these animals had gained even more visceral adipose tissue, which likely counteracts acute-drug effects on insulin sensitivity. Unlike the other atypical drugs, sulpiride is a selective-D2 blocker and, therefore, it is tempting to speculate the olanzapine’s effects on glycemia and glucose tolerance in both sexes are due solely to D2-mediate effects. Furthermore, future studies are needed in which olanzapine or sulpiride are administered centrally to determine whether the target of these effects is centrally or peripherally mediated.

Lastly, the potential implications of prolactin in the pathophysiology of olanzapine-induced obesity are still unclear. All of the atypical antipsychotics are D2-dopamine antagonists and thus have the potential to cause surges in plasma prolactin. These surges in humans differ considerably between the different antipsychotic drugs, with risperidone causing the most severe
hyperprolactinemia clinically. The atypical antipsychotics, including olanzapine, also cause surges in prolactin in rodents, including olanzapine, which is considered clinically to be ‘prolactin-sparing’. The studies examining plasma prolactin levels have done so after chronic treatment. Our laboratory has conducted similar studies in chronically treated female rats, as well as in both sexes after acute drug administration. Cooper et al concluded that prolactin was not involved in the pathogenesis of olanzapine-induced obesity because, unlike weight gain, there was lack of dose-dependence of olanzapine on prolactin (103). Consistent with these observations, we have also examined prolactin elevation after chronic olanzapine (21d) and observed similar results (Fig 7-2A). However, we have also looked on day 2 at the same time and dose of olanzapine (4 mg/kg) that other the acute drug effects were detectable. The differences in the acute versus chronic hyperprolactinemia are striking, though not unexpected, as this appears to be a physiologic negative feedback loop. Thus, in light of these findings we believe that future

**Figure 7-2:** Chronic and acute effect of olanzapine on prolactin. (A) Chronic olanzapine (4 mg/kg p.o.) was for 21 days to female rats, blood samples were collected after an overnight fast (n = 8). (B) Olanzapine was administered acutely by oral gavage to male and female rats, and blood samples collected after a 5h fast on the second treatment day (n = 8-12). Data represent the mean ± S.E. Asterisks indicate significant differences from control (**P<0.01, ***P<0.001).
investigation concerning the effects of prolactin in rats is warranted, especially given the recent advances that suggest direct metabolic actions on insulin sensitivity (reviewed in 265).

7.2 Acute Olanzapine Lowers Plasma Leptin

As mentioned above, olanzapine has an acute leptin-lowering effect in males and females that is detectable within the first 24-48h of drug administration. This leptin effect is potentially involved in the drug-induced hyperphagia in the female rats as well as the ‘relative’ hyperphagia exhibited by male rats. Clinical studies have also suggested that olanzapine may ‘modestly’ reduce plasma leptin concentrations (124), indicating this effect may be shared across species. Whether this effect is related to the acute effects on glucose tolerance, glycemia or insulin sensitivity is unknown. Additionally, it is not clear whether the leptin-lowering effect represents a direct effect on the adipocyte or a leptin-mediator. Moreover, the level of regulation (i.e. transcription, translation, secretion) is also unknown. Given the importance of leptin in body weight and food intake regulation (266, 267), further studies to identify the mechanism of this effect are warranted.

7.3 Shifts in Peripheral Fuel Selection and Insulin Resistance

In addition to altering glucose metabolism, olanzapine also affects fatty acid metabolism. From the current studies (Chapter 5), a striking characteristic of olanzapine treatment is its rapid and robust effect on peripheral fuel selection. This response was demonstrated by an almost immediate decrease in RER that was associated with improved clearance of an oral triglyceride load as well as increased fatty acid uptake into almost all peripheral tissues. The only other drugs that we have identified which decrease RER with such rapidly are the acetyl-CoA carboxylase
inhibitors. Of note, the ACC inhibitors have been implicated in the treatment of type II diabetes and the metabolic syndrome (234, 235, 268). ACC catalyzes the irreversible carboxylation of acetyl-CoA to form malonyl-CoA. ACC inhibitors, therefore, decrease intracellular malonyl-CoA, a potent inhibitor of CPT-1. Importantly, CPT-1 is the rate-limiting step in mitochondrial transport of long-chain fatty acids, and relieving this inhibition increases mitochondrial long-chain fatty acid uptake and consequently fatty acid oxidation. The rapid onset (< 10 minutes) of the effect on RER likely reflects a direct action of olanzapine. With this idea in mind, we assayed olanzapine in vitro for effects on ACC, but no inhibition was noted even at high concentrations (> 135 µM). Even though direct inhibition of ACC by olanzapine was not observed, this does exclude the possibility of inhibition by an olanzapine metabolite. To further test for the involvement of ACC in this drug-mediated effect, we examined the activation state of ACC’s upstream mediator, AMPK, as well as the phosphorylation state of ACC itself. In skeletal muscle from fed animals, the phosphorylation state of AMPK or ACC did not show any changes, suggesting that ACC is not involved in the olanzapine-induced increase in fatty acid oxidation.

