Psychoneuroendocrinology 110 (2019) 104413

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Female mice are protected against acute olanzapine-induced hyperglycemia

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ARTICLE INFO

Keywords:
Sex differences
Olanzapine
Blood glucose
Glucagon

ABSTRACT

Olanzapine is a second-generation antipsychotic (SGA) used frequently in the treatment of schizophrenia and a growing list of off-label conditions. Though effective in reducing psychoses, acute olanzapine treatment causes rapid increases in blood glucose that are believed to be mediated by increases in liver glucose output, skeletal muscle insulin resistance, and beta cell dysfunction. Further, the acute lipidemic response to olanzapine has been largely unexplored. While females have been reported to be more susceptible to olanzapine-induced weight gain, there is little known about the impact of sex on the acute response to SGAs. The purpose of this study was to determine if the acute effects of SGAs on glucose and lipid metabolism display a sexually dimorphic response in C57BL/6 J mice and examine potential mechanisms mediating this effect. Age matched male and female C57BL/6 J mice were treated with olanzapine (5 mg/ kg, IP) or vehicle control and blood glucose was measured at baseline, 15, 30, 60, 90, and 120 min post-treatment and tissues and serum harvested. These experiments were repeated, and mice underwent an insulin (0.5 IU/kg) or pyruvate tolerance test (2 g/kg) following 60 min of olanzapine treatment. Females were protected against olanzapine-induced increases in blood glucose and pyruvate intolerance compared to male mice, and this occurred despite the development of severe insulin resistance. In male mice olanzapine increased the glucagon:insulin ratio whereas in females this ratio was reduced. When challenged with exogenous glucagon (1 mg/kg IP), females were less responsive than males. Male and female mice displayed similar increases in whole body fatty acid oxidation, serum fatty acids and liver triglyceride accumulation. Our findings provide evidence that while there are no apparent sex differences in the lipid metabolism response to olanzapine, that females are protected from acute olanzapine-induced excursions in blood glucose. This is likely due in part to reductions in the glucagon:insulin ratio and glucagon responsiveness which could impact olanzapine induced increases in liver glucose production.

1. Introduction

Second generation antipsychotic (SGA) drugs such as olanzapine are widely used in the treatment of schizophrenia and other severe mental illnesses and are increasingly prescribed for the management of a number of off-label conditions such as attention deficit hyperactivity disorder, behavioral disruptive disorders, and anxiety (Devlin and Panagiotopoulos, 2015). Unfortunately, prolonged SGA use is associated with dysregulated lipid metabolism (Rojo et al., 2015), weight gain (Allison and Casey, 2001), impaired glucose homeostasis and hepatosteatosis (Coccurello et al., 2009), and increases the risk of developing type 2 diabetes (Rojo et al., 2015). Unlike first generation, conventional antipsychotics which chiefly target dopamine receptors, SGAs antagonize a combination of receptors including dopaminergic, serotonergic, adrenergic, and cholinergic receptors (Hahn et al., 2011; Starrenburg and Bogers, 2009).

While the effects of SGAs on glucose metabolism were initially thought to be due to weight gain, there is convincing pre-clinical and clinical data which demonstrate rapid and direct effects of acutely administered SGAs on glucose regulation. For instance, in rodents, olanzapine induces whole-body insulin resistance during a hyperinsulinemic-euglycemic clamp (Houseknecht et al., 2007; Boyda et al., 2012), an effect that occurs within minutes to hours of administration. In humans, acute olanzapine administration can impair indices of glucose metabolism, in otherwise healthy subjects (Hahn et al., 2013; Albaugh et al., 2011). Currently, the exact mechanisms which mediate

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https://doi.org/10.1016/j.psyneuen.2019.104413
Received 25 June 2019; Received in revised form 7 August 2019; Accepted 18 August 2019

olanzapine-induced hyperglycemia are unclear, but is thought to include increases in hepatic glucose production (Chintoh et al., 2009; Klingerman et al., 2013), decreases in pancreatic insulin release (Boyda et al., 2010; Hahn et al., 2011), and peripheral insulin resistance (Chintoh et al., 2008; Boyda et al., 2010).

There is evidence of a sexually dimorphic response with chronic antipsychotic treatment. Specifically, olanzapine-induced weight gain occurs in both sexes in humans but may be exacerbated in females after four weeks of treatment (Jain et al., 2006), with female sex considered to be a risk-factor for SGA-induced weight gain (Gebhardt et al., 2009). On the other hand, in healthy individuals, females typically display enhanced glucose homeostasis compared to males (Varlamov et al., 2015; Macotela et al., 2009). Given these discrepant findings, it is not clear if the acute administration of olanzapine would impact glucose homeostasis to a similar extent in males and females, and if so, what could explain a potential sexual dimorphic response. To the best of our knowledge, a direct comparison of the acute effects of olanzapine on indices of glucose and lipid homeostasis has never been reported. Consequently, the current study aimed to identify the acute effects of olanzapine on markers of carbohydrate and fat metabolism in male and female C57BL/6 J mice. To that end, we challenged male and female mice with a single weight-adjusted bolus of olanzapine and assessed changes in indices of whole-body glucose and lipid metabolism. We also aimed to assess potential underlying mechanisms that contribute to these differences in glucoregulatory control.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the University of Guelph Animal Care Committee and followed Canadian Council on Animal Care guidelines. Approximately 8-week old male and female C57BL/6 J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and housed 1 per cage with a 12:12 h light dark cycle and were given free access to water and standard rodent chow (7004-Teklad S-2335 Mouse Breeder Sterilizable Diet; Teklad Diets Harlan Laboratories, Madison WI). Mice were given 1 week to acclimate to our facilities before experimentation. All olanzapine experiments occurred at the beginning of the animals' light cycle (~0900).

