Olanzapine-induced weight gain: an animal model.

By

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ABSTRACT

Introduction: Using an animal model, we examined weight gain in rats exposed to olanzapine, as well as whether increased weight was associated with food intake, visceral fat and/or locomotion. Methods: Sprague-Dawley rats were chronically treated with olanzapine while being offered diets including standard chow, a high fat (60% fat) diet, and a high fat/high carbohydrate (42% fat; 42.7% carbohydrate) diet. Body weight, food intake, visceral fat and locomotor activity were measured. Results: Our findings related to weight gain are in line with other reports indicating that while olanzapine-induced weight gain can be observed, it does not mirror what is observed in humans on two levels: (i) it is not of the same magnitude, and (ii) it is more gender specific i.e., females greater than males. Conclusions: These data confirm that chronic treatment with olanzapine has varying effects on weight gain, food intake, visceral fat and locomotor activity.
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Introduction

Schizophrenia

Schizophrenia is arguably the most incapacitating and distressing of the mental illnesses, affecting both men and women across all cultures and occurring in approximately 1% of the general population (Williamson, 2004). Its impact on affected individuals and their families is profound, as schizophrenia represents a chronic disease typically striking individuals in their prime i.e., young adulthood, and its course frequently characterized by periods of relapse and/or symptom exacerbation. Approximately 10% of those with schizophrenia commit suicide during the course of the illness.

Reports describing patients who presented with paranoid and catatonic symptoms first arose in the early 19th century (Zuckerman, 1999). In the early 1900’s, Emil Kraepelin (1919) first labelled these patients with ‘dementia praecox’ – a diagnosis which grouped together the symptoms noted by earlier psychiatrists. Kraepelin’s terminology i.e., ‘early dementia’, spoke to how debilitating the illness was and highlighted early onset with progressive decline. Following Kraepelin’s labelling, Eugene Bleuler (1950), a Swiss psychiatrist, introduced the term ‘schizophrenia’ which loosely translates to the splitting of the mind. During the 1930’s, an attempt was made to address the complexity of presenting psychotic and affective symptoms by creating the diagnosis of ‘schizoaffective’ disorder.

Currently, schizophrenia is characterized clinically by the presence of visual and auditory hallucinations, delusions, disorganized speech/behaviour, and negative
symptoms including flat affect, alogia and avolition (American Psychiatric Association, 2000) (Table 1). Those with the illness routinely experience great difficulties in interpersonal relationships, self-care, education and employment. This is reflected in its financial burden, with estimated direct/indirect health care costs of approximately $6.85 billion in Canada during 2004 (Goeree et al., 2005).

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<th>Table 1. Diagnostic criteria for Schizophrenia (American Psychiatric Association, 2000)</th>
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A. **Characteristic symptoms:** Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated):

1. delusions
2. hallucinations
3. disorganized speech (e.g., frequent derailment or incoherence)
4. grossly disorganized or catatonic behavior
5. negative symptoms, i.e., affective flattening, alogia, or avolition

**Note:** Only one Criterion A symptom is required if delusions are bizarre or hallucinations consist of a voice keeping up a running commentary on the person's behaviour or thoughts, or two or more voices conversing with each other.

B. **Social/occupational dysfunction:** For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).

C. **Duration:** Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).
Antipsychotics

The unexpected discovery of chlorpromazine’s antipsychotic properties in the early 1950’s revolutionized the treatment and understanding of schizophrenia. Chlorpromazine, initially being investigated for its sedating properties, led to a new class of medications which have been referred to as ‘antipsychotics’, ‘major tranquilizers’ and ‘neuroleptics’ (Miyamoto et al., 2005). These medications provided evidence that schizophrenia is linked, at least in part, to biological mechanisms.

The dopamine hypothesis

While the discovery of antipsychotic properties with chlorpromazine was serendipitous, by the late 1950’s it was theorized that schizophrenia represented a disease of hyperdopaminergic activity, with the so-called ‘neuroleptics’ alleviating symptoms through their dopamine blockade (van Rossum, 1966), therefore linking dopamine and psychosis (Denicker, 1990). Shortly thereafter, it was identified that blockade of the D₂ receptor specifically accounted for the therapeutic effect of antipsychotics (Seeman, 1987).

Support for the dopamine hypothesis stems from evidence involving the administration of dopamine agonists, i.e. amphetamine, which produce psychotic symptoms (Ellison and Eison, 1983). In addition, the administration of drugs that increase the availability and activity of dopamine have been shown to worsen symptoms in individuals with schizophrenia (Lieberman et al., 1987). As a result, drug development focussed on selective D₂ antagonists such as haloperidol and pimozide, rather than
medications such as chlorpromazine which bind to multiple receptors. Clinically, this was reflected in a transition from low-potency, pharmacologically heterogeneous compounds like chlorpromazine and thioridazine (therefore requiring high doses for therapeutic efficacy e.g. 300-1000 mg/day) to high-potency selective D₂ antagonists like haloperidol, where the total daily dose would be measured in mg/day e.g. 10 mg.

**The atypical antipsychotics**

Clozapine was the first of the so-called ‘atypical’ antipsychotics that strayed from the dopamine hypothesis, challenging the premise that dopamine antagonism was central to antipsychotic response. Clinically, they have been purported to exhibit a reduced risk for extrapyramidal symptoms (EPS) and tardive dyskinesia (TD), as well as greater efficacy in the treatment of negative and cognitive symptoms when compared to typical antipsychotics (Remington et al., 2000, Freedman et al., 2003), although more recent, larger scale effectiveness trials have tempered these claims (Jones et al., 2006; Meyer et al., 2005). As a class, clozapine and the other atypicals have been shown to have a lower affinity for the D₂ receptor and a broader receptor binding profile. Meltzer et al. (1992) has argued that it is clozapine’s greater 5-HT₂ vs. D₂ antagonism that underlies its clinical advantages. Currently, atypical antipsychotics currently represent the first line treatment for schizophrenia (Lieberman et al., 2005).
Side effects of antipsychotics

Unfortunately, the early neuroleptics, later referred to as ‘typical’ antipsychotics were also linked to troublesome side effects, in particular EPS and TD. Acute EPS i.e. dystonia, akathisia, parkinsonism, has been linked to D2 antagonism at the level of the nigrostriatal pathway (Kapur et al., 1995), while chronic antipsychotic exposure may lead to TD, which is characterized by repetitive, involuntary movements affecting the orofacial region, although the trunk extremities may also be affected. Prevalence rates of individuals chronically treated with conventional antipsychotics are in the range of 20-25%, figures which rise notably in the geriatric population (Remington et al. 2002).

Despite their purported clinical advantages and their more benign EPS profile, the atypical antipsychotics are not without adverse side effects. Shortly after their introduction, the atypical antipsychotics were noted to exhibit greater liability for metabolic disturbances and weight gain (Taylor and McAskill, 2000), with reports of type 2 diabetes in as many as 20% of patients with schizophrenia (DeHart et al., 2006). To address this problem, Canada, Japan, and Britain each issued warnings regarding reports of diabetes, ketoacidosis and death in conjunction with the use of atypical antipsychotics during 2001 and 2002. By 2003 the United States FDA (Food and Drug Administration) felt compelled to demand a monograph warning regarding the relationship between atypical antipsychotics as a class and risk of glucose abnormalities. Here in Canada, product monographs for the available atypical antipsychotics now also acknowledge this risk (Canadian Pharmacists Association, 2006).
Atypical antipsychotics and weight gain

While weight gain has been identified as a class effect with the atypical antipsychotics, it is now widely accepted that certain atypical carry a greater risk in this regard (Allison et al., 1999; Ganguli, 1999) (Figure 1). The mechanism(s) that contributes to atypical antipsychotic-induced weight gain in humans is/are currently poorly understood, not surprising given the complex interplay of behavioural, neural and endocrine systems (Goudie et al., 2005). At present, there is no clear consensus as to the mechanism(s) causing weight gain (American Diabetes Association, 2004).

![Figure 1](image-url)

**Figure 1.** The propensity of typical and atypical antipsychotics to induce weight gain in humans (Allison et al., 1999).

Given the secondary effects of obesity, including diabetes and cardiovascular disease, it is disconcerting that obesity is twice as prevalent in people with schizophrenia when compared to the general population (Allison et al., 1999, Wirshing, 2004). This is especially true given that this is a population a) already linked with increased morbidity and mortality (Harris and Barraclaugh, 1998; Haupt, 2007; Newman and Bland, 1991;
Osborn et al., 2007; Osby et al., 2000); and b) without access to the same level of medical care as the general population (Bradford, 2008).

That atypical antipsychotics induce weight gain, albeit to varying degrees, has been confirmed through a comprehensive meta-analysis (Allison et al., 1999). Clozapine and olanzapine are the major contributors to weight gain, citing weight increases of 3.99 kg and 3.51 kg respectively over 10 weeks, while risperidone and quetiapine show moderate effects whereas ziprasidone and aripiprazol exhibit minimal weight gain (Allison et al., 1999).

Although clozapine use represents a ‘niche’ market i.e., refractory schizophrenia, olanzapine remains one of the two most widely used antipsychotics in North America (Allesi-Severini et al., 2008; Sernyak and Rosenheck, 2008). Not surprisingly, considerable attention has turned to the relationship between olanzapine and weight gain. McIntyre et al. (2003) reported weight increases of 18.3% in patients being administered olanzapine diagnosed with bipolar disorder, while a second study involving schizophrenia reported a mean 2-year weight gain of 15.4 kg in olanzapine-treated individuals (Zipursky et al., 2005). Other data confirm that individuals treated with olanzapine routinely gain >7% of their initial body weight (Bobes et al., 2003). Although certain atypicals have been linked to a greater propensity for weight gain, lack of data and/or a clear understanding as to underlying mechanisms have resulted in the United States Food and Drug Administration (FDA) and Health Canada’s Therapeutic Product Division (TPD) designating atypical antipsychotic-induced weight gain as characteristic of all atypical antipsychotics i.e., a class effect.
Baptista et al. (2008) suggested that antipsychotic-induced weight gain may well be caused by an increase in food intake and sedation. In terms of intake and food preference, antipsychotics in general appear to increase total intake versus controls (Strassnig et al., 2003), possibly related to changes in pattern of consumption i.e., binge eating (Theisen et al., 2003). With conventional antipsychotics, studies have shown a preference for carbohydrate and fat (Brown et al., 1999); for the atypicals, reports are few. A recent study involving inpatients treated with olanzapine noted an increase in caloric intake (589 kcal/day or 27.7% over 4 weeks) without change in diet composition (Gothelf et al., 2002). In other studies individuals with schizophrenia were shown to consume less fibre and more saturated fat than controls (Ryan et al., 2004; Brown et al., 1999), although the latter report was based on a community sample and information regarding antipsychotic use was not provided.

