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# Effects of olanzapine on the elevation of macrophage infiltration and pro-inflammatory cytokine expression in female rats

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# Effects of olanzapine on the elevation of macrophage infiltration and pro-inflammatory cytokine expression in female rats

## **Abstract**

The metabolic side-effects of olanzapine have undermined drug compliance and increased concern for this otherwise-effective treatment for schizophrenia. As obesity and type 2 diabetes are associated with low-grade inflammation, and olanzapine-induced weight gain has three typical stages, the current study investigated the inflammatory effects of olanzapine in three treatment stages. Female Sprague-Dawley rats were treated orally with olanzapine (1 mg/kg three times daily) or vehicle for one week, two weeks, and five weeks. Olanzapine significantly increased body weight and white visceral fat deposition in all three treatment stages compared to control. Olanzapine enhanced average adipocyte size and level of macrophage infiltration in white adipose tissue (WAT) compared to control, with levels of macrophage infiltration increased over time. There was a high correlation between adipocyte size and macrophage infiltration rate. Olanzapine also caused increased macrophage infiltration in brown adipose tissue (BAT), but not liver. Additionally, pro-inflammatory cytokines tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin (IL)-1 $\beta$ , and IL-6 were upregulated by olanzapine in the hypothalamus, WAT, and BAT compared to control, but not the liver. Finally, plasma triglycerides were elevated by olanzapine compared to control, but not total cholesterol, high density lipoprotein (HDL) or low density lipoprotein (LDL). These findings indicate that olanzapine-induced inflammation and adiposity are closely related, and that peripheral low-grade inflammation develops during olanzapine treatment.

## **Keywords**

Antipsychotics, olanzapine, inflammation, adiposity, macrophage infiltration

## **Disciplines**

Medicine and Health Sciences

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# Effects of olanzapine on the elevation of macrophage infiltration and pro-inflammatory cytokine expression in female rats

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## **Abstract**

The metabolic side-effects of olanzapine have undermined drug compliance and increased concern for this otherwise-effective treatment for schizophrenia. As obesity and type 2 diabetes are associated with low-grade inflammation, and olanzapine-induced weight gain has three typical stages, the current study investigated the inflammatory effects of olanzapine in three treatment stages. Female Sprague-Dawley rats were treated orally with olanzapine (1 mg/kg; t.i.d.) or vehicle for 1 week, 2 weeks, and 5 weeks. Olanzapine significantly increased body weight and white visceral fat deposition in all three treatment stages compared to control. Olanzapine enhanced average adipocyte size and level of macrophage infiltration in white adipose tissue (WAT) compared to control, with levels of macrophage infiltration increased over time. There was a high correlation between adipocyte size and macrophage infiltration rate. Olanzapine also caused increased macrophage infiltration in brown adipose tissue (BAT), but not liver. Additionally, pro-inflammatory cytokines TNF $\alpha$ , IL-1 $\beta$ , and IL-6 were upregulated by olanzapine in hypothalamus, WAT, and BAT compared to control, but not liver. Finally, plasma triglycerides were elevated by olanzapine compared to control, but not total cholesterol, HDL or LDL. These findings indicate that olanzapine-induced inflammation and adiposity are closely related, and that peripheral low-grade inflammation develops during olanzapine treatment.

## **Keywords:**

Antipsychotics; olanzapine; inflammation; adiposity; macrophage infiltration

## **1. Introduction**

Although second generation antipsychotics such as olanzapine and clozapine have been widely used to treat schizophrenia and other psychiatric disorders, they have been associated with an increased risk of metabolic side-effects such as obesity, abnormal adiposity, and type 2 diabetes (De Hert et al., 2012; Fountaine et al., 2010; Weston-Green et al., 2013). This has caused significant concern in terms of reduced drug compliance and increased morbidity and mortality (De Hert et al., 2012; Deng, 2013; Weston-Green et al., 2013). In addition, both clinical (Haupt, 2006; McEvoy et al., 2005; Pai et al., 2012) and animal studies (He et al., 2014; Huang et al., 2006; Zhang et al., 2014a) suggest that there are three typical stages of olanzapine-induced weight gain: (1) initial stage with a rapid increase of body weight accompanied by an elevated food intake; (2) middle stage with slow body weight gain and no elevation of food intake; and (3) late stage with maintenance of heavy body weight without an elevated food intake.

Accumulating evidence has suggested that obesity and type 2 diabetes are in fact connected to the development of low-grade inflammatory responses (Ouchi et al., 2011). In particular, cafeteria diet-induced obesity (animals self-selecting from highly palatable, readily available foods including cookies, candy, cheese, and processed meats, designed to simulate the human Western diet) and metabolic syndrome have been reported to be associated with liver and adipose tissue inflammation and macrophage infiltration (Sampey et al., 2011). In addition, hypothalamic inflammation has also been suggested to contribute to the pathogenesis of obesity in response to high-fat feeding (Kleinridders et al., 2009).

The effects of olanzapine on central or peripheral inflammation have been scarcely reported. One animal study reported that female rats receiving olanzapine treatment (2 mg/kg) had elevated plasma levels of IL-8 and IL-1 $\beta$ , while both male and female rats receiving olanzapine treatment demonstrated increased adiposity and macrophage infiltration into

adipose tissue (Davey et al., 2012). Another study using male rats revealed that chronic olanzapine treatment can induce extensive macrophage infiltration and increase TNF $\alpha$  mRNA expression in epididymal white adipose tissue (WAT) but not in the liver (Victoriano et al., 2010). A clinical study reported that 14 days of olanzapine treatment (5-10 mg/d) led to increased serum levels of tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) and plasminogen activator inhibitor type 1 compared to the control in healthy male volunteers (Fountain et al., 2010). However, these studies did not explore the potential differential effects of olanzapine on these inflammatory markers along the time course of the treatment, nor did they analyse the relationship between adiposity (hypertrophy/hyperplasia) and inflammation in a female rat model of olanzapine-induced weight gain.