2.2. Materials

Olanzapine (CAT# 11937) was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Dimethylsulfoxide (DMSO) was from Wako Pure Chemical Industries (cat. no. 67-68-5; Richmond, VA, USA). Kolliphor EL was from Millipore Sigma (CAT# C5135; Etobicoke, ON, CA). Blood glucose test strips and a Freestyle Lite handheld glucometer were acquired from Abbott Diabetes Care Inc. (Alameda, CA, USA). Injections were carried out using 25 gauge needles purchased from ThermoFisher Scientific (Mississauga, ON, CAN; cat. no. BD B305122) and 29 gauge insulin needles purchased from VWR (Radnor, PA, USA; cat. no. 10799-004). Primary antibodies against phospho-AMPKa (T172) (cat. no. 2535), AMPKa (cat. no. 2532), Hormone Sensitive Lipase (HSL; cat no. 4107), and phospho-HSL Serine563 (cat. no. 4139) were purchased from Cell Signaling (Danvers, MA, USA). Phosphoenolpyruvate carboxykinase (PEPCK; cat. no. 10004943) and glucose 6 phosphatase (G6Pase; cat. no. B1512) primary antibodies were purchased from Cayman Chemical Company (Ann Arbor, MI, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Antibodies against peroxisome proliferator-activated receptor- γ coactivator 1-α (PGC-1α; cat no. AB3242) and Vinculin (CAT# 05386) were purchased from Millipore (Billerica, MA, USA). Antibodies against GAPDH (CAT# Ab8245) and Insulin Degrading Enzyme (IDE; cat. no. 32216) were purchased from Abcam (Toronto, ON, CA). Ponceau S stain was used as a loading control for Western Blot (CAT# P7170, Millipore Sigma). Secondary antibodies (donkey anti-rabbit and goat anti-mouse IgG) were purchased from Jackson ImmunoResearch (West Grove, PA, USA). Reagents for SDS-PAGE, including molecular weight marker, nitrocellulose membranes, and enhanced chemiluminescence, were purchased from Bio-Rad (Mississauga, ON, CA). All additional substances, including those used to homogenize samples, were purchased from Sigma-Aldrich (St. Louis, MO, USA). ELISAs obtained from Mercodia Inc. (Winston-Salem, NC 27103, USA) were used to measure serum glucagon (cat no. 10-1281-01) and insulin (cat no. 10-1247-01) while the ELISA for Prolactin was purchased from ThermoFisher Scientific (Mississauga, ON, CAN; cat no. EMPRL).

2.3. Terminal olanzapine tolerance test

Olanzapine was dissolved in DMSO (1 mg/100 µl) to create a stock solution. Kolliphor EL solution and saline (500 µl/900 ml) was used to dilute 500 µl of the stock olanzapine solution and mice were injected intraperitoneally (IP) with olanzapine (5 mg/kg) or vehicle (DMSO, Kolliphor EL, saline) at a weight-matched volume of 10 ul/gram body weight at the beginning of the light cycle. A fresh stock solution was made up for all experiments. We (Castellani et al., 2017; Townsend et al., 2018; Bush et al., 2018) and others (Klingerman et al., 2013) have previously used this dose as it mimics human dosing requirements based on dopamine-binding occupancy in rats given olanzapine by subcutaneous injections (Kapur et al., 2003). Blood glucose was measured in mice prior to, 15, 30, 60, 90, and 120 min post-olanzapine administration using a handheld glucometer sampled from a drop of blood taken from the tail vein using a distal tail snip. At this time, mice were anesthetized with sodium pentobarbital (5 mg/100 g body weight) then liver and gonadal white adipose tissue (gWAT), an abdominal fat depot, was snap frozen in liquid nitrogen. Cardiac blood was collected with 25 gauge needles, allowed to clot for ~20 min at room temperature, and centrifuged at 5000 g for 10 min at 4 °C. Serum was collected and stored at −80 °C until further analysis.

2.4. Insulin tolerance test

To assess olanzapine-induced insulin resistance, as done by our group previously (Castellani et al., 2017), non-fasted age matched mice were injected IP with olanzapine (5 mg/kg) or vehicle solution. 1 -h post-olanzapine a weight adjusted bolus of insulin (0.5 IU/kg) was injected IP with 29 gauge insulin needle and glucose measured from a tail vein at baseline and 10, 20, and 30 min after injection.

2.5. Pyruvate tolerance test

Non-fasted age matched mice were injected with olanzapine (5 mg/kg) or vehicle solution. At 1 -h post-olanzapine a weight adjusted bolus of pyruvate (2 g/kg) was injected IP with 25 gauge needle and glucose measured from a tail vein at baseline 15, 30, 45, 60, 90, and 120 min post pyruvate-injection (Castellani et al., 2017; Townsend et al., 2018).

2.6. Glucagon tolerance test

To assess the responsiveness of male and female mice to a weight adjusted bolus of glucagon mice were injected IP with glucagon (1 mg/kg; 25 gauge needle) in the morning at ~0900, as per olanzapine experiments. Blood glucose was measured from the tail vein at baseline and 30 min post-injection as blood glucose reaches a peak at this time point after glucagon administration at this dose (Townsend et al., 2019a).

2.7. ELISAs

Serum concentrations of insulin, glucagon, and prolactin were measured using commercially available enzyme-linked immunosorbent

assays (ELISAs). These were done according to the manufacturer's instructions and samples from each experiment were run on the same 96-well plate, in duplicate with an average CV of <10%. Plates were read using Biotek Synergy Mx Multi Format Microplate Reader. The ELISAs for insulin and glucagon were both read at an optical density of 450 nm. The plate for prolactin was read at both 450 nm and 550 nm, and the results were subtracted to correct for optical imperfections.

2.8. Serum olanzapine

Serum levels of SGAs were measured using liquid chromatography and tandem mass spectrometry (Graff-Guerrero et al., 2015) in pooled serum samples (i.e. serum from 3 mice pooled to obtain 300 µl, n = 4/group).