The problem associated with increased intake is further exacerbated by identified changes in activity and activity/energy expenditure. Olanzapine has been associated with significant sedation, as has clozapine (Haddad and Sharma, 2007), raising this as a potential contributing factor in decreased activity, independent of diet (Casey et al., 1996, Richelson et al., 1999). In fact, decreased activity has been reported in association with antipsychotic use (Brown et al., 1999; Sharpe et al., 2006; Faulkner et al., 2006), possibly related to their sedating properties. The fact that the greatest weight gain with atypicals is observed in those receiving olanzapine or clozapine supports this line of thinking for it is these two agents which appear to be most sedating (Remington et al., 2001). While resting energy expenditure may not be influenced by atypical antipsychotics (Graham et
al., 2005; Gothelf et al., 2002), they have been shown to decrease total daily energy expenditure (Sharpe et al., 2006).

There is a large body of work linking atypical antipsychotic-induced weight gain to a variety of neurotransmitter receptors including dopamine, histamine and serotonin (Richelson, 1999). All antipsychotics block, to varying degrees, D₂ receptors (Kapur and Remington, 2001; Seeman, 2002), offering support for the theory that D₂ receptors may play a role in atypical antipsychotic-induced weight gain. However, the greater propensity for weight gain in atypical antipsychotics, which exhibit a comparatively lower D₂ receptor binding affinity in clinically relevant doses, argues against a predominant role for the D₂ receptor (Kapur et al., 2000).

Atypical antipsychotics also block histamine (Richelson et al., 1999), and it has been established that histamine blockade stimulates appetite which may lead to significant weight gain (Orthen-Gambill, 1988). Olanzapine is a potent histamine antagonist, as is clozapine, and H₁ antagonism has been identified as the most reliable predictor of weight gain with the atypicals (Wirshing et al., 1999). However, this conclusion does not account for ziprasidone’s weight gain neutrality, given its high affinity for histamine (Nasrallah, 2008; Richelson and Souder, 2000).

Serotonin (5-HT₂C) has also been implicated as a possible contributor to atypical antipsychotic-induced weight gain due to its role in food intake. Olanzapine and clozapine have high affinity for 5-HT₂C receptors, and reports indicate that 5-HT₂C knockout mice exhibit hyperphagia and obesity (Heisler et al., 1998; Nonogaki et al., 1998). Once again though, ziprasidone challenges this line of investigation given it’s high affinity for 5-HT₂C receptors (Richelson et al., 2000).
Rat models of antipsychotic-induced weight gain

An animal model would prove valuable in better understanding the mechanism(s) contributing to antipsychotic-induced weight gain, and considerable attention has been given to this topic. Published studies involving rodents have shown that weight gain can be induced by various antipsychotics including sulpiride (Baptista et al., 1998; Baptista et al., 2004; Fell et al., 2005), haloperidol (Fell et al., 2004; Fell et al., 2005; Pouzet et al., 2003), risperidone (Baptista et al., 2002; Baptista et al., 2004; Fell et al., 2004; Fell et al., 2005; Fell et al., 2008; Ota et al., 2002), clozapine (Albaugh et al., 2006) and ziprasidone (Fell et al., 2005; Kalinichev et al., 2006). Greater than 50% of published studies (see Table 2) have investigated olanzapine-induced weight gain, of which the most recent have reported weight gain (Cooper et al., 2008; Fell et al., 2007; Raskind et al., 2007).

Weight gain is regulated by both food energy intake and energy expenditure (Casey and Zorn, 2001). Antipsychotics have long been associated with increased food intake (Goudie et al., 2005), and the most recent studies have reported increased food intake in rats being treated with sulpiride (Fell et al., 2005b), risperidone (Baptista et al., 2004), clozapine (Lee, 2002a) and olanzapine (Albaugh, 2006). Thornton-Jones and Reynolds (2002) have suggested that olanzapine-induced weight gain may be due to suppressed satiety measured by increased food intake and a delay in reduction in runway speed over trials. Olanzapine has also been shown to increase feed efficiency i.e., weight gain/food consumed (Arjona et al., 2004).

Few published studies have investigated the role of energy expenditure i.e., locomotor activity, in conjunction with antipsychotic administration. Arjona et al. (2004) observed decreased activity levels over a 24-hour period and specifically the 12-hour
dark cycle in conjunction with olanzapine treatment. Similarly, Fell et al. (2007) observed decreased activity levels in rats being treated with olanzapine, risperidone and ziprasidone. A more detailed analysis of locomotor activity has indicated increased resting behaviour and decreased exploratory behaviour in rats treated with clinically relevant doses of risperidone and haloperidol via mini-pump (Karl et al., 2006). In a recent report, it was observed that treatment with haloperidol and clozapine resulted in suppression of locomotor activity in juvenile, adolescent and adult rats (Wiley, 2008). Wiley (2008) also noted that juvenile rats were more sensitive to antipsychotic treatment than adolescent and adult rats, leading to the conclusion that age should be considered when investigating antipsychotic properties in rat models. Karl et al. (2006) have encouraged studies with osmotic mini-pumps, given the rapid metabolism of these drugs in rodents (Chiu and Franklin, 1996), to achieve clinically relevant doses over time i.e., D₂ occupancy.

**Shortcomings of the rat model**

The most significant criticism of such a model relates to the weight gain itself. As noted, there have been numerous reports indicating antipsychotic-related weight gain in rats (see Table 2). However, it has also been argued that the degree of weight gain is not comparable to what is seen in humans (Baptista et al., 1987; Allison et al., 1999). As an aside, this is not a criticism that is confined to rodents – the same limitation has also been reported with other species, including dogs (Ader et al., 2005).

Further, antipsychotic-induced weight gain in rodents also appears to be gender-dependent. In humans, both males and females experience significant weight gain with
exposure to atypical antipsychotics (Bergman and Ader, 2005). However, in the rat literature (Table 2), there is a marked propensity for the females to gain weight. Minet-Ringuet et al. (2006b) are the only group to claim significant weight gain in male rats administered olanzapine while Ota et al. (2002) observed significant weight gain in male rats while being exposed to risperidone. Several groups (Albaugh et al., 2006; Choi et al., 2007; Cooper et al., 2007; Lin et al., 2006; Minet-Ringuet et al., 2006a; Pouzet et al., 2003) failed to observe weight gain in males exposed to both olanzapine and clozapine. In fact, several published articles suggest that antipsychotic-induced weight gain in humans may be more common in females (Aichhorn et al., 2006; Homel et al., 2002; Russell and Mackell, 2001), and Aichhorn et al. (2006) provide reasons for this conflicting data including study duration and baseline body mass indices. However, it remains that in rodents the distinction between males and females is much more prominent.

In light of these concerns, it has been argued that a rat model of antipsychotic-induced weight gain is not tenable (Pouzet et al 2003). The lack of a reliable animal model may be explained by varying methodologies, including dosing, route of administration and diet. For example, in the animal literature antipsychotic doses are frequently specified without stating reason or rationale; more recently, Kapur et al. (2000a) have suggested that doses used in rat models be chosen based on D₂ receptor occupancy that ties dose to therapeutic response in humans.

The variability that exists with antipsychotic-induced weight gain in rats may also be due to route of administration, half-life (T₁/₂) of the antipsychotic being administered and the rats’ rapid metabolism of these drugs. In humans, antipsychotics are routinely
administered once or twice per day based on the antipsychotic’s half-life. For example, Tauscher et al. (2002) have shown that the mean half life of olanzapine in olanzapine-treated patients is 19.5 hours and this is a drug that, in humans, is given once daily as a rule. In the vast majority of published related rodent studies olanzapine (and other antipsychotics) are administered once, or at most, twice daily. However, the $T_{1/2}$ of olanzapine in rodents have been shown to be only 3.3 hours (Chiu and Franklin 1996), accounted for by species differences in the metabolism of these drugs (Aravagiri, 1999). Such differences could account for the failure to mirror in rats the degree of antipsychotic-induced weight gain observed in humans.

Finally, studies have suggested that individuals with schizophrenia consume more fat than controls (Brown 1999). A review of the animal literature reveals that the majority of related studies involving rats have employed standard chow, which yields a relatively high percentage of kilocalories from protein and a relatively low percentage of kilocalories from fat. To date, only three published studies have used a diet that approximates that of individuals with schizophrenia. Baptista (1998) reported that rats receiving sulpiride 20 mg/kg for 3 weeks and fed a high fat diet gained significant body weight when compared to controls. Minet-Ringuet (2006b) also showed that a diet high in fat and carbohydrate (31% fat, 54% carbohydrate) leads to significant increases in body weight when exposed to antipsychotics. Finally, Hartfield (2003) offered rats a high calorie fat emulsion along with standard chow to investigate feeding patterns; however, weight gain was not reported.
Olanzapine-induced weight gain in a rat model.

As mentioned previously, olanzapine is one of the atypicals with the greatest risk of weight gain in humans (Allison et al 1999), and as such the focus of numerous animal studies investigating the increased risk in this class of antipsychotics. Pharmacologically, it demonstrates affinity for multiple receptors including dopamine D₂, serotonin 5-HT₂A, 5-HT₂C and histamine H₁, each implicated in influencing food intake (Berridge, 1996; Masaki and Yoshimatsu, 2006; Reynolds et al., 2002). Investigations to deconstruct underlying mechanism(s) of action have though been complicated though by differences in route of administration, diet, gender and dose.

Olanzapine-induced weight gain has been established with once-daily i.p. injection (Fell et al., 2004a; Fell et al., 2005b; Fell et al., 2007), twice-daily i.p. injection (Cooper et al., 2005; Goudie et al., 2002; Patil et al., 2006) and administration by gavage (Arjona et al., 2004; Pouzet et al., 2003). Albaugh et al. (2006) administered olanzapine via food, which resulted in significant weight gain in only female rats, while Minet-Ringuet et al. (2005) failed to achieve significant weight gain when olanzapine was administered via Fortimel.

In rat models, olanzapine doses ranging from 0.01mg/kg to 20mg/kg have been used, with varying results. To date, the only dose which has not produced significant weight gain in both male and female rats is 20 mg/kg, administered via gavage twice daily (Pouzet 2003). However, only one published study investigating olanzapine-induced weight gain in male rats has been able to induce significant weight gain at a dose of 0.5 mg/kg and 2.0 mg/kg (Minet-Ringuet et al 2006b).
Hyperphagia is widely cited in the literature when olanzapine is administered to female rats (Albaugh et al., 2006; Arjona et al., 2004; Choi et al., 2007; Cooper et al., 2005; Fell et al., 2004a; Fell et al., 2005b; Fell et al., 2007; Hartfield et al., 2003; Kalinichev et al., 2005; Patil et al., 2006; Pouzet et al., 2003). Currently though, there is only one investigation reporting increased food intake defined by increased meal size, increased ingestion rate, increased meal duration and decreased intermeal intervals in male rats (Minet-Ringuet et al. 2006b).