Another mechanistic possibility of this drug-induced increase in fatty acid oxidation is the malonyl-CoA decarboxylation is increased independent of changes in ACC activity. This potential lowering of malonyl-CoA could be secondary to olanzapine-induced activation of malonyl-CoA decarboxylase (MCD), the enzyme that catalyzes the decarboxylation of malonyl-CoA to acetyl-CoA. Because cellular malonyl-CoA concentration primarily determines the flux of long-chain fatty acids into the mitochondria, future studies should measure tissue malonyl-CoA content, or alternatively MCD activity, which should provide insight into the mechanism of this drug effect. Furthermore, in an effort to better characterize the cellular location of these effects by olanzapine, it would be desirable to test the effects of olanzapine on isolated mitochondria to determine any direct mitochondrial metabolic effects.
Alternatively, these fatty acid oxidation effects of olanzapine could potentially be independent of changes in cellular malonyl-CoA concentration. It is reasonable to hypothesize that olanzapine may stimulate CPT-1 directly to increase mitochondrial fatty acid flux, independent of cellular malonyl-CoA concentration. This is the case with C75, a known inhibitor of fatty acid synthase, which stimulates fatty acid oxidation without displacement of CPT-1 bound malonyl-CoA (269). However, C75 also decreases food intake and does not increase body weight as seen by olanzapine (270).

Currently, the mechanism of this fuel switching from carbohydrate to fatty acid oxidation is unknown, but the rapid onset, high degree of effect, and seemingly global tissue distribution suggests that it involves a major metabolic player important to most if not all tissues. Another mechanistic pathway that could reasonably lead to the observed effects of olanzapine on fatty acid oxidation could involve pyruvate dehydrogenase (PDH), the enzyme which links glycolysis and the Krebs Cycle by catalyzing the irreversible decarboxylation of pyruvate to acetyl-CoA. In times of food-deprivation and starvation this inactivation functionally preserves glucose and other three-carbon sugars for the brain. In the short-term, PDH is regulated by reversible phosphorylation and allosteric effects. There are a number of tissue-specific PDH kinases and PDH phosphatases that are potential targets. As mentioned in Chapter 5, direct inhibition of PDH is unlikely since that would inhibit the use of glucose as a metabolic fuel by neurons, leading to detrimental effects.

Regardless of the mechanism, our data demonstrate that olanzapine increases whole-body fatty fuel utilization. Superficially, this appears counterintuitive, since burning fats for energy instead of packaging for storage is generally thought to be beneficial. However, recent data suggest that excessive mitochondrial fatty acid oxidation may cause incomplete oxidation of acyl-CoA species that produce insulin resistance (243). In line with this idea, future measurement of diacylglyceride species in the skeletal muscle of olanzapine-treated animals may provide further
insight into the extent of lipid oxidation in these animals. Regardless of the status of diacylglycerides in the cell, the idea of excessive fatty acid oxidation makes considerable sense, since unregulated mitochondrial oxidation would mostly likely lead to excessive free radical formation and subsequent oxidative stress. The widespread affect of olanzapine on metabolic fuel selection necessitates future studies to identify the mediator of this effect, as it may play an important role in maintaining normal cellular metabolic flexibility.