The present study aimed to investigate the inflammatory effects of olanzapine treatment in an established female rat model of olanzapine-induced weight gain through its three stages, and examine the relationship between inflammatory signals and body weight gain or adiposity. We hypothesized that olanzapine can cause time-related increases in macrophage infiltration, and in pro-inflammatory cytokine expression in WAT, brown adipose tissue (BAT), the liver and hypothalamus. We also hypothesized that olanzapine can cause morphological changes in WAT, BAT, and the liver, which are correlated to changes in inflammatory markers. We measured and compared the potential morphological changes of WAT, BAT, and the liver, including individual fat pad weights, adipocyte size, and macrophage infiltration, under olanzapine or vehicle treatments after each of the three treatment stages. In addition, we also measured the mRNA expression of proinflammatory markers TNF $\alpha$ , IL-1 $\beta$ , and IL-6 at the hypothalamus, WAT, BAT, and the liver were also measured. Finally, we analysed plasma lipid profiles, including plasma triglycerides, total cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL). To the best of our knowledge, this is the first study reporting on a time-related effect of olanzapine on inflammation, and the effect of

olanzapine on the mRNA expression of inflammatory markers in the hypothalamus, in an established female rat model of olanzapine-induced weight gain.

## **2. Materials and methods**

### ***2.1 Animals, diets, and drug administration***

Female Sprague-Dawley rats (Animal Resource Centre, Perth, WA, Australia), initially weighing 201-225 g, were individually housed at 22°C, 12-h light-dark cycle (lights on at 07:00 h). All animals had *ad libitum* access to water and a standard laboratory chow diet (3.9 kcal/g; 10% fat, 74% carbohydrate, and 16% protein). (Since the main goal of the present study was to **investigate the effect of the drug (olanzapine), but not diet, we used a standard lab chow diet for all of our animals.**) After one week of acclimatization, animals were trained to self-administer the placebo sweet cookie-dough to ensure the timely delivery of drugs or vehicle on the experimental days. Rats were randomised into either olanzapine (O) or control (C) treatment groups, with three treatment duration cohorts: short-term (8 days), mid-term (16 days), and long-term (36 days) (6 groups; n=6/group). All of the experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia, and complied with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (Australian Government National Health and Medical Research Council, 2004).

A cookie-dough (3.5 kcal/g; 62% carbohydrate, 22% protein, 6% fibre, 10% vitamins and minerals) method was employed as previously reported (Deng et al., 2012; Weston-Green et al., 2011; Zhang et al., 2014a). The cookie-dough method is a non-invasive and effective way to administrate olanzapine, with reported sensitivity to olanzapine-induced side-effects and superior response compared to oral gavage or injection methods (Minet-Ringuet et al., 2006; Shobo et al., 2011). Briefly, a mixture of cornstarch (30.9%), sucrose (30.9%), gelatine (6.3%), casein (15.5%), fibre (6.4%), minerals (8.4%), and vitamins (1.6%) was produced. Three times per day, a small cookie-dough pellet (approximately 0.3 g) mixed with either

olanzapine (1 mg/kg body weight) (Eli Lilly, Indianapolis, IN, USA) or placebo was served to the animals. The cookie dough contained a negligible amount of calories compared to the daily food intake for the rats (accounting for approximately 3-4% of total daily calorie intake). Animals were observed during the administration period to ensure their complete consumption of the pellets. The dosage of olanzapine (1 mg/kg body weight, three times per day) was based on our prior studies (Deng et al., 2012; Weston-Green et al., 2011). This dosage is clinically relevant based on D2 receptor occupancy (Kapur et al., 2003), which is equivalent to a human dosage of approximately 10 mg/day (for a 60 kg person), according to dosage translation between species based on body surface area, following an FDA guideline for clinical trials (Center for Drug Evaluation and Research FDA, 2005; Reagan-Shaw et al., 2008). Body weight and food intake measurements were obtained every second day as we reported earlier (Zhang et al., 2014a).

In this study, rats were closely housed in a room occupied by only female rats. Under this rearing condition, our pre-experiments have shown that the ovarian cycles of all of the female rats are synchronized. Therefore, although we did not directly measure the sex hormone levels, we should expect no significant difference between the groups due to synchronized their ovarian cycles.

## ***2.2 Euthanasia and tissue collection***

Two hours after the last 07:00 h treatment, the rats were euthanized by fast CO<sub>2</sub> infusion (Deng et al., 2012; Han et al., 2008; Weston-Green et al., 2011). Brains were dissected on an ice plate immediately after euthanasia, snap-frozen in liquid nitrogen, and stored at -80°C. Liver, periovary WAT, perirenal WAT, omental WAT, inguinal WAT, and interscapular BAT were dissected and weighed. Liver BAT and periovary WAT (as a representative for visceral WAT) were cut into two halves: one half (for mRNA analysis) was snap-frozen and

stored at -80°C, and the other half (for immunohistochemistry and histology analysis) was fixed overnight by immersion at 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The fixed samples were then dehydrated, cleared, and embedded in paraffin.

### **2.3 Quantitative real-time PCR (qRT-PCR)**

The qRT-PCR protocol was adopted from a previous report from our group (Yu et al., 2013). Briefly, the total RNA from the hypothalamus, BAT, WAT, and the liver was extracted using the Aurum total RNA mini kit (Bio-Rad Laboratories, Hercules, CA, USA) and reverse-transcribed using the high-capacity cDNA reverse transcription kit (AB Applied Biosystems, CA, USA). qRT-PCR was performed for TNF $\alpha$ , IL-1 $\beta$ , and IL-6 in a 20  $\mu$ l final reaction volume using SYBR green I master in a Lightcycler 480 (F. Hoffmann-La Roche Ltd, Switzerland). Primers used are listed in Supplementary Table 1. Amplification was carried out with 45 cycles of 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The results were normalized to GAPDH, which served as the internal control. The experiments were performed in triplicate.