2.9. CLAMS (Comprehensive lab animal monitoring system)

Mice were placed in CLAMS caging at the beginning of their light phase for an ~ 24 -h acclimatization period. At the beginning of the following light cycle (~0900), mice were injected with olanzapine (5 mg/kg), immediately placed back into the CLAMS, and measurements were taken over the following 2 h. VCO2, VO2, respiratory exchange ratio (RER; VCO2/VO2), and physical activity (total beam breaks) were measured. Total energy expenditure (TEE), carbogydrate and fat oxidation were calculated (Peronnet and Massicotte, 1991).

2.10. Liver glycogen

Liver samples were freeze dried and chipped into 0.1–5.0 mg pieces. The addition of 0.1 M NaOH degraded glucose and hexose monophosphates (incubated for 10 min at 80 degrees Celsius). 0.1 M HCl, 0.2 M C6H8O7·H2O, and 0.2 M Na2HPO4·7H2O buffer then neutralized samples. Amyloglucosidase (cat no. A7095; Sigma Aldrich, Oakville, ON, Canada) was added to hydrolyze glycogen and glucose content was directly measured. Subsequently, weight-matched volumes of HK (cat no. H-4502; Sigma Aldrich, Oakville, ON, Canada) and G6PDH (G-5885; Sigma Aldrich, Oakville, ON, Canada) were added to all samples and the absorbance was repeatedly measured at 37 degrees Celsius in duplicate.

2.11. Liver TAGs

To quantify the hepatic TAG content, snap frozen liver was chipped into ~30 mg pieces, was homogenized in 1 ml of 1:2 methanol:-chloroform, and agitated overnight at 4 °C (Rector et al., 2008; Townsend et al., 2019b). One mL of 4 mM MgCl was added the following day, vortexed, and centrifuged for 1 h at 1000 g at 4 °C. The organic infranatant was extracted, evaporated overnight, and reconstituted in a 3:2 butanol-Triton X-114 mix. TAG content was measured with a commercially available kit (Sigma-Aldrich, CAT#F6428) in duplicate.

2.12. Immunoblotting

Liver and gonadal adipose were homogenized, protein extracted, quantified and immunoblotting was completed as we have described in detail previously (Snook et al., 2016; Peppler et al., 2019). In short, membranes were incubated overnight at 4 °C with gentle rocking in antibodies diluted (1:1000) with TBST and 5% BSA. The following morning membranes were briefly washed in TBST and incubated for 1 h at room temperature with horseradish peroxidase conjugated secondary antibodies (1:2000). Proteins of interest were expressed relative to Ponceau S, GAPDH or vinculin, all of which were from the same respective gel as the protein of interest.

2.13. Real time PCR

Liver (10–30 mg) was homogenized in 1 mL of QIAzol (Cat # 15596018; ThermoFisher

Scientific, Mississauga, ON) in a bead mill followed by RNA extraction using a RNeasy mini kit as per the manufacturer's instructions (Cat # 74104; Qiagen, Toronto, ON), including DNase free treatment with a commercially available kit (Cat # AM1906; ThermoFisher Scientific, Mississaugua ON) as per Peppler et al. (2019). Synthesis of cDNA was completed using SuperScript II (Cat # 18064014; ThermoFisher Scientific, Mississauga, ON). PCR was run with Sso Advanced Universal SYBR Green Supermix (BioRadCAT#1725271) using PCR primers listed in Table 1 on a Bio-Rad CFX connect system. All markers are expressed relative to Ppib, which did not change between groups. Relative differences in mRNA expression were determined using the $2^{-\Delta\Delta CT}$ method and normalized to the respective control group.

2.14. Statistical analyses

Statistical tests were completed using GraphPad Prism v.8.0 (GraphPad Software, La Jolla, CA, USA). Differences between two groups were determined using an unpaired, 2-tailed *t*-test (body weight, serum delta values). The effects of olanzapine in male and female mice were analyzed by two-way ANOVA (AUC, glycogen, absolute serum measures) followed by Tukey's post-hoc analysis if there was a significant interaction between sex and drug. Data sets were analyzed for outliers with Grubbs' test using Graphpad Outlier Calculator and values were excluded if identified as outliers. Glucose curves were displayed for context but were not statistically analyzed as they are represented in statistics for AUC values. Normality was assessed using the Shapiro-Wilk test unless a sample size was large enough to use the D'Agostino & Pearson test, as per the recommendation of Graphpad Statistics Guide. If normality tests failed, data were logarithmically transformed ($\log_{10}$) to ensure equal variance and normal distribution. A relationship was considered significant when $p < 0.05$.

3. Results

3.1. Females are protected from acute olanzapine-induced hyperglycemia

We first wanted to determine if there was a sexually dimorphic response to acute olanzapine treatment. Male and female mice were dosed with a weight-matched bolus of olanzapine (5 mg/kg) or an equivalent volume of vehicle control and changes in blood glucose were tracked for 120 min. Expectedly, male mice (27.2 ± 0.32 g) were significantly heavier ($p < 0.0001$) than female mice (20.6 ± 0.61 g). Olanzapine significantly increased blood glucose area under the curve (AUC) in males ($p < 0.0001$) but not females (Fig. 1C). There were no differences in glucose AUC between vehicle treated males and females. These data demonstrate a clear sexually dimorphic response to olanzapine-induced hyperglycemia. The differences in the glucose response to olanzanpine were not accounted by differences in circulating olanzapine concentrations at 2 h following treatment (females 98.67 ± 14.20 ng/mL, males 123.91 ± 14.45 ng/mL in olanzapine-treated animals; n = 4/group analyzed by 2-tailed *t*-test; $p = 0.2593$).