Olanzapine has been shown to suppress activity levels over a 24-hour period and the 12-hour dark cycle (Arjona et al., 2004). Fell et al. (2007) also report decreases in activity levels in rats being treated with olanzapine during weekly 60-minute sessions.

Studies investigating adjunct therapies are warranted given that: i) the mechanism(s) of action of atypical antipsychotic-induced weight gain are still unclear, ii) 90% of patients diagnosed with schizophrenia are being treated with atypical antipsychotics (Agid et al., 2008), and iii) olanzapine, an atypical antipsychotic, does induce significant weight gain. It has been reported that mifepristone, a glucocorticoid antagonist both reduces and prevents olanzapine-induced weight gain (Beebe et al., 2006). Beebe et al. (2006) also conclude that co-treatment with olanzapine and mifepristone resulted in significantly less visceral fat than those rats treated with only olanzapine. Raskind et al. (2007) report significantly lower nocturnal plasma melatonin resulting from olanzapine administration. They report that rats treated with melatonin and olanzapine gained significantly less weight than rats treated with olanzapine but more than rats treated with only melatonin. Visceral fat pad weight was also significantly
higher in the olanzapine treated rats. Snigdha et al. (2008) co-administered olanzapine with ziprasidone or aripiprazole and reported decreases in food intake in both groups.

In the present studies, we set out to establish an animal model of antipsychotic induced weight gain which would be evident in both male and female rats when administered olanzapine. Other variables of interest (i.e., locomotor activity, food intake, visceral fat) were also investigated. To this end, we introduced rats to various diets including standard chow, a high fat paste and a high fat/high carbohydrate diet, which is commonly referred to as a typical North American diet consumed by humans (TD88137, Harlan Teklad, Wisconsin, USA). In addition, we investigated different routes of administration including once daily i.p. injections, once daily s.c. injections and continuous delivery via osmotic mini-pumps.
### Table 2. Weight gain studies (CLOZ-clozapine; HAL-haloperidol; OLZ-olanzapine; RIS-risperidone; SUL-sulpiride; ZIP-ziprasidone)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Sex</th>
<th>Drug &amp; route of administration</th>
<th>Duration</th>
<th>Diet</th>
<th>Results &amp; comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albaugh et al., 2006</td>
<td>MF</td>
<td>OLZ 4mg/kg titrated up to 20 mg/kg in food</td>
<td>33 days</td>
<td>Chow</td>
<td>↑ weight gain in female OLZ treated group</td>
</tr>
<tr>
<td></td>
<td>MF</td>
<td>CLOZ 4mg/kg titrated up to 8 mg/kg + OLZ 4 mg/kg in food</td>
<td>27 days</td>
<td>Chow</td>
<td>↓ weight gain with CLOZ day 0-12 (females)</td>
</tr>
<tr>
<td>Arjona et al., 2004</td>
<td>F</td>
<td>OLZ 2.1 mg/kg gavage</td>
<td>30 days</td>
<td>80% C:0% P and 20% P: 60% C offered concurrently</td>
<td>↑ weight gain - returned to control levels after withdrawal</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>HAL 0.04 mg/kg gavage</td>
<td>16 days</td>
<td>Chow</td>
<td>↓ weight gain</td>
</tr>
<tr>
<td>Baptista et al., 1993</td>
<td>MF</td>
<td>SUL .5,1.2,5,5,10 or 20 mg/kg i.p.</td>
<td>3 weeks</td>
<td>66% chow + 33% corn oil</td>
<td>↓ weight gain in females</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>HAL 0.04 mg/kg gavage</td>
<td>16 days</td>
<td>Chow</td>
<td>ø weight gain</td>
</tr>
<tr>
<td>Baptista et al., 2002</td>
<td>MF</td>
<td>SUL 20 mg/kg i.p.</td>
<td>21 days</td>
<td>66.6% F</td>
<td>↑ weight gain</td>
</tr>
<tr>
<td>Baptista et al., 2004</td>
<td>F</td>
<td>RIS 0.5 mg/kg s.c. or SUL 20 mg/kg s.c.</td>
<td>12 days</td>
<td>20% F</td>
<td>↑ weight gain in RIS and SUL groups (&gt; in SUL group)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>RIS 0.5 mg/kg s.c., SUL 20 mg/kg s.c.</td>
<td>12 days</td>
<td>3 pure macronutrient diets</td>
<td>higher weight gain in SUL group but not significant</td>
</tr>
<tr>
<td>Choi et al., 2007</td>
<td>MF</td>
<td>OLZ 5mg/kg mini-pump or CLOZ 10 mg/kg mini-pump</td>
<td>2 weeks</td>
<td>Chow</td>
<td>↑ weight gain in female OLZ group</td>
</tr>
<tr>
<td>Cooper et al., 2005</td>
<td>F</td>
<td>OLZ 1.2, or 4 mg/kg i.p.</td>
<td>20 days</td>
<td>Chow</td>
<td>↑ weight gain in all groups</td>
</tr>
<tr>
<td>Cooper et al., 2008</td>
<td>F</td>
<td>CLOZ 6 or 12 mg/kg i.p.</td>
<td>20 days</td>
<td>Chow</td>
<td>↓ weight gain in both CLOZ groups</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>CLOZ 1.2 or 4mg/kg</td>
<td>20 days</td>
<td>Chow</td>
<td>ø weight gain</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>CLOZ .25 or .5mg/kg</td>
<td>12 days</td>
<td>Chow</td>
<td>↓ weight gain in .5mg/kg group</td>
</tr>
<tr>
<td>Cooper et al., 2007</td>
<td>M</td>
<td>OLZ 1, 2, or 4mg/kg i.p.</td>
<td>3 weeks</td>
<td>Chow</td>
<td>↓ weight gain in 4mg/kg group</td>
</tr>
<tr>
<td>Fell et al., 2004</td>
<td>F</td>
<td>OLZ 0.5,1.0, or 4.0 mg/kg i.p.</td>
<td>3 weeks</td>
<td>Chow</td>
<td>↑ weight gain in 4 mg/kg group days 3 to 10</td>
</tr>
<tr>
<td>Fell et al., 2004</td>
<td>F</td>
<td>RIS 0.1, 0.5, or 1.0 mg/kg i.p.</td>
<td>3 weeks</td>
<td>Chow</td>
<td>↑ weight gain in 1 mg/kg group days 3 to 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HAL 0.1, 0.5, or 1.0 mg/kg i.p.</td>
<td></td>
<td></td>
<td>↑ weight gain in all HAL groups</td>
</tr>
<tr>
<td>Fell et al., 2005</td>
<td>F</td>
<td>OLZ 4 mg/kg i.p., RIS 0.5 mg/kg i.p., ZIP 2.5 mg/kg i.p., SUL 10 mg/kg i.p., or HAL 0.5 mg/kg i.p.</td>
<td>3 weeks</td>
<td>Chow</td>
<td>↑ weight gain in all groups except ZIP group</td>
</tr>
</tbody>
</table>
### Table 2. Weight gain studies (CLOZ-clozapine; HAL-haloperidol; OLZ-olanzapine; RIS-risperidone; SUL-sulpiride; ZIP-ziprasidone)

<table>
<thead>
<tr>
<th>Authors</th>
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<th>Diet</th>
<th>Results &amp; comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fell et al., 2005</td>
<td>F</td>
<td>ZIP 1 or 2.5mg/kg i.p.</td>
<td>4 weeks</td>
<td>Chow</td>
<td>• ↑ weight gain on day 28 only in 2.5 mg/kg group</td>
</tr>
<tr>
<td>Fell et al., 2007</td>
<td>F</td>
<td>OLZ 2mg/kg, RIS 0.5mg/kg, or ZIP 2.5mg/kg i.p.</td>
<td>3 weeks</td>
<td>pure macronutrient diets</td>
<td>• ↑ weight gain from day 1 to end in OLZ group</td>
</tr>
<tr>
<td>Fell et al., 2008</td>
<td>F</td>
<td>OLZ 2mg/kg, RIS 0.5mg/kg, or ZIP 2.5mg/kg i.p.</td>
<td>4 weeks</td>
<td>20% P: 35% F: 45% C</td>
<td>• ↑ weight gain on day 2 and 4 in RIS group</td>
</tr>
<tr>
<td>Goudie et al., 2002</td>
<td>F</td>
<td>OLZ 4mg/kg i.p.</td>
<td>20 days</td>
<td>Chow</td>
<td>• ↑ weight gain</td>
</tr>
<tr>
<td>Kalinichev et al., 2005</td>
<td>F</td>
<td>OLZ 5.0 mg/kg p.o.</td>
<td>2 weeks</td>
<td>Chow</td>
<td>• ↑ weight gain</td>
</tr>
<tr>
<td>Kalinichev et al., 2006</td>
<td>F</td>
<td>OLZ 2.0mg/kg, ZIP 2.0, 6.0, or 10mg/kg p.o.</td>
<td>1 week</td>
<td>Chow</td>
<td>• ↑ weight gain in Zip 2.0mg/kg, ZIP 6.0mg/kg and OLZ 2.0mg/kg groups</td>
</tr>
<tr>
<td>Lin et al., 2006</td>
<td>M</td>
<td>RIS 2.13mg/kg or HAL 4mg/kg mini-pump</td>
<td>4 weeks</td>
<td>Chow</td>
<td>• ↓ weight gain in RIS group</td>
</tr>
<tr>
<td>Minet-Ringuet et al., 2005</td>
<td>M</td>
<td>OLZ 1 mg/kg or HAL 1 mg/kg in Fortimel</td>
<td>6 weeks</td>
<td>pure macronutrient diets</td>
<td>• ø weight gain</td>
</tr>
<tr>
<td>Minet-Ringuet et al., 2006</td>
<td>M</td>
<td>OLZ 1.0 mg/kg, HAL 1.0 mg/kg, or ZIP 10 mg/kg in food</td>
<td>6 weeks</td>
<td>pure macronutrient diets</td>
<td>• ø weight gain</td>
</tr>
<tr>
<td>Minet-Ringuet et al., 2006</td>
<td>M</td>
<td>OLZ 0.01, 0.1, 0.5, or 2.0 mg/kg in food</td>
<td>6 weeks</td>
<td>14% P, 31% F, 54% C</td>
<td>• ↑ weight gain in 0.5 and 2.0 mg/kg groups</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>OLZ 1.0 mg/kg, HAL 1.0 mg/kg, or ZIP 10 mg/kg in food</td>
<td>3 weeks</td>
<td>14% P, 31% F, 54% C</td>
<td>• ↑ weight gain in OLZ treated group</td>
</tr>
<tr>
<td>Ota et al., 2002</td>
<td>M</td>
<td>RIS 0.005, 0.05, or 0.5 mg/kg s.c.</td>
<td>3 weeks</td>
<td>Chow</td>
<td>• ↑ weight gain in 0.005 mg/kg group</td>
</tr>
<tr>
<td>Patil et al., 2006</td>
<td>F</td>
<td>OLZ 1.0 or 2.0 mg/kg i.p.</td>
<td>20 days</td>
<td>Chow</td>
<td>• ↑ weight gain in both groups</td>
</tr>
<tr>
<td>Parada et al., 1989</td>
<td>M</td>
<td>SUL 20 mg/kg i.p.</td>
<td>3 weeks</td>
<td>66% chow + 33% corn oil</td>
<td>• ø weight gain</td>
</tr>
<tr>
<td>Pouzet et al., 2003</td>
<td>MF</td>
<td>HAL 0.08, HAL 0.31, OLZ 5, or OLZ 20 mg/kg gavage</td>
<td>3 weeks</td>
<td>Chow</td>
<td>• ↓ weight gain in OLZ 20.0 mg/kg males</td>
</tr>
<tr>
<td>Raskind et al., 2007</td>
<td>F</td>
<td>OLZ 2mg/kg in drinking water</td>
<td>8 weeks</td>
<td>Chow</td>
<td>• ↑ weight gain in OLZ group</td>
</tr>
</tbody>
</table>
Objectives