### **2.4 Histology and F4/80 Immunohistochemistry (IHC)**

The paraffin-embedded tissues (in particular, liver, BAT, and WAT; n=6/group) were section-sliced (4  $\mu$ m/section) at 40  $\mu$ m intervals, mounted on charged glass slides, deparaffinized in xylene, stained for F4/80 as described by Weisberg et al. (2014), and counter-stained briefly in hematoxylin (F4/80 is a well-characterized and extensively referenced membrane protein for rodent macrophage analysis). Ten fields per section (per rat) from each of the target tissues were randomly captured with a Syntec STK1160 CCD camera (Syntec Semiconductor Co. Ltd., Taipei, Taiwan) at 10x objective as described in Zhang et al. (2014). The total number of nuclei and the number of nuclei of F4/80-positive cells were counted for each field using the Image J 1.44 p software (Wayne Rasband, National Institutes

of Health, USA). The percentage of F4/80-positive cells for each sample was calculated as the total number of F4/80-positive nuclei over the total number of nuclei in the 10 randomly selected fields. For WAT, the average adipocyte cross-sectional area was also analysed using the ImageJ 1.44 p software.

### ***2.5 Plasma lipid profile***

Blood samples were obtained by puncturing the right ventricle of the heart, collected in EDTA coated tubes, and centrifuged at 3,000 rpm at 22°C for 25 minutes. HDL was isolated from the plasma with dextran sulphate and magnesium chloride, based on a modified method from Sjoblom and Eklund (Sjoblom et al., 1989). Total plasma cholesterol (TC), HDL and triglycerides (TG) were measured using the Konelab® 20XT automatic analyser. The TC, HDL, and TG were analysed with Infinity™ reagent (Thermo Fisher Scientific, Auburn, NSW, Australia). LDL was calculated using the Friedewald formula:  $LDL = (TC - HDL) - (TG/2.19)$  (Friedewald et al., 1972).

### ***2.6 Statistics***

The statistical software SPSS (version 19, SPSS, Chicago, IL, USA) was used to perform the analysis. Two-way ANOVA was used to determine the olanzapine and time-related effects for tissue weight and body weight gain, TNF $\alpha$ , IL-1 $\beta$ , and IL-6 mRNA expression, percentage of F4/80-positive cells, average adipocyte size, and plasma lipid profile. The post-hoc Tukey-HSD test was used for multiple comparisons between the three time points of interest (short-, mid- and long-term cohorts). Levene's tests of equality of error variance were performed to check for heteroscedasticity. One-way ANOVA was conducted to compare olanzapine and the control at the three time points of interest (short-, mid- and long-term cohorts). Correlations were identified by Pearson's correlation. Data were expressed as mean  $\pm$  SEM, and  $P < 0.05$  was considered statistically significant.

### 3. Results

#### *3.1 Olanzapine increases visceral WAT weight and percentage body weight gain*

Olanzapine treatment led to a significant increase of percentage body weight gain in the short-, mid- and long-term cohorts compared to the control (Table 1). Two-way ANOVA revealed that there was a significant main effect of olanzapine ( $F_{(1,30)} = 25.936$ ;  $P < 0.001$ ), as well as a time-related effect ( $F_{(2,30)} = 3.449$ ;  $P < 0.05$ ). There was also a significant interaction between olanzapine and time ( $F_{(2,30)} = 8.895$ ;  $P < 0.01$ ).

Total body adipose tissue weight as well as the total WAT weight were significantly elevated by olanzapine ( $F_{(1,30)} = 11.593$  and  $F_{(1,30)} = 11.711$  for total fat and total WAT, respectively; both  $P < 0.01$ ). There was no significant difference in BAT weight (Table 1). There was no significant time-related effect on total adipose tissue weight or total WAT weight. Furthermore, the visceral WAT weight was significantly increased by olanzapine ( $F_{(1,30)} = 10.863$ ;  $P < 0.01$ ), while the subcutaneous inguinal WAT weight was only significantly increased in the short- and mid-term olanzapine treatment cohorts (Table 1). For visceral fat, the periovary WAT weight was constantly elevated by olanzapine throughout the three treatment cohorts ( $F_{(1,30)} = 10.368$ ;  $P < 0.01$ ), perirenal WAT weight was increased by olanzapine in the short- and mid-term cohorts, and omental WAT weight was not significantly changed in any of the treatment cohorts (Table 1).

#### *3.2 Olanzapine increases adipocyte size and macrophage infiltration in WAT*

The average adipocyte cell area in WAT was significantly increased by olanzapine treatment in all of the cohorts ( $F_{(1,30)} = 61.756$ ;  $P < 0.01$ ; Figure 1A, 1B), but with no time-related effect. In addition, as detected by IHC of F4/80, macrophage infiltration was evident in the olanzapine treatment groups during the three treatment cohorts ( $F_{(1,30)} = 56.400$ ;  $P < 0.001$ ; Figure 1A, 1C). There was a significant time-related effect ( $F_{(2,30)} = 4.528$ ;  $P < 0.05$ ), and the

interaction effect approached significance ( $F_{(2,30)} = 3.142$ ;  $P = 0.058$ ). The percentage of F4/80-positive cells in WAT was significantly elevated by olanzapine (with a difference of 8%,  $P < 0.05$ ; 13%,  $P < 0.001$ ; and 20%,  $P < 0.01$  between olanzapine and control for short-, mid- and long-term, respectively) (Figure 1C).

Interestingly, Pearson's correlation showed that the percentage of F4/80+ cells was positively correlated with the average adipocyte size in WAT ( $r = 0.942$ ,  $P < 0.001$ ; Figure 1D). This significant positive correlation persisted when the data were analysed separately by treatment cohorts ( $r = 0.915$ ,  $P < 0.001$  and  $r = 0.860$ ,  $P < 0.001$  for the control group and olanzapine group, respectively), suggesting a close relationship between WAT inflammation and adiposity. The percentage of F4/80+ cells in WAT was not significantly correlated with cumulative body weight gain ( $P = 0.079$ ) (data not shown).

