A preclinical evaluation of antipsychotic drug-induced metabolic side-effects: a focus on glucose dysregulation and insulin resistance

by

Heidi Noel Boyda

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Abstract

Second generation antipsychotic (SGA) drugs represent the pharmacotherapeutic drug treatment of choice for patients affected by psychosis. Despite their clinical benefits, some SGA drugs are associated with significant metabolic side-effects including weight gain, glucose intolerance, insulin resistance and hypertriglyceridemia that ultimately increases patient risk for the development of cardiometabolic disorders. Although abundant, preclinical literature on SGA drug-induced metabolic side-effects has failed to characterize an underlying mechanism to glucose derangements. We therefore completed seven studies to validate the use of a rat model of SGA drug-induced glucose dysregulation that will establish a consistent approach for future mechanistic studies of insight. We employed the intraperitoneal glucose tolerance test, the hyperinsulinemic-euglycemic clamp, as well as surrogate measures of metabolic indices to ascertain the pharmacological effects of SGA drugs specifically on hyperglycemia, glucose intolerance and insulin resistance. Antipsychotic drugs of higher metabolic liability (olanzapine and clozapine) parallel what is observed in humans and induced substantial glucose intolerance and insulin resistance that was both dose and time dependent. Antipsychotic drugs of lower metabolic liability (risperidone and haloperidol) were associated with modest effects on metabolic indices, compared to controls. The newer SGA drugs also demonstrated drug-dependent effects: iloperidone was associated with profound glucose intolerance and insulin resistance, while asenapine-induced effects were minimal, compared to controls. As for antipsychotic polypharmacy (the practice of when two antipsychotic drugs are administered together), either administrations of clozapine and haloperidol or clozapine and risperidone were found

to cause substantially greater glucose intolerance, relative to the administration of a single drug. Chronic treatment with olanzapine produced sustained glucose intolerance and insulin resistance; whereas intermittent olanzapine treatment led to a first-ever report of the sensitization of glucose metabolism. Lastly, different classes of antidiabetic drug treatments or daily exercise intervention was found to partially ameliorate the metabolic side-effects of olanzapine. These data confirm that our current rodent paradigm parallels clinical literature. Our studies have provided insight into the metabolic influence of SGA drugs on peripheral target tissues such as hepatic, pancreatic and skeletal muscle. Ultimately, these results will further understanding of the underlying mechanisms by which SGA drugs cause glucose dysregulation and insulin resistance.

Preface

Several chapters in this dissertation contain portions that have been published in peer review journals, and are presented with some modifications. My contributions to the chapters are outlined below.

Chapter 1: Introduction

Boyda, H.N., Tse, L., Procyshyn, R.M., Honer, W.G., Barr, A.M. (2010). Preclinical models of antipsychotic drug-induced metabolic side effects. *Trends Pharmacol Sci*. 31(10): 484-97

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Chapter 3: Investigation of pharmacological ameliorative treatments on antipsychotic drug-induced metabolic dysregulation

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Chapter 4: Investigation of non-pharmacological intervention of antipsychotic drug-induced metabolic side-effects

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Chapter 5: General Discussion

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H.N.B performed the literature searches, designed the figures and wrote the manuscript. R.M.P, C.C.P and A.M.B revised and approved the final version of the manuscript.