Objective 1: To determine the effect of clinically relevant doses of olanzapine on weight gain, food intake, visceral fat amassment and locomotor activity.

Objective 2: To determine if different routes of administration i.e. osmotic mini-pump vs. i.p. injection vs. s.c. injection, affect olanzapine-induced weight gain, food intake and visceral fat amassment.

Objective 3: To evaluate the effect of a high fat (60% fat) on weight gain, food intake, visceral fat and locomotor activity while being treated chronically with olanzapine.

Objective 4: To evaluate the effect of a high fat/high carbohydrate diet (42% fat, 42.7% carbohydrate) on weight gain, food intake, visceral fat and locomotor activity while being treated chronically with olanzapine.

Hypotheses

Hypothesis 1: Female rats exposed to chronic olanzapine will, compared to vehicle-treated rats: a) gain significantly more weight, b) exhibit decreased locomotor activity levels, c) consume significantly more kilocalories; and d) amass significantly more visceral fat.

Hypothesis 2: Female rats exposed to chronic olanzapine via osmotic mini-pump, compared to female rats exposed to chronic olanzapine via once daily i.p. injection and
once daily s.c. injection, will: a) gain significantly more weight, b) consume significantly more kilocalories compared to vehicle treated rats; and c) amass significantly more visceral fat compared to vehicle rats.

Hypothesis 3: Male and female rats exposed to chronic olanzapine and a high fat diet (60%) will, compared to vehicle-treated rats: a) gain significantly more weight, b) exhibit decreased locomotor activity levels, c) consume significantly more kilocalories; and d) amass significantly more visceral fat.

Hypothesis 4: Male rats exposed to a high fat/high carbohydrate diet (42% fat, 42.7% carbohydrate) will, compared to vehicle-treated rats: a) gain significantly more weight, b) exhibit decreased locomotor activity levels, c) consume significantly more kilocalories; and d) amass significantly more visceral fat.
Experiment 1:

Effects of clinically relevant doses on olanzapine-induced weight gain
Overview

In this experiment, we examined the effects of olanzapine on weight gain, locomotor activity, food intake and visceral fat amassment. We chose to include two doses: 2.0 mg/kg and 7.5 mg/kg. The 7.5 mg/kg dose is premised on in vivo dopamine D2 occupancy which represents D2 occupancy levels that would be in keeping with clinically therapeutic doses in humans i.e., ≥ 65%. The 2.0 mg/kg dose was chosen to reflect what is observed in the clinical setting based on plasma olanzapine levels which represents what is observed in the clinical setting (Perry et al., 2001; Kapur et al., 2003). Chronic infusion via osmotic mini-pump was chosen as the route of administration due to the rat’s rapid metabolism of these medications (Chiu and Franklin, 1996). Female rats were used given the strong evidence in the literature that female rats are more susceptible to atypical antipsychotic-induced weight gain compared to males (Goudie et. al., 2002; Minet-Ringuet et. al., 2005).

We hypothesized that female rats exposed to chronic olanzapine would: a) gain significantly more weight than vehicle treated rats, b) exhibit decreased locomotor activity levels compared to vehicle treated rats, c) consume significantly more kilocalories compared to vehicle treated rats; and d) amass significantly more visceral fat compared to vehicle treated rats.

Materials and Methods

Animals

Twenty-eight female Sprague-Dawley rats (Harlan, Indianapolis, USA), initially weighing 200 – 225g at the start of the experiment, were singly housed in 19x10.5x8 inch...
transparent polycarbonate cages (Lab Products Inc., Seaforth, Delaware, USA) on a 12-hour light: 12-hour dark cycle with lights on at 0800hrs in a temperature controlled room at 21 ±2°C. Rats had free access to standard rodent chow (Lab Diet, Indiana, USA; 3.02 Kcal/g) and water throughout the duration of the experiment. All procedures conformed to the guidelines of the Canadian Council on Animal Care and were approved by the Centre for Addiction and Mental Health Animal Care Committee.

**Drug treatments**

Three groups of rats were randomly assigned to receive one of the following treatments: 7.5 mg/kg/day of olanzapine, 2.0 mg/kg/day of olanzapine (Toronto Research Chemicals, Toronto, ON) or vehicle for 28 days via Alzet mini-pumps (Alzet model 2ML4, Durect Corp., Cupertino, CA). Olanzapine was dissolved in 2% acetic acid solution, then buffered with 1 N NaOH. Vehicle was a 2% acetic acid solution, then buffered with 1 N NaOH.

**Osmotic mini-pump surgery**

After one week of habituation to the animal facility, each rat was anaesthetized briefly using the inhalant anesthetic isoflurane. Once anaesthetized, a small portion of the animal’s back was shaved and sterilized with both isopropyl alcohol and betadine solution. An incision was made within the shaved area followed by blunt dissection of connective tissue with blunt-tipped forceps. The mini-pumps were sterilized with isopropyl alcohol and then inserted subcutaneously, slightly posterior to the scapulae, according to the manufacturer’s specifications. The incision was closed using 9mm
surgical staples. Post-operative animals recovered from the anaesthetic in a heated Plexiglas cage.

Body weight, food intake and body composition

Food intake and body weight were measured daily between 1000hrs and 1200hrs. Food intake was calculated as the difference between the weight of the food that was placed in the hopper and the weight of the food remaining at the time of measuring the following day. On day 28, the animals were sacrificed by CO2 inhalation. To measure visceral adiposity, omental fat pads were dissected out and weighed.

Locomotor activity

Locomotor activity was measured at baseline and weekly thereafter on days 7, 14, 21 and 28 for one hour beginning at 1000hrs. All experiments were conducted in clear polycarbonate cages, measuring 25-cm-wide, 20-cm-high, and 45-cm-long. An array of six infrared photocells was attached outside the longer sides of the cages. The photocells were spaced 7.5 cm apart and 2 cm above the floor of the cage. The equipment was housed in a room different to the colony room.

Data Analysis

Statistical analyses were performed using SPSS Version 15.0 and SAS System v.9.1.3. Results are expressed as mean ± SD and statistical significance for all analyses was set at p<0.05. Analysis of variance (ANOVA) was used to determine the effect of treatment on the dependent variables, total food intake and visceral fat. Mixed models
repeated measures (MMRM) analysis was used to determine the effect of treatment on the dependent variables, weight gain and locomotor activity.

Results

Weight gain

A MMRM was conducted to examine the relationship between treatment and weight gain, expressed as percentage of baseline body weight. The MMRM revealed a significant time main effect, $F(3,63) = 24.53$, $p<0.0001$, a nonsignificant treatment main effect, $F(2,25) = 1.40$, $p=0.266$ and a marginally significant time x treatment interaction, $F(6,63) = 2.29$, $p=0.046$.

Bonferroni-adjusted pairwise comparisons revealed that when the groups were pooled, weight gain was significantly greater in weeks 2-4 than in week 1 ($p=0.0101$, $p<0.0001$, and $p<0.0001$ respectively). Weight gain was also significantly greater in week 4 than in week 2 ($p<0.0001$).

Bonferroni-adjusted multiple comparisons revealed no significant differences between groups; however, there was a dose-dependent trend towards an increase in weight gain in both olanzapine treated groups, more so in the $7.5 \text{ mg/kg}$ group than the
2.0 mg/kg group (Figure 2).

![Graph showing weight gain during 4 weeks of treatment with Olanzapine (2.0 mg/kg and 7.5 mg/kg) and Vehicle.](image)

**Figure 2.** Weight gain during 4 weeks of treatment with Olanzapine (2.0 mg/kg and 7.5 mg/kg) and Vehicle.

**Food intake**

A one-way ANOVA was conducted to evaluate the relationship between treatment and total food intake, expressed in grams. The one-way ANOVA revealed no effect of 7.5mg olanzapine or 2.0mg olanzapine on total food intake compared to the vehicle treated group, F(2,27) = 2.04, p=0.151. Therefore, further follow-up tests were not performed. However, there was a trend towards greater food intake in both olanzapine treated groups, more so in the 7.5 mg/kg group than the 2.0 mg/kg group (Figure 3).
Locomotor activity

A MMRM was conducted to determine the relationship between treatment and locomotor activity, expressed as total beam breaks. The MMRM revealed a significant treatment main effect, $F(2,25) = 18.68$, $p<0.0001$, a significant time main effect, $F(4,92) = 30.66$, $p<0.0001$ and a significant time x treatment interaction, $F(8,92) = 2.91$, $p=0.006$.

Pooling the time points, Bonferroni-adjusted pairwise comparisons revealed that activity scores in the vehicle group were significantly higher than those in both the 2mg ($p<0.0001$) and 7.5mg ($p<0.0001$) groups. The 2mg and 7.5mg groups did not differ from each other ($p=1.0$).

Pooling the groups, Bonferroni-adjusted pairwise comparisons revealed that baseline activity values were significantly higher than those at weeks 1-4 ($p<0.0001$ in each case). Week 3 activity values were also significantly higher than week 4 values ($p=0.0196$).