### ***3.3 Olanzapine causes macrophage infiltration in BAT but not liver tissue***

The percentage of F4/80-positive cells in BAT was significantly increased by olanzapine treatment ( $F_{(1,30)} = 35.303$ ;  $P < 0.001$ ), with a difference of 6% ( $P < 0.05$ ), 8% ( $P < 0.05$ ) and 15% ( $P < 0.01$ ) in the short-, mid- and long-term, respectively (Figure 2A, 2B). There was a significant time-related effect ( $F_{(2,30)} = 3.685$ ;  $P < 0.05$ ), but no interaction effect. However, in the liver, there was no significant difference in terms of the percentage of F4/80-positive cells (Figure 3A, 3B).

### ***3.4 Olanzapine increases the mRNA expression of TNF $\alpha$ , IL-1 $\beta$ , and IL-6 in the hypothalamus, WAT, and BAT, but not in the liver***

In the hypothalamus, the mRNA expression of TNF $\alpha$  was significantly elevated by olanzapine treatment ( $F_{(1,30)} = 33.319$ ;  $P < 0.001$ ), with a difference of 47% ( $P < 0.05$ ), 69% ( $P < 0.05$ ) and 91% ( $P < 0.01$ ) in the short-, mid- and long-term, respectively; Figure 3a). In addition, hypothalamic IL-1 $\beta$  mRNA expression was significantly elevated by olanzapine by

95% ( $P < 0.05$ ), 101% ( $P < 0.01$ ) and 133% ( $P < 0.01$ ) in the short, mid- and long-term, respectively ( $F_{(1,30)} = 46.853$ ;  $P < 0.001$ ; Figure 3a). Hypothalamic IL-6 mRNA expression was also elevated by olanzapine by 44% ( $P < 0.05$ ), 62% ( $P < 0.01$ ) and 89% ( $P < 0.001$ ) in the short, mid- and long-term, respectively ( $F_{(1,30)} = 48.665$ ;  $P < 0.001$ ; Figure 4A).

Similarly, in WAT, the mRNA expression of TNF $\alpha$ , IL-1 $\beta$ , and IL-6 were significantly elevated by olanzapine treatment throughout the three treatment stages ( $F_{(1,30)} = 25.225$ ,  $F_{(1,30)} = 24.419$  and  $F_{(1,30)} = 36.815$ , respectively; all  $P < 0.001$ ; Figure 4B). Similar results were found in BAT, where TNF $\alpha$ , IL-1 $\beta$ , and IL-6 mRNA expression were significantly upregulated by olanzapine treatment ( $F_{(1,30)} = 30.236$ ,  $F_{(1,30)} = 22.071$  and  $F_{(1,30)} = 25.391$ , respectively; all  $P < 0.001$ ; Figure 4C). However, there was no significant difference in the liver mRNA expression of TNF $\alpha$ , IL-1 $\beta$ , or IL-6 between olanzapine and the control (Figure 4D).

Pearson's correlation revealed that the mRNA levels of TNF $\alpha$  ( $r = 0.521$ ,  $P < 0.01$ ), IL-1 $\beta$  ( $r = 0.487$ ,  $P < 0.01$ ), and IL-6 ( $r = 0.415$ ,  $P < 0.05$ ) were positively correlated with the percentage of F4/80+ cells in WAT (data not shown).

### ***3.5 Olanzapine increases plasma triglycerides, but not total cholesterol, HDL, or LDL levels***

Plasma lipid analysis reveals that olanzapine treatment caused an elevation of plasma triglyceride levels throughout the three treatment stages (Table 2). Two-way ANOVA revealed that there was a significant olanzapine treatment effect ( $F_{(1,30)} = 30.364$ ;  $P < 0.001$ ), although there were no significant time-related or interaction effects. However, olanzapine did not change the plasma levels of total cholesterol, HDL, or LDL (Table 2). The plasma triglyceride level was positively correlated with the percentage of F4/80+ cells ( $r = 0.379$ ,  $P$

< 0.05), average adipocyte size in WAT ( $r = 0.475$ ,  $P < 0.01$ ), and cumulative body weight gain ( $r = 0.611$ ,  $P < 0.001$ ) (data not shown).

#### **4. Discussion**

In the present study, we showed that chronic and sub-chronic olanzapine treatment had a significant effect on body weight gain, adiposity, and inflammatory parameters in WAT and BAT but not in the liver. It is well-known that second generation antipsychotics, including olanzapine, can cause numerous metabolic side-effects including body weight gain or obesity in both humans and animal models (Deng, 2013; Weston-Green et al., 2013; Zhang et al., 2013). Obesity has also been reported to be associated with “low-grade” chronic inflammation (Cancello et al., 2006). Previous studies have reported on the effect of olanzapine on macrophage infiltration and the elevation of pro-inflammatory markers in rodents (Davey et al., 2012; Victoriano et al., 2010). However, the present study showed for the first time the time-related effects of olanzapine treatment on macrophage infiltration in WAT and BAT in an established female rat model of olanzapine-induced obesity. We also showed that the mRNA expression of pro-inflammatory markers TNF $\alpha$ , IL-1 $\beta$ , and IL-6 were upregulated by olanzapine treatment compared to the control in the hypothalamus, WAT, and BAT, but not in the liver. Previous studies have shown that olanzapine treatment is associated with increased CD68 cells in WAT in male rats (Victoriano et al., 2010), and increased plasma IL-8 and IL-1 $\beta$  levels, and elevated WAT CD68 and IL-6 mRNA expression in female rats (Davey et al., 2012). These findings are consistent with the olanzapine-induced elevation of peripheral inflammation and macrophage infiltration found in the present study.