3.2. Males and females develop similar olanzapine-induced insulin resistance

We next wanted to determine if differences in olanzapine-induced hyperglycemia between males and females could be explained, at least in part, by olanzapine-induced whole-body insulin resistance. Mice were challenged with insulin 60 min following olanzapine treatment or vehicle control and blood glucose was measured for 30 min post-insulin. There were main effects of both drug and sex to increase glucose AUC. Male mice had greater glucose AUC with both olanzapine and

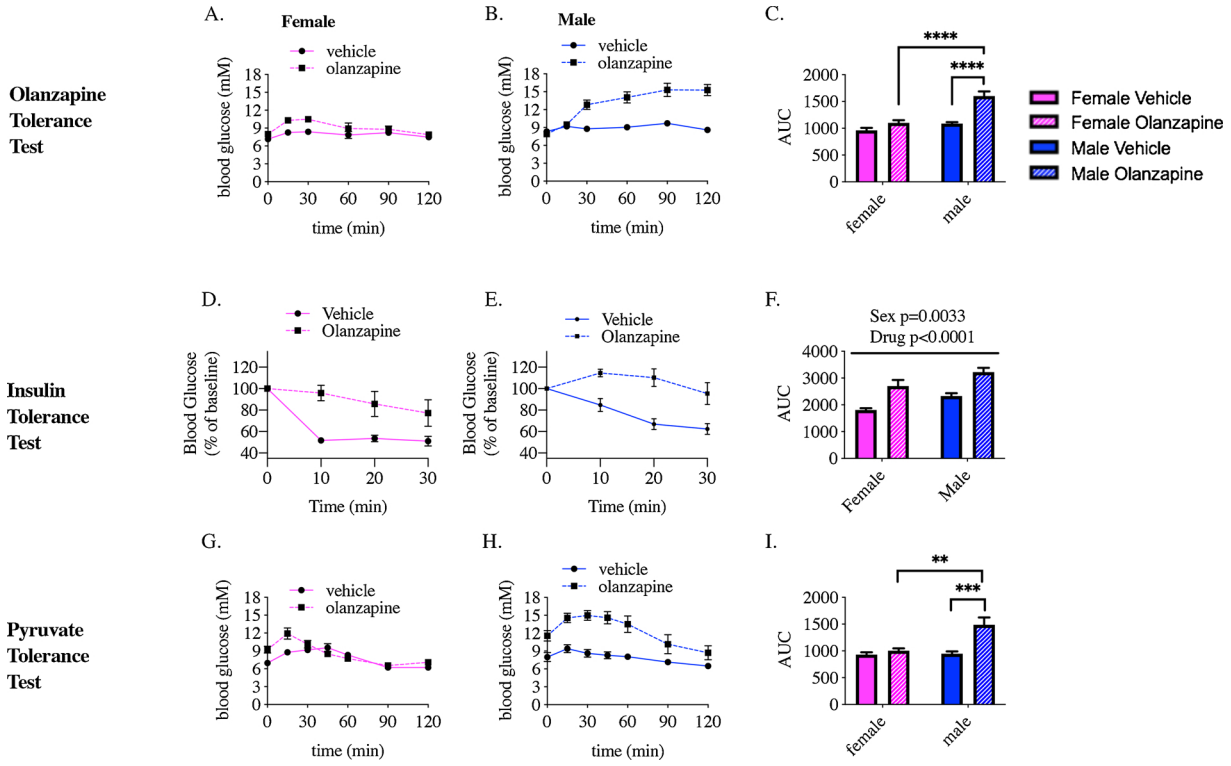

Fig. 1. (A–C) Females are protected from acute olanzapine-induced hyperglycemia. Males and females were injected IP with a weight-matched bolus of olanzapine (5 mg/kg) or vehicle and changes in blood glucose measured 30, 60, 90, and 120 min post injection (A and B) and the glucose area under the curve (AUC) calculated (C). The olanzapine induced increase in blood glucose was greater in male mice (n = 14 mice/group). **(D–F)** Males and females display similar olanzapine-induced insulin resistance. Mice were injected with either olanzapine (5 mg/kg) or vehicle and 1 h later injected IP with insulin (0.5 IU/kg). Blood glucose measured for 30 min post-insulin are represented as a percentage of baseline for both females (D) and males (E) (n = 7/group). Blood glucose area under the curve for the insulin tolerance tests (F) had main effects of both sex (p = 0.0033) and drug (p < 0.0001) determined using a two-way ANOVA. **(G–I)** There is a sexually dimorphic blood glucose response to pyruvate following olanzapine treatment. Mice were injected with either olanzapine (5 mg/kg) or vehicle and 1 h later injected IP with pyruvate (2 g/kg). Blood glucose was measured over the following 2 h for females (G; n = 6/group) and males (H; n = 7/group). Blood glucose area under the curve was calculated for each pyruvate tolerance test (I). **P < 0.01, ***p < 0.001 between groups determined using a two-way ANOVA. All data are presented as mean ± SEM.

vehicle treatment (Fig. 1F). Importantly, there was no interaction of drug and sex (p = 0.99) to affect glucose AUC. These findings provide evidence that olanzapine induces insulin resistance to a similar extent in both male and female mice and that the sexually dimorphic response to olanzapine-induced hyperglycemia is not explained by sex differences in whole-body insulin action.

3.3. Pyruvate-induced liver glucose output is potentiated by olanzapine in male but not female mice

As olanzapine-induced differences in hyperglycemia between male and female mice were not explained by a sexually dimorphic development of insulin resistance, we tested whether the impact of olanzapine on a marker of liver glucose production was different between sexes. We examined this using a pyruvate tolerance test, a rough proxy for hepatic glucose output (Gray et al., 2015). Female (Fig. 1G) and male (Fig. 1H) mice were challenged with pyruvate (2 g/kg) 60 min following treatment with either olanzapine or vehicle and blood glucose was measured for 2 h post-pyruvate administration. Glucose AUC was significantly increased in olanzapine-treated male mice compared to vehicle treated males (p = 0.0004) and female mice (p = 0.0022; Fig. 1I). There were no differences in blood glucose AUC between sexes in the vehicle treated groups. These findings provide evidence of increases in hepatic glucose output in male compared to female mice with olanzapine treatment.