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Table of contents

List of tables

List of figures

Abbreviations

AMPK: Adenosine monophosphate protease-dependent kinase

APDs: Antipsychotic drugs

AUC: Area under the curve

CATIE: Clinical Antipsychotic Trial of Intervention Effectiveness

DA: Dopamine

D_2: Dopamine 2 receptor

DM: Diabetes Mellitus

ELISA: Enzyme-Linked ImunoSorbent Assay

EPS: Extra-pyramidal symptoms

FDA: Food and Drug Administration

FFAs: Free fatty acids

FGA: First-generation antipsychotic

HGC: Hyperglycemic clamp

GIR: Glucose infusion rate

GLP-1: Glucagon-like peptide-1

GLUT: Glucose transporter protein

HIEC: Hyperinsulinemic-euglycemic clamp

HDL: High-density lipoprotein

HGO: Hepatic glucose output

HOMA-IR: Homeostatic model assessment of insulin resistance

IGTT: Intraperitoneal glucose tolerance test

ISI: Insulin sensitivity index

ITT: Insulin tolerance test

PCP: Phencyclidine

PPAR: Peroxisome proliferator-activated receptor

PPI: Prepulse inhibition

SGA: Second-Generation antipsychotic

SOHO: Schizophrenia Outpatient Health Outcomes

WAT: White adipose tissue

Additional abbreviations are given in the legend to Table 1.2.

Equations

HOMA-IR equation

$$(I_0 \times G_0) \div 22.5$$

ISI equation

$$\left(10^4\right) \div \left(\left(G_0 \times I_0 \times G_m \times I_m\right)^{1/2}\right)$$

I_0 = Fasting insulinemia (unit = μU/ml)

G_0 = Fasting glycemia (unit = mmol/L)

G_m = Mean glycemia (unit = μU/ml)

I_m = Mean insulinemia (unit = mmol/L)

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"Strategy will compensate the talent. The talent will never compensate the strategy."

-Marco Pierre White

Chapter 1: Introduction

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1.1 Schizophrenia

Recognized as one of the more severe psychotic disorders, schizophrenia is a lifelong debilitating psychotic disorder that affects about 1% of the population [1]. Schizophrenia presents itself as an enigmatic disorder with varied symptomology that typically begins in late adolescent or early adulthood years. Clinical characteristics of schizophrenia consist of overt psychotic manifestations known as 'positive symptoms,' which include disorganized thoughts, sensory hallucinations, and delusions as well as 'negative' symptoms comprised of alogia, anhedonia, avolition, blunted affect and social withdrawal. Additional impairments in cognitive function (attention and memory) and behavior deficits are also common diagnostic features of the illness [1]. Interestingly, mounting evidence suggests that schizophrenia discriminates between genders, where an earlier age of onset is more prevalent in males, while women with chronic illness display more severe positive symptoms [2]. To date, however, schizophrenia still remains a disorder of unknown etiology with several theories underlying the pathology and symptomology.

1.2 Dopamine hypothesis

The dopamine (DA) theory of schizophrenia was first conceptualized in the mid-20th century [3-5], and for decades now, has remained a predominant biological hypothesis of the illness due to the robust pharmacological data that supports augmentation of DA transmission. The DA hypothesis postulates that psychotic tendencies develop from a hyperdopamineric state in various regions of the brain, such as the basal ganglia [6]. In addition, dopaminergic agonists such as stimulant drugs (e.g. amphetamine) exacerbate psychosis [7], while dopaminergic

antagonists including the synthetic phenothiazine class exert antipsychotic properties [8]. In spite of pharmacological evidence, the DA hypothesis has several flaws. Firstly, complete blockade of DA neurotransmission does not fully diminish all symptoms of schizophrenia, and suggests that other neurotransmitter pathways are at play. Additionally, antipsychotic medications decrease DA neurotransmission, however, no notable decreases in DA or its metabolites are found in patients treated with antipsychotic drugs (APDs) [1]. Nevertheless, perhaps the strongest support in favor for this theory relies on well-known evidence that the therapeutic efficacy of all APDs is partially attributed to their D_2 receptor antagonistic properties.

1.3 Pharmacological treatment

1.3.1 Antipsychotic drugs

The development of APDs used to treat psychosis represents one of the major success stories in psychiatry. These compounds have improved the lives of millions of patients with schizophrenia. Furthermore, APDs are now increasingly being used to treat additional psychiatric conditions, such as mood and anxiety disorders with perturbations of acute psychotic, manic and psychotic-depressive episodes [9, 10], often in non-traditional populations such as adolescents. APDs continue to represent one of the most profitable areas in the pharmaceutical industry, with global sales of approximately $22 billion in 2008.