**Figure 3.** Total food intake during 4 weeks of treatment with Olanzapine (2.0 mg/kg and 7.5 mg/kg) and Vehicle.
Bonferroni-adjusted pairwise comparisons revealed that the groups did not differ from each other at baseline ($p=1.0$ for each pairwise comparison). At week 1, vehicle scores were significantly higher than both active treatment groups ($p<0.0001$ in each case), and treatment arms did not differ from each other ($p=1.0$). Similarly at week 2, vehicle scores were significantly higher than those in both the 2.0mg and 7.5mg groups ($p=0.0090$ and $p=0.0015$, respectively). Once again, the olanzapine groups did not differ from each other ($p=1.0$). This was also the case in week 3 (vehicle vs. 2.0mg: $p<0.0001$, vehicle vs. 7.5mg $p<0.0001$, 2.0mg vs. 7.5mg: $p=1.0$). By week 4 there was no evidence of a difference across the three treatment groups (vehicle vs. 2.0mg: $p=0.1575$, vehicle vs. 7.5mg: $p=0.1140$, 2.0mg vs. 7.5mg: $p=1.0$).

**Figure 4.** Locomotor activity during 4 weeks of treatment with Olanzapine (2.0 mg/kg and 7.5 mg/kg) and Vehicle.
Visceral fat

A one-way ANOVA was conducted to determine the relationship between treatment and visceral fat, expressed in grams. Results revealed that visceral fat mass significantly increased after 28 days of olanzapine treatment, $F(2,23) = 14.83, p<0.001$ (Figure 5). Bonferroni-adjusted post hoc tests revealed significantly larger visceral fat mass in the 7.5mg olanzapine treated group compared to the 2.0mg/kg group ($p<0.001$) and the vehicle group ($p<0.001$), but no significant difference between the olanzapine 2.0 mg/kg and vehicle groups ($p=1.00$).

![Figure 5. Visceral fat after 4 weeks of treatment with Olanzapine (2.0 mg/kg and 7.5 mg/kg) and Vehicle.](image)
Experiment 2:

Effects of different routes of administration on olanzapine-induced weight gain
Overview

In this experiment, we examined the effects of olanzapine via once daily i.p. injections, once daily s.c. injections and osmotic mini-pumps on weight gain, food intake and visceral fat over fourteen days. We chose to administer 7.5mg/kg olanzapine via osmotic mini-pump, in keeping with the doses employed in humans to establish therapeutic blockade of dopamine D2 receptors (Kapur et al., 2003). As in the previous experiment, female rats were investigated given their liability for antipsychotic-induced weight gain (Goudie et. al., 2002; Minet-Ringuet et. al., 2005).

We hypothesized that female rats exposed to chronic olanzapine via osmotic mini-pump, compared to female rats exposed to chronic olanzapine via once daily i.p. injection and once daily s.c. injection, would: a) gain significantly more weight, b) consume significantly more kilocalories; and, c) amass significantly more visceral fat.

Materials and Methods

Animals

Forty-eight female Sprague-Dawley rats (Harlan, Indianapolis, USA), initially weighing 200 – 225g, were singly housed in 19x10.5x8 inch transparent polycarbonate cages (Lab Products Inc., Seaforth, Delaware, USA) on a 12-hour light: 12-hour dark cycle with lights on at 0800hrs in a temperature controlled room (21 ±2° C). Rats had free access to standard rodent chow (Lab Diet, Indiana, USA; 3.02 Kcal/g) and water throughout the duration of the experiment. All procedures conformed to the guidelines of the Canadian Council on Animal Care and were approved by the Centre for Addiction and Mental Health Animal Care Committee.
**Drug treatments**

Three groups of rats were randomly assigned to receive 7.5 mg/kg/day of olanzapine (Toronto Research Chemicals, Toronto, ON) or vehicle for 14 days via Alzet mini-pumps (Alzet model 2ML2, Durect Corp., Cupertino, CA), once-daily s.c. injection and once-daily i.p. injection. Both s.c. and i.p. injections were given daily between 1000hrs and 1200hrs after body weight and food intake were measured. Olanzapine was dissolved in 2% acetic acid solution, buffered with 1 N NaOH. Vehicle was a 2% acetic acid solution, buffered with 1 N NaOH.

**Osmotic mini-pump surgery**

After one week of habituation to the animal facility, each rat was anaesthetized briefly using the inhalant anesthetic isoflurane. Once anaesthetized, a small portion of the animal’s back was shaved and sterilized with both isopropyl alcohol and betadine solution. An incision was made within the shaved area followed by blunt dissection of connective tissue with blunt-tipped forceps. The MPs were sterilized with isopropyl alcohol and then inserted subcutaneously slightly posterior to the scapulae, according to the manufacturer’s specifications. The incision was closed using 9mm surgical staples. Post-operative animals recovered from the anaesthetic in a heated Plexiglas cage.

**Body weight, food intake and body composition**

Food intake and body weight were measured daily between 1000hrs and 1200hrs. Food intake was calculated as difference between the weight of food that was placed in the hopper and weight of food remaining at the time of measurement the following day.
On day 14, the animals were sacrificed by CO2 inhalation. To measure visceral adiposity, omental fat pads were dissected out and weighed.

Data Analysis

Statistical analyses were performed using SPSS Version 15.0 and SAS System v.9.1.3. Results are expressed as mean ± SD and statistical significance for all analyses was set at p<0.05. ANOVAs were used to determine the effect of treatment on the dependent variables, total food intake and visceral fat. Mixed models repeated measures (MMRM) analysis was used to determine the effect of treatment on the dependent variable weight gain.

Results

Weight gain

A MMRM was conducted to determine the effect of route of administration (i.e. osmotic pump, s.c. injection, i.p. injection) and treatment on weight gain. The MMRM revealed a significant route main effect, F(2,42) = 7.91, p=0.0012, a significant treatment main effect, F(1,42) = 38.33, p<0.0001, a significant time main effect, F(13,546) = 10.23, p<0.0001, a significant route x time interaction, F(26,546) = 1.87, p=0.0058, and a significant treatment x time interaction, F(13,546) = 3.28, p<0.0001.

Controlling for treatment and time, Bonferroni-adjusted pairwise comparisons revealed that weight gain was significantly higher in the osmotic mini-pump group than in both the s.c. and i.p. injection groups (p=0.0166 and p=0.0014, respectively). The s.c. injection and i.p. injection groups did not differ from each other (p=1.0).
Controlling for route and time, weight gain was significantly greater in the olanzapine treated group than in the vehicle treated group ($p<0.0001$).

The 3-way interaction between route, treatment, and time shows trend-level significance, but was not investigated further through pairwise comparisons, however, the olanzapine treated mini-pump group shows the most weight gain, followed by the olanzapine treated s.c. injection group and the olanzapine treated i.p. injection group.

**Figure 6.** Weight gain during 2 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle administered via osmotic mini-pump, once daily i.p. injection and once daily s.c. injection.
Food intake

A 3 x 2 ANOVA was conducted to determine the effects of the three routes of administration and treatment on total food intake. The 3 x 2 ANOVA indicated a significant treatment main effect (p<0.001), a significant route of administration main effect (p<0.001), but no significant treatment x route of administration interaction (p=0.470). The significant treatment main effect suggested that the olanzapine treated group consumed more kilocalories than the vehicle treated group. Bonferroni-adjusted post hoc tests revealed that the osmotic mini-pump groups consumed more kilocalories than the i.p. injection groups. Summarizing, rats treated with olanzapine and administered via osmotic mini-pumps yield the greatest food intake.

Figure 7. Total food intake during 2 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle administered via osmotic mini-pump, once daily i.p. injection and once daily s.c. injection.
Visceral fat

A 3 x 2 ANOVA was conducted to determine the effects of the three routes of administration and treatment on visceral fat amassment. There was a significant main effect for treatment \( (p<0.01) \), but no significant main effect for route of administration \( (p=0.709) \) or interaction effect \( (p=0.439) \). Thus, olanzapine treated group amassed significantly more visceral fat.

**Figure 8.** Visceral fat after 2 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle administered via osmotic mini-pump, once daily i.p. injection and once daily s.c. injection.
Experiments 3 and 4:

Effects of diet on olanzapine-induced weight gain
Overview

In two experiments, we examined the effects of olanzapine on weight gain, locomotor activity, food intake and visceral fat amassment. However, we attempted to mirror the human condition by offering rats a diet which resembles that of a typical individual diagnosed with schizophrenia (Brown, 1999). In addition, we chose to administer 7.5mg/kg olanzapine via osmotic mini-pump which is in keeping with the therapeutic blockade of dopamine D2 receptors (Kapur et al., 2003). In the first experiment, we chose to include females because of existing literature that suggests females are more prone to develop antipsychotic-induced weight gain (Goudie et al., 2002; Minet-Ringuet et al., 2005). In the second experiment, we chose only to include males given the fact that numerous studies had already achieved significant weight gain in females, as previously cited. To the best of our knowledge, this is the first study to investigate the chronic administration of a therapeutic dose of olanzapine, coupled with the offering of a typical Western diet to male rats.

We hypothesize that in the first experiment, male and female rats exposed to chronic olanzapine and a high fat diet (60%) will: a) gain significantly more weight than vehicle treated rats, b) will exhibit decreased locomotor activity levels compared to vehicle treated rats, c) will consume significantly more kilocalories compared to vehicle treated rats and d) amass significantly more visceral fat compared to vehicle treated rats. In the second experiment, we hypothesize that male rats exposed to a high fat/high carbohydrate diet (42% fat, 42.7% carbohydrate) will: a) gain significantly more weight than vehicle treated rats, b) will exhibit decreased locomotor activity levels compared to
vehicle treated rats, c) will consume significantly more kilocalories compared to vehicle treated rats and d) amass significantly more visceral fat compared to vehicle treated rats.

**Experiment 3**

**Materials and methods**

*Animals*

Sixteen female and sixteen male Sprague-Dawley rats (Harlan, Indianapolis, USA), initially weighing 200 – 225g and 300 – 325g respectively, were singly housed in 19x10.5x8 inch transparent polycarbonate cages (Lab Products Inc., Seaforth, Delaware, USA) on a 12-hour light: 12-hour dark cycle with lights on at 0800hrs in a temperature controlled room (21 ±2° C). Rats had free access to water and a high fat diet: 60% of Kcal from fat, 22% from carbohydrate and 18% from protein, which provided 5.3 Kcal/g (Dyets Inc., Pennsylvania, USA) throughout the duration of the experiment. Rats were habituated to the diet seven days prior to baseline. All procedures conformed to the guidelines of the Canadian Council on Animal Care and were approved by the Centre for Addiction and Mental Health Animal Care Committee.