3.4. Liver gluconeogenic enzymes and glycogen content do not account for the observed differences in blood glucose response to olanzapine

To further examine possible factors in the observed sexual dimorphism of glucose homeostasis in response to acute olanzapine we examined the content of indices of hepatic gluconeogenic machinery. Surprisingly, there were no significant differences in the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK; p = 0.09 for main effect of sex) and glucose 6 phosphatase (G6Pase), nor in the transcriptional regulator of these enzymes (Yoon et al., 2001), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (Fig. 2A). Additionally, there were no differences in total content or phosphorylation status of AMPK (Fig. 2A), a signaling enzyme that has been suggested to attenuate hepatic glucose production (Johanns et al., 2016). Similarly, liver glycogen content was not significantly affected by either drug (p = 0.0908) or sex (p = 0.1033) (Fig. 2C) at 2 -hs post olanzapine treatment.

3.5. Serum concentrations of glucoregulatory hormones display a sexually dimorphic response to acute olanzapine treatment

To gain insight into potential sex differences in the endocrine response to olanzapine we measured serum insulin and glucagon in mice 2 h following treatment. Olanzapine significantly increased serum insulin in females (p = 0.0499) but not males (Fig. 3A). There was also a significant difference in serum insulin between vehicle treated males and females (Fig. 3A; p = 0.0057) thus the delta of vehicle to

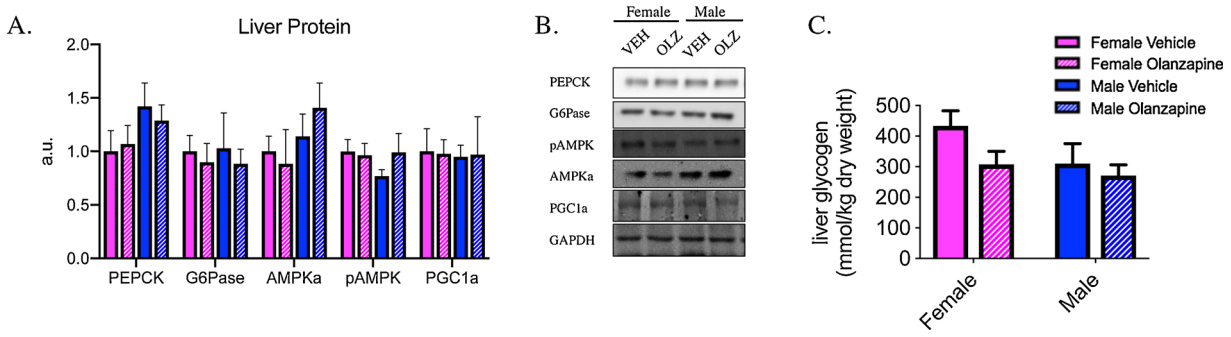

Fig. 2. Liver gluconeogenic proteins and glycogen content are not different between male and female mice. Quantified Western blots for liver protein content of PEPCK, G6Pase, AMPKa, phospho-AMPKa (n = 6/group), and PGC1a (A; n = 3/group) with representative images for Western blots (B). Only GAPDH loading control is shown, though each protein of interest was normalized to its respective loading control. Liver glycogen (C) did not show main effects of drug (p = 0.0908) or sex (p = 0.1033; n = 14/group) determined using a two-way ANOVA. All data are presented as mean ± SEM.

olanzapine treated animals increased in females while it decreased in males (Fig. 3D). Conversely, glucagon increased in olanzapine treated males (Fig. 3B; p = 0.0451) but not females and there was a significant difference in the change in glucagon with olanzapine treatment between sexes (Fig. 3E). Consequently, the ratio of glucagon to insulin was decreased by olanzapine in females and increased by olanzapine in males (Fig. 3F). Collectively these findings provide evidence of clear sex differences in the glucagon and insulin response to olanzapine. Of some relevance, liver content of insulin degrading enzyme (IDE) was significantly increased in male compared to female mice (main effect of sex p = 0.0222, female vehicle 1.00 ± 0.09, female olanzapine 1.09 ± 0.05, male vehicle 1.41 ± 0.21, male olanzapine 1.39 ± 0.13; n = 6/group). To gain further insight into potential sex differences in the endocrine response to olanzapine we measured serum prolactin, as hyperprolactinemia is believed to play a role in metabolic side effects associated with some atypical antipsychotics including olanzapine (David et al., 2000). Female mice had significantly higher serum prolactin concentrations compared to males (main effect of sex p < 0.0001) and olanzapine treatment significantly increased serum prolactin in females (p = 0.0011), while male mice had low prolactin concentrations in both treatment conditions (female vehicle 13.83 ± 2.19 ng/mL, female olanzapine 30.29 ± 3.82, male vehicle 2.69 ± 0.47, male olanzapine 1.56 ± 0.25; n = 5–9/group).

3.6. Males are more responsive to acute glucagon-induced hepatic glucose production

Glucagon increases liver glucose production and we have previously provided evidence that it is a key player in olanzapine-induced hyperglycemia (Castellani et al., 2017). As there were clear sex differences in serum glucagon concentrations with olanzapine treatment, we wanted to examine if the response to an endogenous glucagon challenge would also be different in male and female mice. Age matched male and female C57BL/6 J mice were injected IP with a weight-matched bolus of glucagon (1 mg/kg) or vehicle and blood glucose was measured at baseline and 30 min post glucagon. Female mice (Fig. 4A) displayed a main effect of time (pre-injection to 30 min) to increase blood glucose in both the glucagon and vehicle groups, however there was no significant effect of glucagon in females. Males (Fig. 4B) displayed a significant difference pre- and 30 min post-glucagon injection, while vehicle injected animals did not. These findings provide evidence that the in vivo response to glucagon is different between male and female mice, though it should be noted that this interpretation is muddied as vehicle treatment increased blood glucose in female mice.