The time-related effects of olanzapine on body weight gain and inflammation observed in the present study are interesting. Over the duration of the treatments, olanzapine led to a gradual reduction in the percentage cumulative weight gain compared to the control, while the levels

of peripheral inflammation (as indicated by macrophage infiltration in WAT and BAT) were gradually elevated. These findings indicate that the peripheral inflammatory response may be the result, rather than the cause, of the accumulating adiposity and body weight gain induced by olanzapine. To the best of our knowledge, this is the first study reporting on the time-related effects of olanzapine on inflammation. In the present study, adipocyte size (Fig. 1B) and adipose tissue weight (Table 1) increased throughout the duration of the olanzapine treatments with no time-related effects observed, while macrophage infiltration increased from the first week of treatment and continued to rise until the final observed time point (week 5). Future research into the molecular basis of these time-related effects is warranted.

The present study also showed for the first time that hypothalamic TNF $\alpha$ , IL-1 $\beta$ , and IL-6 mRNA expression were upregulated by olanzapine treatment, suggesting that olanzapine affects central inflammation. However, it is unclear in the present study whether the elevated mRNA expression of hypothalamic pro-inflammatory cytokines is the cause or result of body weight gain or adiposity induced by olanzapine. It has been suggested that low-grade hypothalamic inflammation could be a possible pathogenic condition for body weight gain, through the impairment of the central leptin and insulin signalling pathways (Thaler et al., 2010). Nevertheless, the cause-effect relationship is still being debated (Cancello et al., 2006; Velloso et al., 2008; Wisse et al., 2009).

Hypothalamic inflammation has also been implicated in undermining thermogenesis (Arruda et al., 2011). Intriguingly, in the same batch of animals, we have previously reported that olanzapine treatment can induce a reduction in BAT thermogenesis in the mid- to long-term (Zhang et al., 2014b). Future studies are required to elucidate the cause-effect relationship between weight gain and inflammation in the context of olanzapine treatment. In addition, given the positive results on macrophage infiltration in WAT, BAT, and the hypothalamus under olanzapine treatment, future studies are warranted that investigate the effect of

olanzapine on different types of macrophages (eg. M1, M2) and their relationship to body weight gain.

Interestingly, in the liver, there was no significant difference in terms of either macrophage inflammation or the mRNA expression of TNF $\alpha$ , IL-1 $\beta$ , or IL-6 between the olanzapine and the control cohorts. Although one study has reported that hepatosteatosis and inflammation were found in the liver of high-fat diet-fed (compared to low-fat diet-fed) rats (Sampey et al., 2011), another study found no significant difference in the F4/80-positive Kupffer cells within the liver between lean C57BL/6J mice and *Lep<sup>ob/ob</sup>* mice (Weisberg et al., 2003). This inconsistency may be due to the different causes of obesity in the animal models used in these studies. The results of the present study suggest that obesity which is induced by olanzapine treatment is not associated with liver inflammation, without the choices between high-fat and low-fat diets. Future studies are warranted to investigate the mechanism for this difference.

Plasma triglyceride levels were elevated, while total cholesterol, HDL, and LDL levels were unaffected throughout the three olanzapine treatment time courses. Consistently, a previous study reported that a mid-term study (2 weeks) of olanzapine treatment led to elevated serum triglyceride levels but not elevated total cholesterol, HDL, or LDL levels in female rats (Skrede et al., 2012). An acute study showed that olanzapine treatment resulted in increased serum triglyceride levels but reduced cholesterol and LDL levels in female rats, while HDL levels were not affected (Jassim et al., 2012). A long-term (6-week) study suggested that olanzapine treatment increased plasma cholesterol and HDL levels, but did not change triglyceride levels in male rats (Minet-Ringuet et al., 2006). Compared to the present study, these animal studies showed consistent results for the effect of olanzapine on blood triglyceride levels, except that an elevation effect is also evident in the long-term (5 weeks) in the present study; while the effects on cholesterol, HDL, and LDL levels may vary, which could be due to different gender of animals, different drug administration routes and/or dosages

used in different studies. Interestingly, clinical studies have shown that chronic antipsychotic treatment (including olanzapine) is associated with elevated triglyceride levels and hypercholesterolemia (increased total cholesterol and LDL levels, and decreased HDL cholesterol levels) (Albaugh et al., 2011). While elevated triglyceride levels were evident in the present study, hypercholesterolemia was absent.

While there were significant time-related effects in olanzapine-induced body weight gain and plasma triglyceride levels, the effect of olanzapine on adipose tissue weight, adipocyte size, macrophage infiltration, and the mRNA expression of TNF $\alpha$ , IL-1 $\beta$ , and IL-6 were time-independent (although there was a differential time-related effect of olanzapine on macrophage infiltration in WAT). These differences in time-related effects, along with the fact that WAT macrophage infiltration is significantly correlated with adipocyte size but not cumulative body weight gain, suggest that the effects of olanzapine on weight gain and plasma lipid profiles may be through a different pathway compared to its effects on adiposity and inflammation. In fact, previous studies have evidenced the effect of olanzapine on elevating adiposity without causing weight gain in male rats (Cooper et al., 2007; Victoriano et al., 2010).

In WAT, olanzapine causes a significant upregulation of TNF $\alpha$ , IL-1 $\beta$ , and IL-6 mRNA expression, along with macrophage infiltration and increased average adipocyte size. The macrophage infiltration rate was highly correlated with the average adipocyte size, suggesting a link between inflammation and adiposity in the context of olanzapine treatment. In fact, a positive correlation between the percentage of F4/80+ cells and adipocyte size has been indicated in a previous report investigating macrophage accumulation and adiposity in rodents due to either diet (DIO mice) or obesity-related mutations ( $A^y/+$  and  $Lep^{ob/ob}$  mice) (Weisberg et al., 2003). This strong correlation between adipocyte size and macrophage infiltration in visceral adipose tissue indicates that the adipose tissue macrophage paracrine

pathway may play a role in the regulation of adipocyte function under olanzapine treatment, a hypothesis warrants future studies. In addition, there is a significant correlation between macrophage infiltration and adipocyte size in WAT, but not between macrophage infiltration and cumulative body weight gain. This suggests that inflammation in adipose tissue may be more closely related to adiposity than body weight gain under olanzapine treatment.