*Drug treatments*

Animals were randomly assigned to receive 7.5 mg/kg/day of olanzapine (Toronto Research Chemicals, Toronto, ON) or vehicle for 28 days via Alzet mini-pumps (Alzet model 2ML4, Durect Corp., Cupertino, CA). Olanzapine was dissolved in 2% acetic acid solution, then buffered with 1 N NaOH. Vehicle was a 2% acetic acid solution, then buffered with 1 N NaOH.
Osmotic mini-pump surgery

After one week of habituation to the animal facility, each rat was anaesthetized briefly using the inhalant anesthetic isoflurane. Once anaesthetized, a small portion of the animal’s back was shaved and sterilized with both isopropyl alcohol and betadine solution. An incision was made within the shaved area, followed by blunt dissection of connective tissue with blunt-tipped forceps. The MPs were sterilized with isopropyl alcohol and then inserted subcutaneously, slightly posterior to the scapulae, according to the manufacturer’s specifications. The incision was closed using 9mm surgical staples. Post-operative animals recovered from the anaesthetic in a heated Plexiglas cage.

Body weight, food intake and body composition

Food intake and body weight were measured daily between 1000hrs and 1200hrs. Food intake was calculated as difference between the weight of food that was placed in the hopper and the weight of food remaining at the time of measurement the following day. On day 28, the animals were sacrificed by CO2 inhalation. To measure visceral adiposity, omental fat pads were dissected out and weighed.

Locomotor activity

Locomotor activity was measured at baseline and weekly thereafter on days 7, 14, 21 and 28 for one hour beginning at 1000hrs. All experiments were conducted in clear polycarbonate cages, measuring 25-cm-wide, 20-cm-high, and 45-cm-long. An array of six infrared photocells was attached outside the longer sides of the cages. The photocells
were spaced 7.5 cm apart and 2 cm above the floor of the cage. The equipment was housed in a room different to the colony room.

Data Analysis

Statistical analyses were performed with SPSS Version 15.0 and SAS System v.9.1.3. Results are expressed as mean ± SD and statistical significance for all analyses was set at p<0.05. Data were analyzed according to gender. T-tests were used to determine the effect of treatment on the dependent variables, total food intake and visceral fat. Mixed models repeated measures (MMRM) analysis was used to determine the effect of treatment on the dependent variables, weight gain and locomotor activity.

Results

Weight gain

Females

A MMRM was conducted to determine the effect of treatment on weight gain. The MMRM revealed a significant time main effect, F(3,42) = 21.27, \( p < 0.0001 \), a significant treatment main effect, F(1,14) = 6.93, \( p = 0.02 \) and a nonsignificant time x treatment interaction, F(3,42) = 2.67, \( p = 0.06 \). When pooling the time points, weight gain in the olanzapine group was significantly higher than that in the vehicle group (\( p = 0.0197 \)).

When pooling the study groups, Bonferroni-adjusted pairwise comparisons revealed that weight gain was significantly higher in weeks 2-4 than in week 1 (\( p < 0.0001 \).
in each case). There was no significant change in weight gain between weeks 2 and 3 
\((p=1.0)\), 2 and 4 \((p=0.2513)\) or 3 and 4 \((p=0.6834)\).

Bonferroni-adjusted pairwise comparisons were not conducted for the time x 
treatment interaction; however, there was a trend towards greater weight gain in the 
olanzapine treated group (Figure 9).

*Males*

A MMRM was conducted to determine the effect of treatment on weight gain.
The ANOVA revealed a significant time main effect, \(F(3,42) = 56.72, p<.0001\), a 
nonsignificant treatment main effect, \(F(1,14) = 1.07, p=0.32\) and a nonsignificant time x 
treatment interaction, \(F(3,42) = 1.07, p=0.37\).

Pooling the study groups, Bonferroni-adjusted pairwise comparisons revealed that 
weight gain was significantly higher in weeks 2-4 than in week 1 \((p<0.0001\) in each 
case). Weight gain was significantly greater at weeks 3 and 4 than at week 2 \((p=0.0044 
and \(p<0.0001\) respectively), and weight gain at week 4 was significantly greater than at 
week 3 \((p<0.0001)\).

Bonferroni-adjusted pairwise comparisons were not conducted for the time x 
treatment interaction; however, unlike the female group, there was a trend towards 
greater weight gain in the vehicle treated group (Figure 9).
Figure 9. Weight gain during 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.

Food intake

Females

An independent samples $t$-test was conducted to determine the effect of treatment on total food intake, expressed in grams. The independent samples $t$-test did not reveal a significant difference, $t(14) = 1.31, p=0.212$; however, there was a trend towards greater food intake in the olanzapine treated group.

Males

An independent samples $t$-test was conducted to determine the effect of treatment on total food intake, expressed in grams. The independent samples $t$-test did reveal a significant difference, $t(14) = 2.68, p<0.05$), indicating that the olanzapine treated group (395.6 ± 19.2 g ) consumed more than the vehicle treated group (372 ± 15.9 g).
Figure 10. Total food intake during 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.

Locomotor Activity

Females

A MMRM was conducted to determine the relationship of treatment on locomotor activity. The MMRM revealed a significant time main effect, $F(4,56) = 9.4, p<0.0001$, a significant treatment main effect, $F(1,14) = 24.54, p=0.0002$ and a significant time x treatment interaction, $F(4,56) = 6.55, p=0.0002$.

When pooling the time points, activity values were significantly higher in the vehicle group than in the olanzapine group ($p=0.0002$).

When pooling the study groups, Bonferroni-adjusted pairwise comparisons revealed that activity is significantly less at week 1 than at baseline ($p=0.0010$), week 3
(p<0.0001), and week 4 (p=0.0348). Activity was also significantly lower at week 2 than at week 3 (p=0.0004).

The olanzapine and vehicle groups did not differ at baseline (p=0.5820). Activity values were significantly higher in the vehicle group than in the olanzapine group at weeks 1-4 (p=0.0040, p=0.0228, p=0.0005, and p<0.0001, respectively).

**Males**

A MMRM was conducted to determine the relationship of treatment on locomotor activity. The MMRM revealed a significant time effect, F(4,56) = 10.96, p<0.0001, a significant treatment effect, F(1,14) = 15.94, p=.0013 and a significant time x treatment effect, F(4,56) = 4.31, p=0.0041.

When pooling the time points, activity values were significantly higher in the vehicle group than in the olanzapine group (p=0.0013).

When pooling the study groups, Bonferroni-adjusted pairwise comparisons revealed that activity was significantly less at week 1 than at baseline (p=0.0004), and at weeks 2-4 (p<0.0001 in each case).

The olanzapine and vehicle groups did not differ at baseline (p=1.0), at week 1 (p=0.3375), or at week 4 (p=0.1845). Activity values were significantly higher in the vehicle group than in the olanzapine group at weeks 2 and 3 (p<0.0001 and p=0.0030, respectively).
Figure 11. Locomotor activity during 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.

Visceral fat

Females

An independent samples $t$-test was conducted to test the hypothesis that the olanzapine treated group amassed more visceral fat than the vehicle treated group. The independent samples $t$-test did not reveal a significant difference, $t(14) = .203, p=0.842$; however, there was a trend towards greater visceral fat mass amassment in the olanzapine treated group.
Males

An independent samples t-test was conducted to test the hypothesis that the olanzapine treated group amassed more visceral fat than the vehicle treated group. The independent samples t-test did not reveal a significant difference, $t(14) = .98, p=0.346$), however, similar to the female group, there was a trend towards greater visceral fat mass amassment in the olanzapine treated group.

![Graph](image)

**Figure 12.** Visceral fat after 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.
Experiment 4

Materials and Methods

*Animals*

Sixteen male Sprague-Dawley rats (Harlan, Indianapolis, USA), initially weighing 300 – 325g were singly housed in 19x10.5x8 inch transparent polycarbonate cages (Lab Products Inc., Seaforth, Delaware, USA) on a 12-hour light: 12-hour dark cycle with lights on at 0800hrs in a temperature controlled room (21 ±2° C). Rats had free access to water and a high fat, high carbohydrate diet: 42.0% of Kcal from fat, 42.7% from carbohydrate and 15.2% from protein, which provided 4.5 Kcal/g (TD 88137, Harlan Teklad, Wisconsin, USA) throughout the duration of the experiment. Rats were habituated to the diet seven days prior to baseline. All procedures conformed to the guidelines of the Canadian Council on Animal Care and were approved by the Centre for Addiction and Mental Health Animal Care Committee.

*Drug treatments*

Animals were randomly assigned to receive either 7.5 mg/kg/day of olanzapine (Toronto Research Chemicals, Toronto, ON) or vehicle for 28 days via Alzet mini-pumps (Alzet model 2ML4, Durect Corp., Cupertino, CA). Olanzapine was dissolved in 2% acetic acid solution, buffered with 1 N NaOH. Vehicle was a 2% acetic acid solution, buffered with 1 N NaOH.
Osmotic mini-pump surgery

After one week of habituation to the animal facility, each rat was anaesthetized briefly using the inhalant anesthetic isoflurane. Once anaesthetized, a small portion of the animal’s back was shaved and sterilized with both isopropyl alcohol and betadine solution. An incision was made within the shaved area followed by blunt dissection of connective tissue with blunt-tipped forceps. The MPs were sterilized with isopropyl alcohol and then inserted subcutaneously slightly posterior to the scapulae, according to the manufacturer’s specifications. The incision was closed using 9mm surgical staples. Post-operative animals recovered from the anaesthetic in a heated Plexiglas cage.

Body weight, food intake and body composition

Food intake and body weight were measured daily between 1000hrs and 1200hrs. Food intake was calculated as the difference between the weight of food that was placed in the hopper and weight of the food remaining at the time of measurement the following day. On day 28, the animals were sacrificed by CO2 inhalation. To measure visceral adiposity, omental fat pads were dissected out and weighed.

Locomotor activity

Locomotor activity was measured at baseline and then weekly during 24-hour sessions in a 15.75”L x 8.5”W x 12.0’D custom-built clear polycarbonate cage (Med Associates, Vermont, USA). Locomotor activity was recorded by EthoVision software (Noldus, The Netherlands) and a Sony CCD video camera. Nocturnal Locomotor activity was recorded with the aid of an infrared LED array (Tracksys, Noldus, The Netherlands)
so as not to disturb the animals. The equipment was housed in a room different to the colony room.

Data Analysis

Statistical analyses were performed with SPSS Version 15.0 and SAS System v.9.1.3. Results are expressed as mean ± SD and statistical significance for all analyses was set at p<0.05. Data were analyzed according to gender. T-tests were used to determine the effect of treatment on the dependent variables, total food intake and visceral fat. Mixed models repeated measures (MMRM) analysis was used to determine the effect of treatment on the dependent variables, weight gain and locomotor activity.