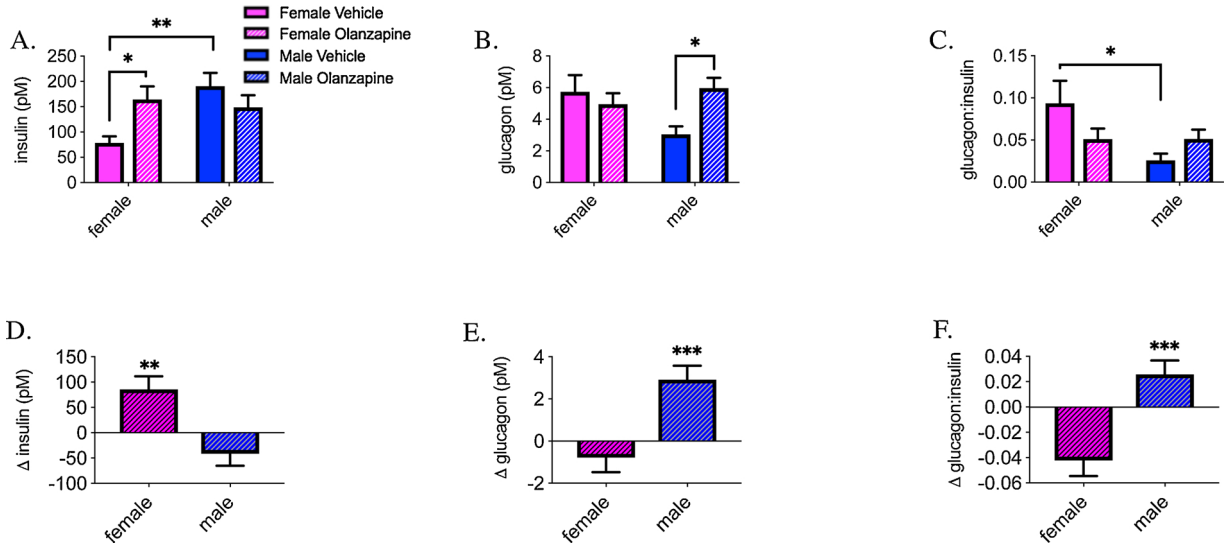

Fig. 3. Acute olanzapine induces a sexually dimorphic response of glucoregulatory hormones. Insulin (A) and glucagon (B) were measured in serum from male and female mice injected with olanzapine (5 mg/kg) or vehicle control 2 h following treatment. The ratio of glucagon to insulin was calculated (C) and the deltas of the respective serum measures were calculated by dividing the olanzapine treated group by the mean of the vehicle group (D–F). Serum measures were analyzed by two-way ANOVA and delta values were analyzed by a two-tailed unpaired T-test (n = 12–14/group). *P < 0.05, **P < 0.01, ***P < 0.001 between indicated groups. All data are presented as mean ± SEM.

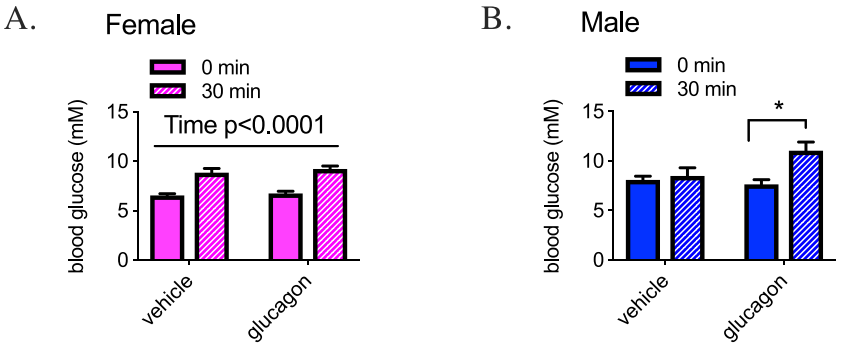

Fig. 4. Glucagon induces a sexually dimorphic response in blood glucose. Mice were injected with either glucagon (1 mg/kg) or vehicle and blood glucose was measured at baseline and 30 min post. *P < 0.05 between groups using a two-way ANOVA (n = 7/group). All data are presented as mean ± SEM.

3.7. Olanzapine increases circulating NEFA and liver TAG concentrations

Alterations in fat utilization is a common side effect of olanzapine treatment (Klingerman et al., 2013). Given the sexually dimorphic response to olanzapine-induced hyperglycemia we aimed to determine if this was associated with changes in indices of lipid homeostasis. It has been previously shown in rats that acute olanzapine treatment is associated with increased circulating fatty acids and liver TAG accumulation (Jassim et al., 2012). Following 2 -hs of olanzapine treatment in male and female mice, serum NEFA was significantly increased ($p = 0.0001$; Fig. 5A) while there was no effect of drug ($p = 0.1541$) or sex ($p = 0.1321$) on serum glycerol concentrations (Fig. 5B). In gonadal white adipose tissue (gWAT), the ratio of phosphorylated to total HSL, one of the primary proteins involved in mediating intracellular lipolysis (Strålfors et al., 1984), was increased in males ($p < 0.0001$) and females ($p = 0.0463$) following olanzapine treatment, with a significant difference between olanzapine treated males and females ($p < 0.0001$; Fig. 5E). Liver TAG accumulation was increased by olanzapine ($p = 0.0003$) and higher in females compared to males ($p = 0.0012$; Fig. 5C) however there was not an interaction of drug and sex ($p = 0.99$) suggesting similar olanzapine-induced liver TAG accumulation between sexes. Liver mRNA expression of lipogenic genes FAS, ACC, and DGAT2 and a transcriptional regulator of these, Srebp1c (Foretz et al., 1999), were higher in female compared to male mice ($p < 0.0001$) (Fig. 5D). Interestingly, there was a main effect of olanzapine to reduce ACC expression ($p = 0.0001$). These findings provide evidence that both males and females largely respond similarly to olanzapine-induced changes in lipid metabolism, at least in the serum and liver. Of note, the increase in serum NEFA occur rapidly and could contribute to the observed increase in liver TAGs.

