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# Olanzapine reduced brown adipose tissue thermogenesis and locomotor activity in female rats

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ABSTRACT

Excessive weight gain has been identified as a serious metabolic side-effect of second-generation antipsychotics (SGAs), including olanzapine. While hyperphagia has been suggested to be the main contributor for this side-effect in the short term, reduced energy expenditure, in particular thermogenesis and locomotor activity, has been considered to contribute to the maintenance of heavy weight under long-term SGA treatments. Recent studies have identified metabolically active brown adipose tissues (BAT) in adult humans, suggesting potential clinical significance for the involvement of BAT thermogenesis in SGA-induced weight gain. However, to date there has been little research elucidating the central neuronal pathways affecting BAT thermogenesis or the morphological changes of the BAT. The present study aimed to investigate the role of BAT thermogenesis and locomotor activity in olanzapine-induced weight gain during the prolonged time courses of olanzapine treatment in an established female rat model. Although short- to mid-term olanzapine treatment had no effect on BAT temperature, we observed that long-term olanzapine treatment (from day 18 to 34) induced a significant reduction in BAT temperature, with an acute effect being observed between 45 and 150 min post-treatment in the long-term cohort. Additionally, in the long-term olanzapine group, the reduced BAT temperature was accompanied by decreased UCP1 and PGC-1α expressions in the BAT. Moreover, TH mRNA expressions in both hypothalamus and brainstem were also downregulated after mid- to long-term olanzapine treatment. Further, olanzapine led to reduced percentage of brown adipocytes in BAT during mid- to long-term treatments. Finally, locomotor activity was reduced throughout the three treatment cohorts. In summary, our results suggest that the reduction of BAT thermogenesis plays an important role during the long-term of olanzapine-induced weight gain, which was accompanied by an earlier onset of BAT adipocyte morphological changes and biochemical changes in the hypothalamus and the brainstem, while locomotor activity contributes to the entire olanzapine treatment courses.


## 1. Introduction

While second-generation antipsychotics (SGAs) have been widely prescribed as the primary treatment for patients with schizophrenia and other psychiatric diseases, excessive body weight gain associated with the use of SGAs, in particular olanzapine and clozapine, has attracted increasing concerns from patients, clinicians, and medical researchers (Zhang et al., 2013). This weight gain side-effect of SGAs, along with other metabolic side-effects, could lead to increased morbidity and mortality, and poor compliance to the antipsychotic drug for patients (Deng, 2013).

Both clinical and animal studies have suggested that along the time course of olanzapine-induced weight gain, there are three typical stages: the initial stage with rapid increase of body weight accompanied with elevated food intake, the middle stage with slow body weight gain and no elevation of food intake, and the late stage with maintenance of the heavy body weight without elevated food intake (Huang et al., 2006; Pai et al., 2012). In recent years, researchers have identified multiple contributing factors for the weight gain side-effect induced by SGAs (Kroeze et al., 2003; Nasrallah, 2008; Reynolds and Kirk, 2010). For example, the roles of antagonism on the histaminergic H1 and H3 receptors (Deng et al., 2010), serotoninergic 5HT2c receptor (Panariello et al., 2011), dopaminergic D2 receptor (Lian et al., 2013) and α-adrenergic receptor (Nasrallah, 2008), and central ghrelin signaling (Zhang et al., in press) have been indicated. However, the exact mechanism of SGA-induced weight gain was not fully understood. One of the unsolved questions of these studies is with maintenance

*Abbreviations:* BAT, brown adipose tissue; IBAT, interscapular brown adipose tissue; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α; qRT-PCR, quantitative real-time PCR; SGAs, second-generation antipsychotics; TH, tyrosine hydroxylase; UCP1, uncoupling protein1.

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of the heavy body weight during the long-term SGA treatments when elevated food intake is absent. Reduced energy expenditure, in particular thermogenesis, has been suggested to play an important role in antipsychotic-induced weight gain (Arjona et al., 2004; Stefanidis et al., 2009; van der Zwaal et al., 2012).

Metabolically active brown adipose tissue (BAT) has been identified in adult humans (Nedergaard et al., 2007, 2010), with its functional thermogenic protein, uncoupling protein1 (UCP1) found in humans (Cinti, 2006), suggesting that the involvement of BAT thermogenesis in SGA-induced weight gain observed in rodents may have clinical relevance in humans. In addition, olanzapine treatment has been reported to reduce locomotor activity in rodents (Deng et al., 2012; van der Zwaal et al., 2010), although the time-dependent effect has not been investigated. The aim of this study is to investigate the effect of olanzapine on BAT thermogenesis and locomotor activity during the prolonged time courses of olanzapine treatment, in an established female rat model of olanzapine-induced weight gain. Besides the physiological changes in BAT thermogenesis, biochemistry changes in the central sympathetic network for BAT thermogenic innervations, as well as morphological changes of the interscapular brown adipose tissue (IBAT), were also examined through the time courses of olanzapine-induced weight gain.