At present, over 60 antipsychotic medications have been developed globally, 21 of which are currently approved for use in the United States by the Food and Drug

Administration (FDA) [11]. Traditionally, APDs have been classified into two main categories known as the first-generation antipsychotics (FGAs), conventional, or 'typical' agents, and the second-generation antipsychotics (SGAs) or 'atypical' compounds. Chlorpromazine, the very first FGA drug, was introduced clinically in the early 1950s; whereas clozapine, the first reported SGA drug, became approved for clinical use by the FDA in early 1990 [12]. In particular, after clozapine was developed a multitude of SGA drugs became available in the United States in the early 1990s and 2000s (Table **1.1**). Accordingly, one main pharmacological difference between FGA and SGA drugs arises from the pharmacodynamic interactions and binding profiles. FGAs preferentially block the D_2 receptor with a high affinity, while SGAs block the D_2 receptor to a lesser degree and exert more potent blockade on the serotonergic 5-HT_{2A} receptors and other non-D_2 receptor-mediated targets [13].

Table 1.1: *Year of Food and Drug Administration approval of antipsychotics drugs in the United States*. Data from U.S. Food and Drug Administration website, accessed February 01, 2013 (http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics).

Class of Antipsychotic Drug	Generic Name of Drug	Year of FDA Approval
First-Generation	Chlorpromazine	1954
	Fluphenazine	1959
	Haloperidol	1967
	Loxapine	1975
	Molindone	1974
	Perphenazine	1957
	Pimozide	1984
	Prochlorperazine	1956
	Trifluoperazine	1959
	Thioridazine	1962
	Thiothixene	1967
Second-Generation	Aripiprazole	2002
	Asenapine	2009
	Clozapine	1989
	Iloperidone	2009
	Lurasidone	2010
	Olanzapine	1996
	Paliperidone	2006
	Quetiapine	1997
	Risperidone	1993
	Ziprasidone	2001

Yet despite the recognized importance and widespread use of these drugs, it may be argued that there has been no fundamental breakthrough in how APDs treat psychosis since the advent of chlorpromazine: all approved, existing drugs require significant D_2 receptor antagonism [14]. Attempts to develop drugs that target alternate molecular substrates, such as the metabotropic glutamate 2/3 receptor, have so far not met with success, indicating that psychiatry is likely to continue using the currently available drugs for the foreseeable future. Thus, the utility of most APDs is now, and indefinitely, based in large part on the unique side-effect profile of the individual compound.

1.4 Antipsychotic drug-induced side-effects

Despite the efficacy of pharmacotherapy with APDs, there are serious treatment burdens which include a range of neurologic, metabolic, cardiovascular, hematologic, musculoskeletal, and endocrine side-effects among others [15-17]. These adverse effects undoubtedly have important implications for patient quality of life and treatment compliance, and accordingly, increased health care costs in dealing with them. Importantly, FGA and SGA drugs differ markedly in their adverse side-effect profiles.

1.4.1 First-generation antipsychotic drugs and extrapyramidal symptoms

The side-effects associated with the earlier FGA drugs are most often attributed to the robust D_2 blockade in the nigostriatial, tuberoinfundibular and mesolimbic systems to produce an array of extrapyramidal symptoms (EPS) including parkinsonism, dystonia and akathisia, tardive dyskinesia, neuroleptic-induced deficit syndrome, and hyperprolactemia [13]. Based on their ability to cause EPS, FGAs are most associated with neurologic adverse events and with it, increased morbidity and diminished medication adherence, which is the main underlying reason for the more frequent use of SGA drugs today.