3.8. Olanzapine reduces RER similarly in male and female mice

Acute treatment with olanzapine has been previously shown to cause rapid and robust increases in whole body fatty acid oxidation. To determine if biological sex impacts this effect mice were housed in CLAMS metabolic caging and whole-body substrate oxidation measured before and for 2 h following drug treatment. There was a main effect of olanzapine to increase VO_2 (Fig. 6A) and a drug by sex interaction for VCO_2, however post-hoc comparisons did not yield significant differences between conditions (Fig. 6B). Olanzapine significantly reduced RER to a similar degree in male and female mice (Fig. 6C). Olanzapine decreased carbohydrate oxidation, increased fat oxidation, and increased total energy expenditure (Fig. 6E-G) with a trend in total energy expenditure for an interaction of drug and sex ($p = 0.0533$). These findings bolster our previous data to show that males and females respond similarly to olanzapine in regards to whole body carbohydrate and lipid oxidation.

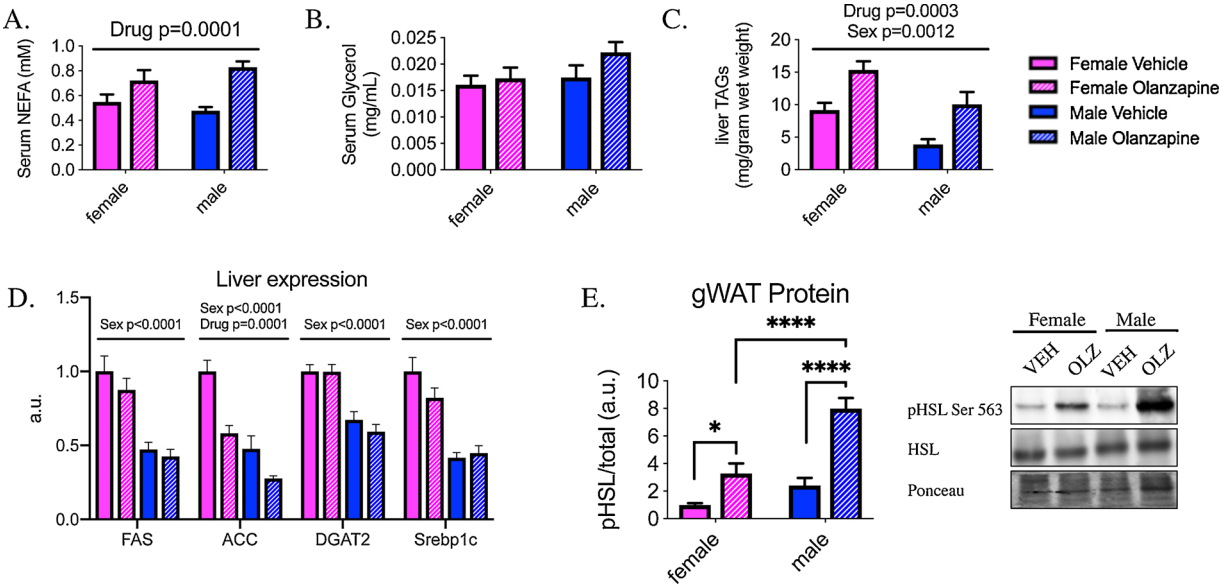

Fig. 5. Olanzapine-increases circulating NEFAs and liver TAG concentrations. Serum NEFA (A), glycerol (B), liver TAGs (C), liver mRNA expression of lipogenic genes (D; n = 6–7/group), and gonadal adipose tissue protein content of HSL and phospho-HSL (E; n = 11–12/group) were measured 2 -hs post olanzapine (5 mg/kg). *P < 0.05, ****p < 0.0001 between groups determined using a two-way ANOVA. All data are presented as mean ± SEM.

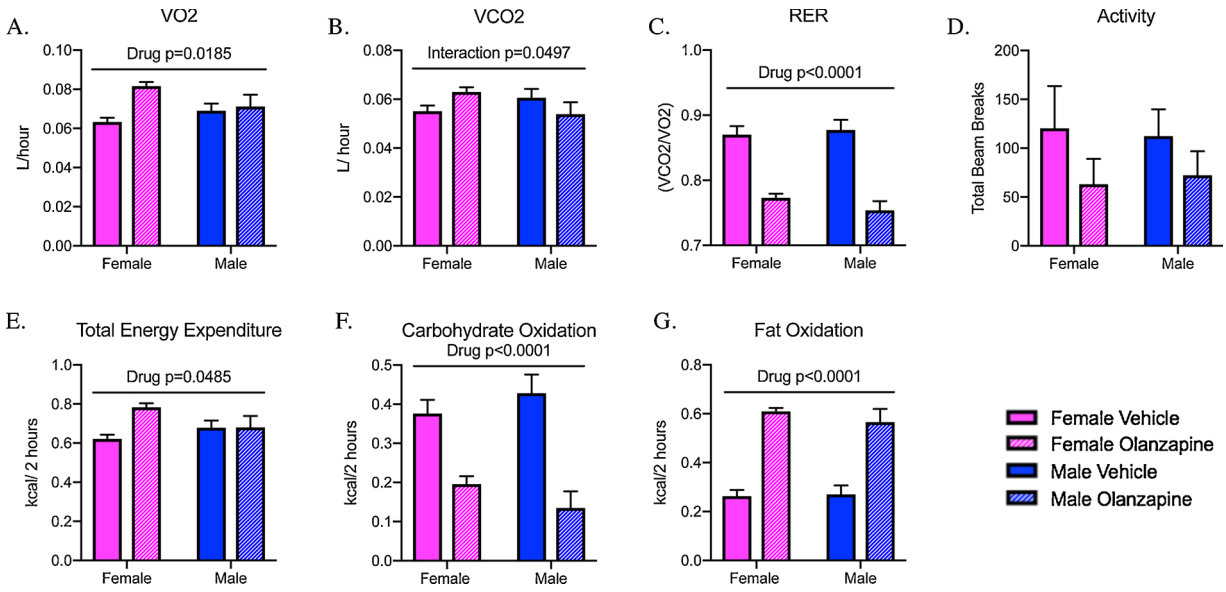

Fig. 6. Male and female mice display similar changes in whole body substrate oxidation following olanzapine treatment. Mice were injected with olanzapine (5 mg/kg) at the beginning of their light cycle and changes in VO2 (A), VCO2(B), RER (C), activity (D), total energy expenditure (E), and carbohydrate (F) and fat (G) oxidation tracked for 2 h (n = 5-6/group). Main effects analyzed using a two-way ANOVA are displayed on graphs. All data are presented as mean ± SEM.