Finally, chronic inflammation, as shown in this study, results in the activation of the pituitary-adrenal axis and the elevation of glucocorticoids. Corticosterone induces changes in glucose, lipid, and protein metabolism and may play a role in olanzapine-induced metabolic disorders. In fact, olanzapine treatment has been reported to increase the plasma/serum levels of corticosterone (Assié et al., 2008; Marx et al., 2006). Therefore, future studies investigating the role of corticosterone signalling in olanzapine-induced metabolic side-effects are warranted.

## **5. Conclusions**

In conclusion, the findings of this study suggest that olanzapine treatment could cause increased adipocyte size in WAT, and a time-independent elevation of the mRNA expression of pro-inflammatory markers  $\text{TNF}\alpha$ ,  $\text{IL-1}\beta$ , and  $\text{IL-6}$  in the hypothalamus, WAT, and BAT. This may contribute to the time-dependent macrophage infiltration in WAT, increasing the levels of macrophage infiltration over time. Moreover, in view of the correlation between periovary WAT adipocyte size and macrophage infiltration, and the lack of correlation between macrophage infiltration and cumulative weight gain, a closer link between inflammation and adiposity compared to the link between inflammation and body weight gain was suggested under olanzapine treatment. These findings confirm the low-grade inflammatory response under olanzapine treatment, and extend our knowledge of inflammation and related abnormal adiposity responses through the three stages of obesity

development under olanzapine treatment. Given the relationship between low-grade inflammation and reduced metabolic rate and insulin resistance, future research into specific inflammatory pathways is warranted, and therapeutic interventions through anti-inflammatory agents could be a new target for olanzapine-induced metabolic disorders. In fact, COX-2 inhibitors have been reported to have favourable effects over placebo in schizophrenia and major depression in animals (Müller et al., 2008). It may be worthwhile to investigate the effects of the co-treatment of COX-2 inhibitors with olanzapine in reducing the metabolic side-effects of this otherwise-effective atypical antipsychotic.

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**Table 1.** Percentage difference from control in adipose tissue weight and body weight gain

	ST	MT	LT
Perirenal WAT	39 ± 8*	36 ± 11*	25 ± 6
Periovary WAT	36 ± 9*	53 ± 12**	35 ± 6*
Omental WAT	24 ± 16	10 ± 12	5 ± 12
Total Visceral WAT	36 ± 4**	42 ± 14*	29 ± 5*
Subcutaneous WAT (Inguinal WAT)	24 ± 3**	44 ± 14*	16 ± 6
Total WAT	33 ± 4**	42 ± 12**	26 ± 6*
BAT	-22 ± 4	19 ± 12	16 ± 7
Total Fat	30 ± 4**	42 ± 11*	26 ± 5*
Total Body Weight Gain	268 ± 37***	76 ± 16***	32 ± 8*

The percentage change in adipose tissue weight and total body weight gain (mean ± SEM) in rats treated with olanzapine for ST, MT, and LT (n=6/group).

ST: short-term, 1 week; MT: mid-term, 2 weeks; LT: long-term, 5 weeks; WAT: white adipose tissue; BAT: brown adipose tissue. Total visceral WAT includes perirenal WAT, periovary WAT, and omental WAT. Total WAT includes visceral WAT and subcutaneous WAT. Total fat includes WAT and BAT. \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001 vs. control. The levels were given relative to vehicle-treated rats that were defined as 100%.

**Table 2.** Percentage difference from control in plasma lipid profile in female rats

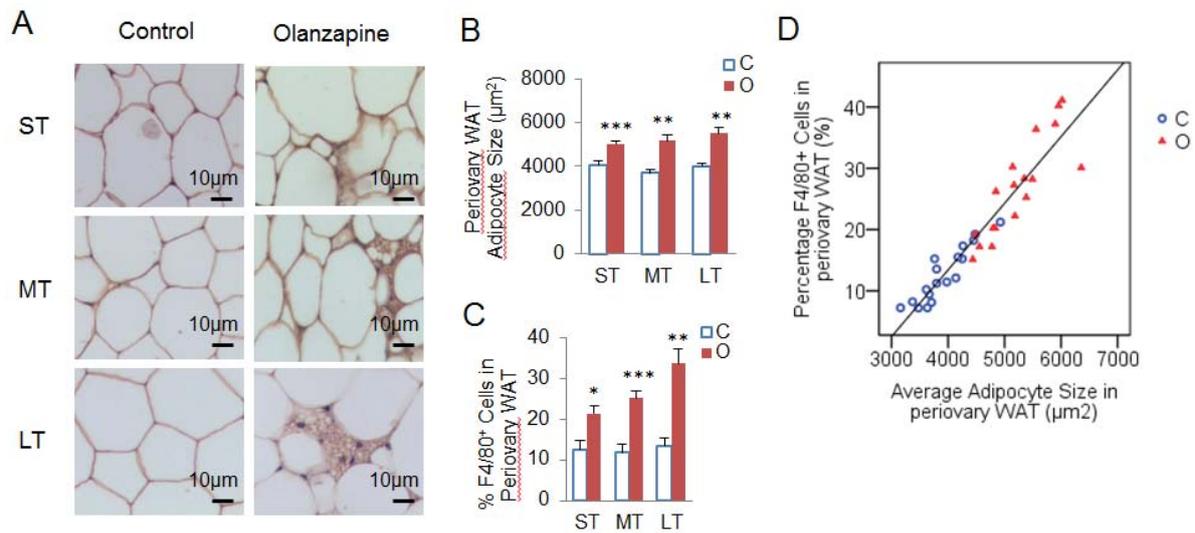
	ST	MT	LT
TC	3 ± 5	19 ± 10	1 ± 3
TG	91 ± 15***	55 ± 15*	27 ± 7*
HDL	8 ± 11	24 ± 11	1 ± 10
LDL	-22 ± 13	2 ± 22	-17 ± 15

The plasma levels (mean ± SEM) of total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were measured in rats treated with olanzapine for ST, MT, and LT (n=6/group).