Results

Weight gain

A MMRM was conducted to determine the effect of treatment on weight gain. The MMRM revealed a significant time main effect, F(3,42) = 110.55, p<0.0001, a nonsignificant treatment main effect, F(1,14) = 0.17, p=0.684 and a significant time x treatment interaction, F(3,42) = 3.54, p=0.023.

Pooling the study groups, Bonferroni-adjusted pairwise comparisons revealed that weight gain was significantly greater at each time point than at all earlier time points (p<0.0001 in each case).

After adjusting for multiple comparisons, the vehicle and olanzapine groups did not differ at any of the time points (p=1.0 in each case).
**Figure 13.** Weight gain during 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.

**Food intake**

An independent samples *t*-test was conducted to test the hypothesis that the olanzapine treated group consumed more kilocalories than the vehicle treated group. The independent samples *t*-test did not reveal a significant difference, \( t(14) = 1.007, p=0.331 \); however, there was a trend greater total food intake in the olanzapine treated group.
Figure 14. Total food intake during 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.

Locomotor activity

24 hours

A MMRM was conducted to determine the effect of treatment on locomotor activity. The MMRM revealed a significant treatment main effect, $F(1,14) = 9.99$, $p=0.007$, a significant time effect, $F(4,56) = 6.62$, $p=0.0002$ and a nonsignificant time x treatment interaction, $F(4,56) = 1.64$, $p=0.178$.

Pooling the time points, 24 hour total activity values were significantly higher in the vehicle group than in the olanzapine group ($p=0.0069$).

When pooling the study groups together, Bonferroni-adjusted pairwise comparisons revealed that 24 hour activity was significantly greater at week 1 than at baseline ($p=0.0003$) and at week 4 ($p=0.0007$). Bonferroni-adjusted pairwise comparisons
were not conducted for the time x treatment interaction; however, activity levels were lower in the olanzapine treated group at week 1 through week 4.

**Figure 15.** Locomotor activity (24 hours) during 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.

12-hour light phase

A MMRM was conducted to determine the effect of treatment on locomotor activity. The MMRM revealed a significant time main effect, $F(4, 56) = 3.44, p=0.014$, a nonsignificant treatment main effect, $F(1, 14) = 1.61, p=0.226$ and a nonsignificant time x treatment interaction, $F(4, 56) = .59, p=0.67$.

Pooling the study groups, Bonferroni-adjusted pairwise comparisons revealed that light phase activity values were significantly higher at week 1 than at baseline and week 4 ($p=0.0224$ and $p=0.0372$, respectively).
Bonferroni-adjusted pairwise comparisons were not conducted for the time x treatment interaction, however, activity levels were lower in the olanzapine treated group at week 1 through week 4.

**Figure 16.** Locomotor activity (12 hour light phase) during 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.

**12- hour dark phase**

A MMRM was conducted to determine the effect of treatment on locomotor activity. The MMRM revealed a nonsignificant time main effect, $F(4,56) = 2.41, p=0.06$, a significant treatment main effect, $F(1,14) = 10.74, p=0.0055$ and a nonsignificant time x treatment interaction, $F(4,56) = 1.74), p=0.154$.

Pooling the time points, dark phase activity values were significantly higher in the vehicle group than in the olanzapine group ($p=0.0055$).
Bonferroni-adjusted pairwise comparisons were not conducted for the time x treatment interaction; however, activity levels were lower in the olanzapine treated group at week 1 through week 4.

**Figure 17.** Locomotor activity (12 hour dark phase) during 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.

*Visceral fat*

An independent samples $t$-test was conducted to test the hypothesis that the olanzapine treated group amassed more visceral fat than the vehicle treated group. A significant difference was not found, $t(14) = .848, p=0.411$; however, there was a trend
towards more visceral fat amassment in the olanzapine treated group.

Figure 18. Visceral fat after 4 weeks of treatment with Olanzapine (7.5 mg/kg) and Vehicle.
Discussion

Summary.

Atypical antipsychotics are currently the first choice of treatment for schizophrenia (Agid et al., 2008). This shift was based on their numerous purported clinical benefits; however, their widespread clinical use soon identified troublesome adverse events in the form of significant weight gain and associated metabolic disturbances which may lead to other ailments including cardiovascular disease, type II diabetes and hypertension. The mechanisms responsible for antipsychotic-induced weight gain are poorly understood, in part related to the diverse pharmacological profiles of these newer drugs.

There are reasons at present as to why this may no longer be perceived as important. First, there are now several atypical agents that claim ‘weight neutral’ status, that is without the notable weight gain i.e., aripiprazole, ziprasidone (Allison et al., 1999). Further, more recent clinical data that has moved the focus from efficacy to effectiveness have tempered the claims of significant gains with these newer medications versus their conventional counterparts (Grunder et al., 2009). This has raised the possibility of returning to first generation antipsychotic use, based on the argument that more judicious dosing may mitigate against their liability of EPS while gaining the benefits of lower weight gain risk and decreased expense.

This is, in fact, unlikely to occur. It remains that the conventional antipsychotics appear to have a greater risk of TD when compared to the atypical agents (Remington, 2007), a compelling argument against their resurgence. Further, there is evidence for modest clinical gains with the newer antipsychotics across important symptom domains.
and as a class they have been associated with greater tolerability and adherence (Lieberman et al., 2005). While the atypicals have not proven to be the panacea that they were initially claimed to represent, they do reflect incremental gains and from the standpoint of drug development it behooves us to build upon these gains while circumventing their pitfalls. To this end, it is critical to clearly establish the mechanisms underlying the weight gain/metabolic abnormalities linked to the atypical antipsychotics and the present series of experiments sought to investigate the effects of olanzapine on weight gain, food intake, locomotor activity and visceral fat while using different routes of administration, doses and diets. Olanzapine was chosen for several reasons. It, along with clozapine, reflect the two atypical antipsychotics with the greatest weight gain liability (Allison, 1999); in addition, despite these concerns it remains among the two most widely prescribed atypical antipsychotic in North America (Allesi-Severini et al., 2008; Sernyak and Rosenheck, 2008). The critical dependent variables in this work included weight gain, pattern of weight distribution, food choice/intake, and locomotor activity.

Experiment 1 examined dose, investigating the effects of olanzapine administered at doses of 2.0 and 7.5 mg/kg on female rats over 4 weeks being maintained on standard chow ad libitum. The lower dose, i.e. 2.0 mg/kg, reflects what is observed in the clinical setting based on plasma olanzapine levels (Perry et al., 2001; Kapur et al., 2003). The higher dose, i.e. 7.5 mg/kg, is based on in vivo dopamine D2 occupancy which represents D2 occupancy levels that would be in keeping with clinically therapeutic doses in humans i.e., ≥ 65%. Rats treated with 7.5 mg/kg olanzapine failed to exhibit increases in weight
gain and food intake; however, locomotor activity was suppressed and visceral fat
deposition was greater than that of rats treated with vehicle. Rats treated with 2.0 mg/kg
olanzapine also failed to exhibit significant increases in weight gain, food intake and
visceral fat deposition but exhibited significant decreases in locomotor activity levels. We
believe this to be the first experiment carried out using a dose i.e., 7.5 mg/kg, which
approximates the 60-75% dopamine D₂ receptor occupancy required for optimal
therapeutic response in humans (Kapur et al., 2000b).

Experiment 2, in turn, examined route of administration, specifically the effects of
this same dose of olanzapine (7.5 mg/kg) on female rats comparing 3 routes of
administration; 1) once daily i.p. injections, 2) once daily s.c. injections, and 3) osmotic
mini-pump over a 2-week period while being maintained on standard chow ad libitum.
This work was driven by the aforementioned findings and an outstanding question as to
whether the lack of weight gain reflected recognized differences in antipsychotic
metabolism between rodents and humans (Chiu and Franklin 1996). Female rats treated
with olanzapine significantly increased body weight regardless of route when compared
to rats treated with vehicle. All three routes of administration yielded greater food intake
in rats treated with olanzapine compared to their respective vehicle groups; however,
there were no significant differences between olanzapine treated groups. Olanzapine
administered via osmotic pump and once-daily s.c. injections yielded significantly more
visceral fat when compared to their respective vehicle groups although there were no
significant differences between the three olanzapine treated groups.

Experiments 3 and 4 investigated the effects of olanzapine (7.5 mg/kg) via osmotic
mini-pump on male and female rats-over a 4-week period while being maintained on a
high fat (60%) diet and a high fat/high carbohydrate diet (42% fat, 42.7% carbohydrate) ad libitum. In female rats, olanzapine was associated with significant weight gain and suppressed locomotor activity in conjunction with a high fat (60%) diet. For this same diet in male rats, olanzapine was associated with an increase in food intake and suppressed locomotor activity. Olanzapine was associated with suppressed locomotor activity over the 24-hour period and the 12-hour dark period in male rats maintained on a high fat/high carbohydrate diet, although there were no significant differences in food intake, weight gain or visceral adiposity. That the olanzapine-treated animals exposed to a high fat/high carbohydrate diet failed to show differences on these measures seemed counterintuitive; however, studies in the existing literature have reached the same conclusions (see Table 2).

**Current data vs. existing literature**

*Weight gain*

Failure to elicit weight gain in Experiment 1 is not consistent with the large body of literature linking olanzapine with weight gain in female rats (Table 2). Having said this, we did observe a significant treatment effect in female rats being maintained on both standard chow (Experiment 2) and a high fat diet (60%) (Experiment 3). Studies that failed to elicit weight gain include Fell et al. (2004a) who treated female rats with olanzapine (0.5 mg/kg) over 3 weeks via i.p. injection, and similarly, Pouzet et al. (2003) who did not observe weight gain after administering a high dose of olanzapine (20 mg/kg) over a 3-week period via gavage. In contrast, Fell et al. (2008) observed significant weight loss in female rats treated with 2.0mg/kg olanzapine over 4 weeks.
while being maintained on a diet consisting of 20% protein, 35% fat and 45% carbohydrate. Fell et al. (2008) suggest that olanzapine-induced weight gain may be influenced by both palatability of the diet used and the decreased preference for fat which resulted in decreased food intake.

Consistent with other studies (Albaugh et al., 2006; Choi et al., 2007; Cooper et al., 2007; Minet-Ringuet et al., 2005; Minet-Ringuet et al., 2006a; Pouzet et al., 2007), we were unable to elicit weight gain in male rats treated with olanzapine exposed to both a high fat diet and a high fat/high carbohydrate diet (Experiments 3 and 4). To date, only one study has reported olanzapine-induced weight gain in male rats (Minet-Ringuet et al., 2006b) by administering olanzapine in a diet consisting of 14% protein, 31% fat and 54% carbohydrate. Minet-Ringuet et al. (2006b) suggest that the ratio between fat and carbohydrate may be important in attempting to create an animal model of weight gain in male rats.