## 2. Materials and methods

### 2.1. Animals and oral drug treatments

The animal model of olanzapine-induced weight gain has been well established and validated in female rats in our and other laboratories (Deng et al., 2012; Lian et al., 2013; Stefanidis et al., 2009; Weston-Green et al., 2011). Rats were closely housed in a room occupied by only female rats, which ensures that the ovarian cycles of all female rats are synchronized (Lian et al., 2013). Briefly, female Sprague–Dawley rats (201–225 g) were obtained from the Animal Resource Centre (Perth, WA, Australia). Rats were individually housed at 22 °C, 12-h light–dark cycle with 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). After 1 week of acclimatization, a Bio-Thermo microchip "LifeChip" (Destron Fearing, South St. Paul, MN, USA) was inserted into the interscapular brown adipose tissue with independent packaged disposable sterilized needles. The positions of the microchips were visually confirmed post-euthanasia (Fig. S1). After 1 week of recovery, animals were trained to self-administer the placebo sweet cookie-dough. Rats were randomized 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; $n = 12$/group). However, for the subsequent biochemistry (Western blot and qRT-PCR) and histology measurements, only 6 rats were randomly selected from each group ($n = 6$/group). All 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 (62% carbohydrate, 22% protein, 6% fiber, 10% vitamins and minerals) method was employed as previously reported (Deng et al., 2012; Han et al., 2008; Weston-Green et al., 2011). Briefly, a mixture of cornstarch (30.9%), sucrose (30.9%), gelatine (6.3%), casein (15.5%), fiber (6.4%), minerals (8.4%) and vitamins (1.6%) was produced. Three times per day, a cookie-dough (0.3 g; 3.36 kcal/g) mixed with either olanzapine (1 mg/kg BW) (Eli Lilly, Indianapolis, IN, USA) or placebo was served to the corresponding animals. Animals were observed during the administration period to ensure complete consumption of the pellets. The dosage of olanzapine (1 mg/kg BW, three times per day) was based on our prior studies (Deng et al., 2012; Han et al., 2008; Weston-Green et al., 2011), which was clinically relevant calculated based on D2 receptor occupancy (Kapur et al., 2003). Body weight was measured every second day.

### 2.2. BAT temperature measurements

The instant BAT temperature data were detected by the microchip and received by a remote pocket reader "Pocket Reader EX" (Digital Angel Corp., South St. Paul, MN, USA). BAT temperature measurements were conducted 2 h and 6 h after the 7 am and 11 pm drug treatments (Fig. S2) every second day. To determine whether there is a circadian effect over the effect of olanzapine on BAT temperature, BAT temperature up to 3 h immediately post-treatment was measured after 7 am (light phase) and 11 pm (dark phase), 2 days before euthanasia.

### 2.3. Open field test

To determine the effect of olanzapine on locomotor activity during the three different treatment courses, open field tests were performed 3 days before euthanization for each cohort (day 5, day 13 and day 31 for the short-, mid- and long-term cohorts, respectively). The open field test protocol was described previously (Deng et al., 2012; Weston-Green et al., 2011). Briefly, each rat was placed in the center of a black rectangular arena ($60 \times 60$ cm$^2$, 40 cm high) with an average light exposure of 25 lx. The behavior of the rats was recorded by a video camera from the top for 30 min. Locomotor activity was analyzed using EthoVision Color-Pro software (Noldus Information Technology, Wageningen, The Netherlands). Total distance moved (cm) and average velocity (cm/s) were measured.

### 2.4. Euthanasia and tissue collection

Two hours after the last 7 am treatment (on day 8, day 16 and day 36, for short-, mid-, and long-term cohorts, respectively), rats were euthanized by fast $CO_2$ 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 in −80 °C. IBAT was dissected and cut into two halves: one half was snap-frozen and stored in −80 °C, and the other half 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.

### 2.5. Western blot

The Western blot protocol was adopted from our previous report (Zhang et al., in press). Briefly, the BAT were homogenized in 10 vol (v/w) homogenizing buffer (containing NP40, Protease Inhibitor Cocktail, 1 mM PMSF and 0.5 mM β-glycerophosphate). Total protein concentrations were determined by DC-Assay (Bio-Rad, Hercules, USA), detected by SpectraMax Plus384 absorbance microplate reader (Molecular Devices, USA). Samples were heat-treated in Laemmli buffer at 95 °C, loaded to 8% SDS–PAGE gels for fractionation, and then transferred onto Immun-BlotTM PVDF membranes (Bio-Rad, Hercules, CA, USA). The block consists of 5% BSA in TBST. The membranes were then incubated with UCP1 or peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) (Santa Cruz Biotechnologies; dilution factor 1:500) antibody in TBST containing 1% BSA overnight at 4 °C. Secondary antibodies were anti-rabbit (for PGC-1α) or anti-goat (for UCP1) IgG conjugated with horseradish peroxidase (Santa Cruz Biotechnologies, USA; dilution factor 1:3000). For visualization, ECL detection reagents were used and films were exposed on the AGFA CP1000 Tabletop Processor (COD Medical, USA). Films were then analyzed using the Quantity One software, connected to GS-690 Imaging Densitometer (Bio-Rad, Hercules, USA).

### 2.6. Quantitative real-time PCR (qRT-PCR)

The qRT-PCR protocol was adopted from our previous report (Zhang et al., in press). Briefly, total RNA was extracted from the hypothalamus