ST: short-term, 1 week; MT: mid-term, 2 weeks; LT: long-term, 5 weeks; TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein. \**P* < 0.05; \*\*\**P* < 0.001 vs. control. The levels were given relative to vehicle-treated rats that were defined as 100%.

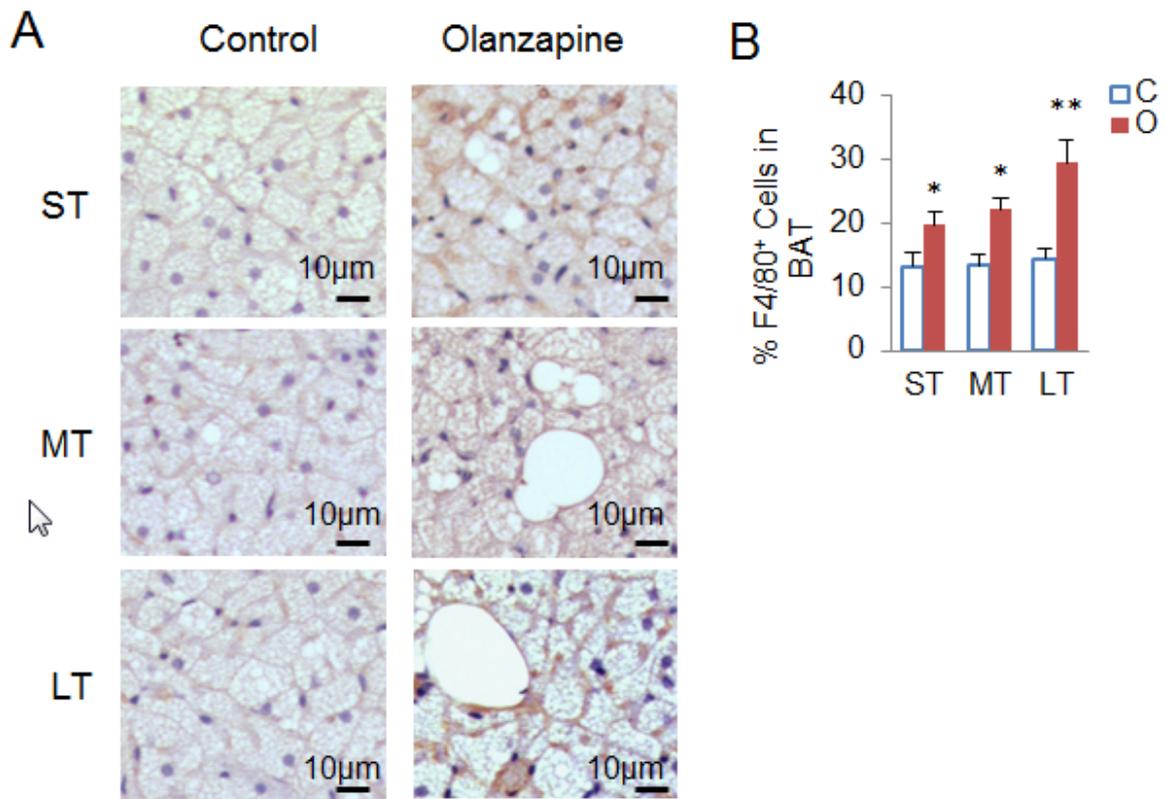
## Figure Legends

Figure 1



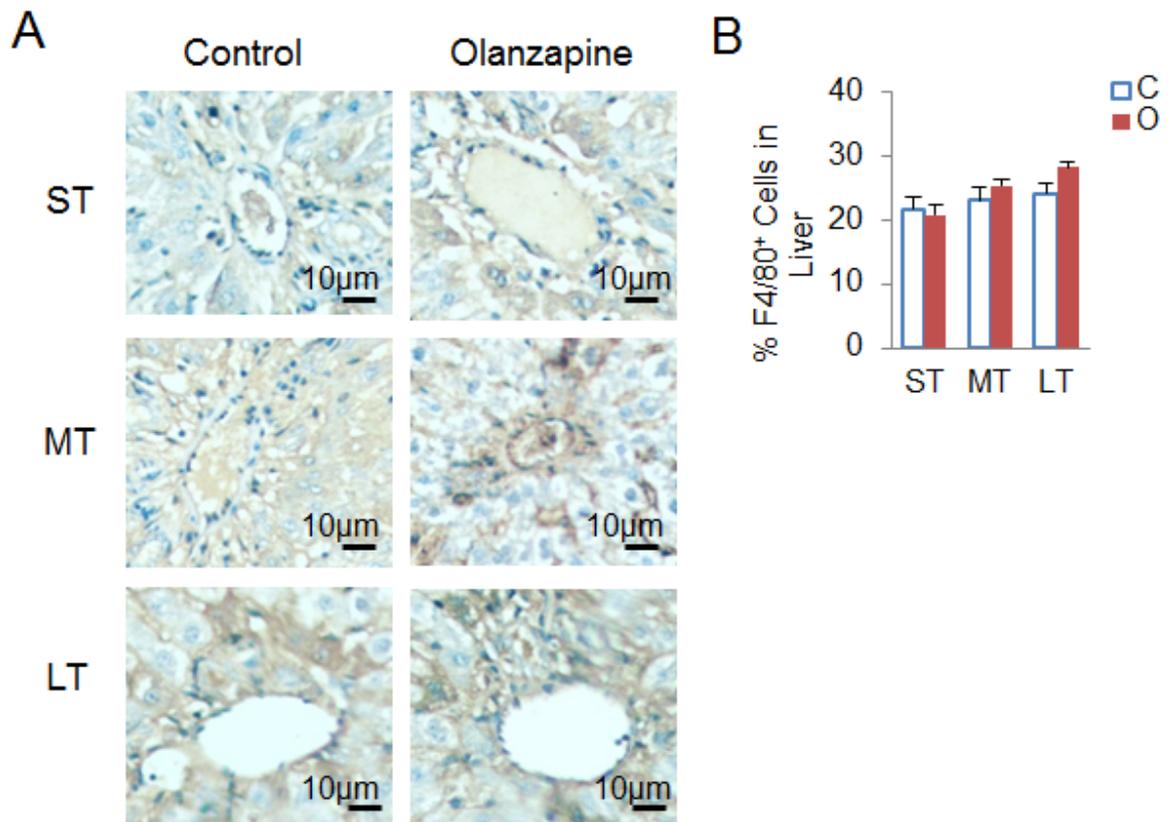
**Figure 1.** Macrophage infiltration and adipocyte enlargement in periovary WAT of female Sprague-Dawley rats treated with olanzapine. (A) Immunohistochemical detection of F4/80-positive cells counter-stained with hematoxylin. (B) Average adipocyte size of periovary WAT. (C) Percentage of F4/80-positive cells in periovary WAT. (D) Correlation between average adipocyte size and percentage F4/80-positive cells in periovary WAT. ST: short-term; MT: mid-term; LT: long-term; C: control; O: olanzapine; WAT: white adipose tissue. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs control.  $n=6$ /group.