1.4.2 Second-generation antipsychotic drugs and metabolic side-effects

Initial excitement for the SGA drugs, with their lower incidence of EPS, was quickly tempered by emerging evidence that they were associated with metabolic disturbances that promote adiposity, obesity and glucose impairments in an already metabolically suseptible population. In particular, SGA drug therapy is linked to increased rates of metabolic syndrome, which places patients at substantial risk for developing cardiometabolic disorders such as Type II Diabetes Mellitus (DM) and cardiovascular disease.

1.5 Metabolic syndrome in humans

Metabolic syndrome, formally identified as 'syndrome x', can generally be described as a cluster of metabolic abnormalities comprising abdominal obesity, insulin resistance, elevated blood pressure and atherogenic dyslipidemia [18]. Five of the most common features of metabolic syndrome in humans are: disturbances in fasting/postprandial plasma glucose levels and improper glucose clearance, insulin resistance, altered plasma lipid levels (including *increased* free fatty acids (FFAs) and/or triglycerides and *decreased* HDL cholesterol), weight gain and hypertension. Furthermore, increased waist circumference, body mass index and waist-to-hip ratio have been strongly associated with the development of metabolic syndrome [19]. Over the past two decades however, the exact clinical definition of metabolic syndrome has been reconsidered, as some characteristics do not consistently fall under the same phenotype [18]. Therefore, patients with metabolic syndrome may have three or more of any of these symptoms. For the purpose of this thesis, we will describe five of the

most common metabolic side-effects associated with SGA drug-use in patients: glucose dysregulation, insulin resistance, lipid and hormone alterations, weight gain and blood pressure effects.

1.5.1 Glucose dysregulation

The common term 'prediabetes' denotes a phase between the premature development of glucose dysregulation and the advanced stages of Type II DM. Most patients diagnosed with glucose dysregulation will exhibit either glucose intolerance (i.e., impaired glucose clearance from the blood) or fasting hyperglycemia. Consistent with the American Diabetes Association [20], individuals with impaired glucose tolerance reflect mild-to-severe insulin resistance in skeletal muscle and defects in pancreatic insulin secretion, whereas individuals with fasting hyperglycemia have moderate hepatic insulin resistance [21].

Many clinical studies report findings on increased fasting hyperglycemia both in Type II DM [22, 23] and SGA drug-treated patients [24, 25], due to the minimally invasive and convenient blood sampling technique. Additionally, fasting glycemia can be used to acquire further surrogate measures, such as the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR; see section 1.5.2 below) [26]. While fasting levels of blood glucose provide a relatively accurate measure of initial fasting hyperglycemia, by no means does this technique allow for the direct confirmation of tissue-specific glucose uptake. A second and more indicative measurement for glucose dysregulation involves the use of the glucose tolerance test (GTT), whereby a fasted subject is given a glucose challenge and blood glucose levels are repeatedly determined afterwards. This test allows for the comparison

between fasting and post-prandial levels of glucose, and evaluates how capable the subject is in clearing exogenous glucose to reinstate euglycemia. A robust amount of clinical literature suggests that the SGA drugs clozapine and olanzapine are associated with significant hyperglycemia and glucose intolerance [27-30], and a significant proportion of these studies describe these effects to be independent of body weight gain [31-34]. Therefore, clinical studies have recently focused their research on SGA drug-induced metabolic measurements on individual side-effect risk factors, rather than the cluster of effects as a whole.

1.5.2 Insulin resistance

Second to increased weight gain, the most consistent reports of SGA drug-induced metabolic side-effects in humans is the development of insulin resistance. Peripheral insulin resistance is characterized by changes in plasma insulin levels (mainly hyperinsulinemia) and the failure of liver, skeletal muscle or adipose cells to respond to insulin signaling via the insulin receptor [35]. Most clinical studies have relied on the reports of fasting hyperinsulinemia as a biomarker for insulin resistance, which has consistently been reported in humans after either clozapine or olanzapine drug treatment [28, 36-38]. However, fasting measurements are only partially indicative for basal activity and do not reflect how insulin-sensitive tissues handle glucose after a meal. More comprehensive metabolic assessments exist, and three in particular are most commonly used in the clinic.