Food intake

Our data in Experiment 1 fall in line with several studies which failed to report an increase in food intake in female rats treated with olanzapine while maintained on standard chow (Cooper et al., 2005; Fell et al., 2004a; Fell et al., 2005b; Patil et al. 2006; Pouzet et al., 2003). In contrast, several studies report an increase in food intake with standard chow (Albaugh et al., 2006; Choi et al., 2007; Cooper et al., 2005; Fell et al., 2004a; Kalinichev et al., 2005; Patil et al., 2006; Pouzet et al., 2003), as did our results in Experiment 2.
We also failed to elicit an increase in food intake while offering female rats a high-fat diet (60%), in agreement with Fell et al. (2008) who report no effect of olanzapine on food intake with a high-fat diet (35%). Our data in Experiments 3 and 4 involving male rats reveal an increase in food intake with a high-fat diet (60%) and a nonsignificant trend towards increased food intake with a high fat/high carbohydrate diet. It is unclear as to why significance was not reached in Experiment 4. Only one other study included a diet high in fat (31%) and carbohydrates (54%) with male rats, and it reported an increase in food intake (Minet-Ringuet et al., 2006b).

**Visceral fat**

The increase in visceral fat with olanzapine 7.5mg/kg, reported in Experiments 1 and 2, concurs with other studies involving olanzapine, where doses range from 2.0mg/kg to 5.0mg/kg (Fell et al., 2004a; Fell et al., 2005b; Kalinichev et al., 2005; Raskind et al., 2007). However, we failed to observe an increase in visceral adiposity (Experiment 1) after treatment with olanzapine 2.0mg/kg. Fell et al. (2004a) also failed to elicit an increase in visceral adiposity in female rats with olanzapine 0.5mg/kg and 1.0mg/kg after 3 weeks of treatment. Our results in Experiment 3 were in keeping with those reported by Minet-Ringuet et al. (2006) who observed increased visceral adiposity in male rats without an increase in body weight.
Locomotor activity was suppressed in each of the four experiments, consistent with other published studies involving both female (Arjona et al., 2004; Fell et al., 2007) and male rats (Karl et al., 2006) treated with olanzapine.

Limitations and future directions

The most significant problem related to the present line of investigation must be seen as the applicability of the rodent model to antipsychotic-induced weight gain. In humans, drugs like olanzapine and clozapine are associated with rapid and dramatic increases in weight that are observed in both males and females. This is not the case when these same drugs are administered to rodents, where the degree of weight gain is modest at best and gender-specific i.e., more consistent in females. There is some evidence in humans that females may gain more weight (Aichhorn et al., 2006, Homel et al., 2002, Russell and Mackell, 2001), but the risk for males is still substantial.

Various explanations have been posited to explain the animal findings. It has been suggested, for example, that this limitation may stem from the species studied and that more robust weight gain could be observed by treating obesity prone rats. These could include: i) the high-fat feeding rat exposed to a cafeteria diet consisting of calories derived from foods such as chocolate and cookies, ii) the obese Zucker rat which, due to a spontaneous mutation in the fa gene, becomes obese when fed standard chow ad libitum, iii) the Zucker diabetic fatty rat which becomes obese when fed a high fat diet, and iv) the Koletsky rat which becomes obese by consuming more kilocalories (Young and Kirkland, 2007).
From a gender standpoint, Baptista et al. (1997) have suggested that the gender difference may be attributed to an interaction between atypical antipsychotics and female hormones. Albaugh et al. (2006) suggest that olanzapine-induced weight gain may be more difficult to determine in male rats because the weight gained due to treatment with olanzapine may be masked by the constant and steady weight gained due to normal growth.

Complicating the picture further is distribution of weight gain, a factor now identified as critical to the cardiovascular risk story (Bays, 2009; Despres and Lemieux, 2006), but one that has received comparatively little attention to date in either the human or animal literature. More specifically, it is the visceral fat deposition, which translates to increased abdominal girth, that seems critical to cardiovascular risk, not weight gain per se. The importance of central obesity is reflected by its inclusion in the criteria for metabolic syndrome (George et al., 2005), and more recent data linking these fat stores to alterations in insulin and glucose (Perrini et al., 2008). Our own work, for example, demonstrated increased visceral fat in the absence of notable weight gain (Experiment 1). This has important implications from a metabolic standpoint, as it indicates that these drugs may influence glucose regulation independent of weight gain that is through changes in fat distribution. Thus, the latter may actually be the more critical measure from the standpoint of metabolic risk, and the rodent model seems to offer a potentially useful investigative tool in this regard.

Finally, in the human condition there is the influence of the illness itself. A sizeable body of literature has identified the presence of weight gain and increased visceral fat deposition in antipsychotic naïve schizophrenic patients (Dixon et al., 2000;
suggestion that the illness itself may carry an increased liability for weight gain. In addition, schizophrenia is linked to a sedentary lifestyle and poor diets, high in fat and low in fibre (Brown, 1999; Faulkner and Cohn, 2006), factors that may contribute to the degree of weight gain observed with antipsychotic treatment.

Just as in humans, it would be valuable to not just examine total body weight in this type of work, but to include more discrete measures such as lean body mass/adiposity in an effort to better understand distribution of weight gain. Further, there would be value in examining basal metabolic rate (BMR) both at baseline and after treatment with olanzapine, as changes here could account for differences in weight gain (or lack thereof) independent of more commonly assessed measures e.g., food intake, activity. This may also shed light on the apparent discrepancies observed with these drugs between humans and rodents; there is, for example, evidence that there are notable species-specific differences in how these drugs are metabolized (Kapur et al., 2003).

While it would be ideal, an animal model is not required to mirror all aspects of the human condition to be relevant. Indeed, it is more common for their utility to be confined to specific aspects of the human condition (Lipska and Weinberger, 2000). Here, it is apparent that the degree of weight gain is a rate-limiting step in drawing comparisons to what is observed in humans following exposure to newer antipsychotics such as olanzapine. However, the model may closely mirror what is observed in terms of activity level changes and, in fact, there is evidence to support this position (Sharpe et al., 2006). Whether it might also prove valuable in examining other parts of the story e.g.,
changes in BMR, distribution of weight gain, requires closer examination in the context of future investigations.

Conclusions

In conclusion, this series of experiments has attempted to investigate the effects of olanzapine administration on weight gain, food intake, the accumulation of visceral fat and locomotor activity in rats. Findings related to weight gain are in line with other reports indicating that while olanzapine-induced weight gain can be observed, it does not mirror what is observed in humans on two levels: (i) it is not of the same magnitude, and (ii) it is more gender specific i.e., females greater than males. The present work suggests that differences in drug metabolism likely explain at least part of this distinction, in that rodents are known to metabolize antipsychotics much more rapidly than what is seen in humans (Chiu and Franklin 1996). Thus, the likelihood of observing weight gain was increased in animals where the olazapine was administered via osmotic mini-pump (see Experiments 2, 3). However, this does not adequately explain the gender effect and we are left to speculate, with others, that notable differences in size, body composition and growth curves between male and female rats contribute to the lack of drug effect in the former.

Our results do speak to the importance of examining distribution of fat i.e., visceral adiposity, as well as weight gain in this type of work. While olanzapine-induced increases in visceral adiposity were observed in conjunction with weight gain (see Experiment 2, 3), this was also seen when weight gain per se was not recorded (see Experiment 1). Ensuring that visceral adiposity is measured therefore takes on
importance for several reasons. First, it offers a more discrete means of examining olanzapine-induced changes in body composition, and in the rodent model where weight gain per se is less obvious, it may prove a valuable alternative. In addition, it is abdominal girth that is critical to cardiovascular risk (Kissebah and Krakower, 1994), making visceral adiposity in the rodent model an important proxy for abdominal girth in humans.

In humans, we continue to struggle with why such weight gain occurs with exposure to atypical antipsychotics like olanzapine and clozapine. There is, of course, considerable interest in direct pharmacological mechanisms e.g., histamine (Kroeze et al., 2003), but the influence of more indirect non-pharmacological factors cannot be ignored. For example, does the sedating effect of these medications translate to diminished activity, which in turn enhances liability for increased weight? Do individuals eat more, or gradually shift intake to diets that enhance risk of weight gain? These represent important questions, not only from the standpoint of understanding underlying mechanisms, but in establishing interventions that might be most effective in the clinical setting. Perhaps the most consistent finding in the present series of experiments was effect of olanzapine on locomotor activity (see Experiments 1, 3, 4), which is in line with its sedating effects in the clinical setting. More recent work, in fact, suggests that antipsychotic-induced decreases in activity, possibly due to sedation, may be critical to the weight gain observed in humans treated with medications such as clozapine and olanzapine (Morrens et al., 2007; Sharpe et al., 2006). While our data suggest that olanzapine’s effects on activity may, at least indirectly, contribute to its weight gain
potential, this does not preclude a direct effect on weight that is pharmacologically mediated.

We did examine dietary differences by looking at patterns of intake, weight gain and visceral fat distribution in olanzapine-treated animals exposed to a high fat and high fat/high carbohydrate diet compared to standard chow. It was the high fat diet that incurred notable weight gain, and the magnitude of weight gain was substantial, at least in females (Experiment 3). While we did not specifically examine food choice per se with this line of investigation, our results suggest that exposure to a high fat diet in conjunction with olanzapine treatment can translate to substantial weight gain. This is important given evidence that this type of diet might better mirror the ‘real world’ situation of those with schizophrenia; that is, for various reasons they find themselves exposed to diets of this sort (Brown et al., 1999).

Concluding, there are clearly features of the rodent model of antipsychotic-induced weight gain that distinguish it from what is observed in humans. While these are important and cannot be ignored, they do not necessarily preclude the use of this model. Rather, we are reminded that precisely because of these differences it is necessary to employ certain strategies to optimize its utility. More specifically, these would include: a) use of females; b) appropriate antipsychotic dosing; c) use of osmotic mini-pump (or depot formulation) for administration; d) access to a high fat diet. To date, many studies have, at best, addressed only several of these factors in their methodology. Under such conditions though, we have been able to establish olanzapine induces notable changes in weight and/or its distribution, as well as behaviours that are likely to contribute to this risk e.g. activity levels, food intake.
Moving forward, the logical next step would be to employ such a paradigm in evaluating other antipsychotics varying in their liability for weight gain in humans. A profile of risk approximating what is observed clinically would further validate this model and, in so doing, add support for its use in better understanding the underlying mechanisms, both pharmacological and non-pharmacological.
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