Figure 2



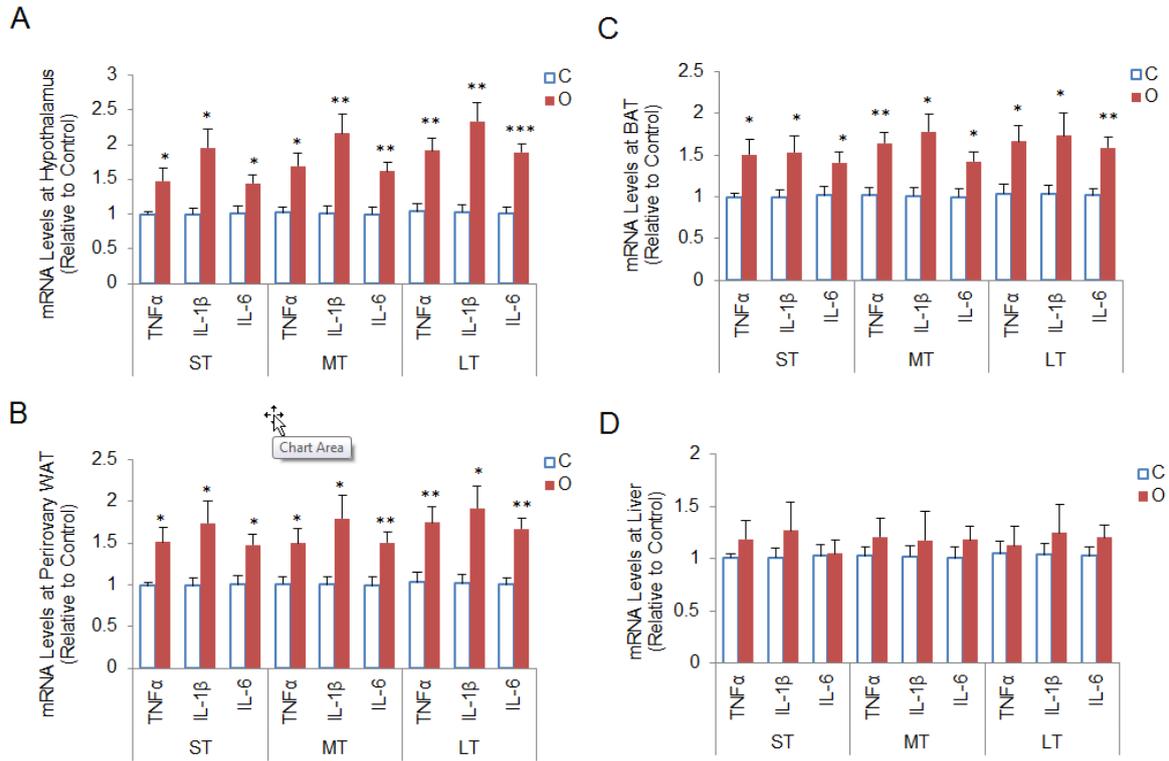
**Figure 2.** Macrophage infiltration in BAT of female Sprague-Dawley rats treated with olanzapine. (A) Immunohistochemical detection of F4/80-positive cells counter-stained with hematoxylin. (B) Percentage of F4/80-positive cells in BAT. ST: short-term; MT: mid-term; LT: long-term; C: control; O: olanzapine; BAT: brown adipose tissue. \* $P < 0.05$ , \*\* $P < 0.01$  vs control.  $n=6$ /group.

Figure 3



**Figure 3.** Immunohistochemistry staining of F4/80 in the liver of female Sprague-Dawley rats treated with olanzapine. (A) Immunohistochemical detection of F4/80-positive cells counter-stained with hematoxylin. (B) Percentage of F4/80-positive cells in the liver. ST: short-term; MT: mid-term; LT: long-term; C: control; O: olanzapine. n=6/group.

Figure 4



**Figure 4.** mRNA expression of TNFα, IL-1β, and IL-6 in (A) hypothalamus, (B) periovary WAT, (C) BAT, and (D) liver. ST: short-term; MT: mid-term; LT: long-term; C: control; O: olanzapine; WAT: white adipose tissue; BAT: brown adipose tissue; TNFα: tumour necrosis factor α; IL-1β: interleukin-1β; IL-6: interleukin-6. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs control. n=6/group.