The first, and most often reported, is the measurement of fasting complementary metabolic parameters such as fasting blood glucose, obtained in parallel to fasting insulin levels. With these two fasting indices, the HOMA-IR score can be calculated to provide a

powerful index of insulin resistance (i.e., the larger the HOMA-IR score, the greater the insulin resistance) [39]. Clinical literature has shown that psychiatric patients who are treated with olanzapine or clozapine for at least 8 weeks display significant increases in HOMA-IR scores and therefore have impaired insulin sensitivity [40, 41]. Furthermore, in a randomized cross-over study in healthy control subjects, 8 days of olanzapine administration elevated HOMA-IR scores along with increased plasma triglyceride levels, which was independent of body adiposity [42].

The second comprehensive metabolic assessment estimates pancreatic secretory function by obtaining plasma insulin levels at multiple time points after a carbohydrate challenge, such as during a standard GTT. Manu and colleagues recently conducted a study with 520 adult psychiatric inpatients treated with clozapine (n = 73), olanzapine (n = 190), quetiapine (n = 91) or risperidone (n = 166) who underwent an assessment for insulin secretion via the oral GTT and plasma insulin measurement technique [43]. Interestingly, only clozapine was a predictor of post-challenge insulin secretion and was found to enhance insulin secretion in patients with normal glucose tolerance.

The third metabolic assessment for insulin resistance, known as the hyperinsulinemic-euglycemic clamp (HIEC), represents the 'gold-standard' methodology and provides a direct measurement of insulin sensitivity *in vivo* (See below, section 1.6.2 for further details) [44]. When tested in healthy control subjects, olanzapine, but not ziprasidone or haloperidol treatment, was associated with significant decreases in whole body insulin sensitivity in response to the HIEC challenge [45, 46]. In a clinical study involving non-diabetic patients with schizophrenia who underwent HIEC clamp procedures, olanzapine and risperidone treatment was associated with small decrements in insulin sensitivity [47]. Altogether,

treatment with SGA drugs of high metabolic liability induces effects on insulin secretion and peripheral insulinotropic actions, which places patients at a much higher risk for metabolic syndrome.

1.5.3 Lipid and hormonal alterations

The effects of SGA drugs on blood lipids generally parallel the effects on glucose dysregulation and insulin resistance [48]. First noted in 1996 with clozapine, significant increases in triglyceride levels have also been reported with olanzapine [49]. Risperidone and quetiapine are associated with moderate risk for hyperlipidemia, whereas ziprasidone and aripiprazole have minimal risk [50]. In a double-blind, randomized, placebo-controlled cross-over trial in healthy volunteers, olanzapine treatment for 3 consecutive days caused a 24% increase in leptin levels, 22% in triglycerides and 42% in glucose area under the curve (AUC), whereas free fatty acids (FFAs) declined by 32% and high-density cholesterol decreased by 11% [51]. Although some studies have reported significant associations between weight gain and increased triglycerides, the effect of weight gain on lipid profiles is less clear than on glucose and requires further study.

1.5.4 Weight gain

Weight gain is the best-recognized metabolic side effect, although this varies markedly among the SGA drugs. Furthermore, reports of clinically relevant weight gain (characterized as ≥ 7% of pretreatment body weight) have been reported in psychiatric patients with the use