4. Discussion

Prolonged olanzapine administration results in exacerbated weight gain in females compared to males (Gebhardt et al., 2009; Jain et al., 2006) but sex differences in the acute metabolic side effects of SGAs, including impaired glucose homeostasis (Coccurello et al., 2009) and dysregulated lipid metabolism (Rojo et al., 2015), have, to the best of our knowledge, never been directly examined. In the current study, we are the first to demonstrate that female, compared to male mice are protected against acute olanzapine-induced hyperglycemia. Importantly, this does not seem to be related to differences in olanzapine clearance or metabolism as serum concentrations of olanzapine, at least at the time point at which serum was obtained, was not different between sexes.

Olanzapine-induced hyperglycemia is associated with increases in liver glucose production (Chintoh et al., 2009; Klingerman et al., 2013) and reductions in both insulin secretion and sensitivity (Boyda et al., 2010; Hahn et al., 2011; Chintoh et al., 2008), at least in male rodents. Interestingly, when mice were treated with olanzapine and then challenged with insulin there was a similar degree of insulin resistance in males and females. This finding provides evidence that differences in insulin resistance did not explain the sexually dimorphic blood glucose response to olanzapine. In contrast to the development of insulin resistance we found that pyruvate-induced excursions in blood glucose following olanzapine treatment were markedly increased in male but not female mice, suggesting that females are protected against increases in olanzapine-induced hepatic glucose output and that this could be a potential mechanism explaining the attenuated hyperglycemic response to olanzapine in female mice. These findings are consistent with previous work from our group in which we demonstrated that despite the development of severe insulin resistance, that glucagon receptor knockout mice were protected against olanzapine-induced hyperglycemia, secondary to decreases in indices of liver glucose production (Castellani et al., 2017).

Past research using male rats has shown that insulin secretion is impaired by olanzapine (Hahn et al., 2014), whereas notably, we observed a distinct endocrine response to olanzapine between sexes. Serum insulin was increased in females but not in males, whereas serum glucagon responded in the opposite fashion, that is, it was increased in males, but not females. In addition, the blood glucose response to a glucagon challenge was increased in male compared to female mice, though it should be noted that the interpretation of this data is somewhat confounded by the finding that blood glucose was increased in females to an equivalent degree by both vehicle and glucagon treatment. As we have previously shown that intact glucagon signaling is required for the acute hyperglycemic effects of olanzapine (Castellani et al., 2017) our data would suggest that the observed sexually dimorphic response to olanzapine is likely driven by increases in liver glucose production. It is our working model that in male mice acute olanzapine treatment increases serum glucagon which stimulates liver glucose production. At the same time, there is the development of profound insulin resistance which is not paralleled by compensatory increases in serum insulin. In female mice, glucagon levels are not increased with olanzapine treatment and indices of liver glucose production are not increased to an appreciable extent. While olanzapine induces insulin resistance to a similar degree as in male mice, this is likely compensated for by increases in serum insulin in females (See graphical abstract).

Since olanzapine is known to alter fat utilization (Klingerman et al., 2013) we also analyzed potential sex-specific differences in olanzapine-induced changes in indices of lipid homeostasis. Olanzapine increased circulating NEFA, liver TAG accumulation, and fat oxidation while reducing carbohydrate oxidation to a similar degree in both sexes. While there were sex differences in the extent of olanzapine-induced phosphorylation of HSL (Ser 563) in adipose tissue, our findings provide evidence that both sexes largely respond to the same extent to olanzapine in regards to lipid metabolism. Of interest, the NEFA response to olanzapine, similar to glucose, is rapid, and could be a contributing factor to the surprisingly precipitous increase in liver TAG accumulation.

Given the known protective effect of female sex hormones on glucose (Stubbins et al., 2012) and lipid metabolism (Palmisano et al., 2018) this is a plausible starting point for future investigations. Supporting this notion, ovariectomized mice that have large reductions in serum estradiol display reductions in serum insulin, and increases in serum glucagon, an effect that was reversed with estradiol treatment (Handgraaf et al., 2018). This model recapitulates the differences observed herein between females and males and could suggest a biological basis for the observed sex differences in the current investigation.

5. Conclusions

Taken together, we show that the acute hyperglycemic effects of olanzapine are exaggerated in male compared to female mice and this occurs despite the development of equivalent insulin resistance and serum olanzapine accumulation in both sexes. Female mice are likely protected against olanzapine induced hyperglycemia due to reductions in the glucagon:insulin ratio, glucagon responsiveness and liver glucose production. Future work will be needed to explain what drives these unique hormonal and metabolic responses between sexes and this could provide insight into new adjunct treatments to offset the metabolic side effects of SGA treatment.

Author contributions

KDM, LKT, and DCW planned the experiments. KDM, and LKT performed the experiments. KDM, LKT, MKH, and DCW drafted the manuscript.

All authors edited and approved the final draft of the manuscript.

Declaration of Competing Interest

The authors have no competing interests to declare.

Funding

KDM was supported by the University of Guelph Graduate Tuition Scholarship. LKT was supported by a Dairy Farmers of Ontario Doctoral Research Assistantship and an NSERC Postgraduate Scholarship. MKH has grant support from the BBDC New Investigator Award, Canadian Institutes of Health Research, PSI foundation, and holds the Cardy Endowment for Schizophrenia Research Chair. This research was supported by a Project Grant (PJT 159538) from the Canadian Institutes of Health Research to DCW (PJT 159538), who is a Tier II Canada Research Chair.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.104413.

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