**Figure 7-3:** Effects of various typical and atypical antipsychotics on respiratory exchange ratio in male mice. Male C57Bl/6J mice were acclimated to the testing conditions and either food-restricted or administered vehicle (Ad Libitum Fed) at the indicated time (arrow). Other mice received either sulpiride (SUL), olanzapine (OLZ), clozapine (CLZ), aripiprazole (ARI), risperidone (RIS), or ziprasidone (ZIP) via oral gavage at the indicated time (arrow). Data represent the mean respiratory exchange ratio ± S.E. during the experiment at 15-min intervals.
7.4 Olanzapine and Sudden Cardiac Death

Recent clinical findings (245) have demonstrated that the atypical antipsychotics increase the risk for sudden cardiac death. This was thought to be due to either direct effects on K⁺-channels or other cardiac electrical currents. In Chapter 6, however, we suggest that olanzapine may potentially put undue metabolic stress on the heart by decreasing fuel availability, by increasing peripheral utilization of fatty fuels and cardiac insulin resistance, while simultaneously increasing cardiac work. Our data suggest that there is an insulin-independent increase in glucose uptake in the insulin resistant hearts following food-deprivation. The mechanism of this increase in glucose uptake is not known and requires future study, but may represent an AMPK-independent increase in glucose uptake observed by another group (255).

The increase in fatty fuel utilization in the peripheral tissues following olanzapine administration leads to detectable decreases in circulating FFAs and triglycerides, and thus these fuels are less available for use by the heart. Cardiac fatty acid uptake was not increased in vivo, however, these studies are confounded by the complexity of whole-body metabolism. Future studies should examine whether this potential effect on heart fatty acid uptake is a direct effect of olanzapine on cardiac FFA extraction or secondary to increased peripheral utilization of fatty fuels. Additionally, it is necessary to determine whether the olanzapine associated decreases in insulin-mediated glucose uptake occur in perfused hearts. Collectively, our data suggest that olanzapine might be increasing metabolic stress in the heart, but further studies are required to examine how olanzapine affects cardiac glucose and lipid metabolism. Obviously, future ex vivo studies in perfused hearts are necessary to isolate the heart and examine the direct effects of olanzapine on cardiac function and metabolism, especially given the potential increased mortality from olanzapine-related sudden cardiac death (245). Our results indicate that in addition to primary increases in cardiac work by increasing heart rate, olanzapine may have secondary
metabolic effects on that strain the heart metabolically by starving it of its preferred fatty fuels, FFA and triglycerides.

Interestingly, all of the antipsychotic compounds described in a recent article from the New England Journal of Medicine (245) that cause sudden cardiac death contain a secondary piperazine or piperidine ring (e.g. fluphenazine, trifluoperazine, thiothixene, clozapine, olanzapine, risperidone, aripiprazole and ziprasidone), which are shared by some antihistaminergics and anticholinergics, but can also found in a number of ligands (e.g., trimetazidine, ranolazine, perhexilene, S-15176) for enzymes in fatty acid oxidation including CPT-1 and long chain 3-ketoacyl Co-A thiolase. Thus, these additional antipsychotic compounds should be screened for the RER effects, as these lipid metabolism effects may represent a novel mechanism leading to cardiac pathology.

**Figure 7-4:** Piperazine and piperidine ring structures. Many pharmaceutical compounds, including several of the atypical antipsychotics, are derivatives of either (Left) piperazine (e.g. aripiprazole, clozapine, olanzapine, ziprasidone) or (Right) piperidine building blocks (e.g. risperidone).
Figure 7-5: Derivatives of Piperidine and Piperazine Rings that are involved in fatty acid metabolism.
7.5 Additional Future Directions

Besides potential studies already discussed, one of the most important future goals for these studies is to better understand and identify the molecular mechanisms and specific tissue metabolic effects of atypical antipsychotics. The non-specific nature of these drugs coupled with complex, intact animal models makes it difficult to isolate the effects \textit{in vivo}. To gain a better understanding of these effects, techniques such as organ perfusion (e.g. hind limb, liver, heart) must be used to understand the tissue and then subsequently the cellular targets of these metabolic effects. By identifying the specific targets of olanzapine and other atypical antipsychotics that lead to these effects, newer more specific antipsychotic drugs could be designed to limit off target adverse effects. Additionally, novel therapeutic targets for diabetes and obesity medications could be designed based on the off-target effects of olanzapine.
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