of *any* APD compound when compared with placebo, and therefore no APD is considered entirely body-weight neutral [52]. In a recent post-hoc analysis of pooled data obtained from the Schizophrenia Outpatient Health Outcomes (SOHO) studies, the mean weight change observed after 3 years of continual APD treatment in adults was: olanzapine 4.2 kg, clozapine 3.3 kg, risperidone 3.1 kg, quetiapine 2.5 kg, depot FGAs 2.5 kg and oral FGAs 2.3 kg [53]. A second prospective study in Asian adult patients treated for 2 years with APDs reported the following weight gain liability: 72.4% for clozapine, 66.7% for olanzapine, 65.0% for risperidone, 56.0% for quetiapine and 66.6% for aripiprazole [54]. Likewise, increased use of APD therapy in children and adolescent patients has reported similar findings in mean weight gain: olanzapine 4.0 kg, clozapine 2.4 kg, risperidone 2.0 kg, quetiapine 1.7 kg, and aripiprazole 0.9 kg [55]. This rank order of weight gain correlates with the risk for hyperglycemia and diabetes, which led to the hypothesis that metabolic changes were largely related to the effects of APDs on body fat. However, a comprehensive review has detailed new-onset diabetes in over 25% of diabetic ketoacidosis cases and 15% new-onset hyperglycemia in patients who did not experience any weight gain or who had lost weight [56]. Furthermore, a study of the acute effects of atypical APDs in non-psychiatric subjects noted marked effects on glucose tolerance in the absence of marked weight gain [45].

1.5.5 Blood pressure

Although hypertension (systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg) is one of the five major risk factors for cardiometabolic disorders, the clinical literature on APD-induced effects is scarce and is often missed due to lack of monitoring [57]. Orthostatic hypotension is, however, one frequently reported acute vascular effect of APDs [58]. APDs such as olanzapine, ziprasidone or haloperidol are considered low-risk, quetiapine and risperidone are moderate in nature, while clozapine and chlorpromazine are considered high-risk for the development of orthostatic hypotension [59, 60]. On the other hand, one cross-sectional study conducted in psychiatric patients on quetiapine or risperidone for at least 3 months indicated that peripheral arterial compliance (defined as the distensibility of large arteries in the leg) was significantly reduced compared to control subjects [61]. Future directions should focus on determining what effects APDs have on vascular indices and if these effects are exacerbated when co-morbidities, such as insulin resistance, are present.

1.6 Metabolic syndrome: a role for animal paradigms?

High rates of metabolic syndrome in patients treated with APDs likely occur as a result of a number of mechanisms, but none of which is necessarily exclusive. Firstly, metabolic changes may be an idiopathic consequence of the psychiatric disorder itself, independent of pharmacological treatment [62]. Secondly, APDs may cause behavioral changes, such as increased appetite and food consumption [63], or decreased activity. Thirdly, treatment with APDs changes one metabolic profile directly, such as hyperlipidemia [48], leading to

secondary changes, such as weight gain and insulin resistance. Finally, there may be acute and direct effects of the drugs on multiple independent metabolic parameters. Clearly, it is important to study each of these potential mechanisms separately before developing complex models to analyze interacting factors. In humans, this is a daunting task, and therefore animal models have been critical in making progress in this area. Hence, understanding the mechanism(s) responsible for mediating APD-induced metabolic side-effects has become an important research topic, and the last few years has witnessed a steady accumulation of results from preclinical studies using animal paradigms.

As the animal literature on the metabolic effects of APDs is broad, the following introduction focuses on five key risk factors by which APD induces metabolic syndrome: glucose dysregulation, insulin resistance, lipid/hormone disturbances, weight gain and blood pressure abnormalities. For most preclinical studies, emphasis is placed on modeling in detail individual symptoms rather than the syndrome in its entirety.

1.6.1 Glucose dysregulation

Numerous studies have shown that APD-induced glucose dysregulation is one of the most consistent findings in rodent paradigms, closely paralleling human observations (Table **1.2**): Elevated and sustained plasma glucose levels can be largely attributable to either decreased insulin-mediated glucose uptake or increased endogenous hepatic glucose production/output (HGO), reflecting gluconeogenesis (anabolic production of glucose) and/or glycogenolysis (catabolic break-down of glycogen to glucose).

I*n vivo* models commonly report elevated fasting plasma glucose levels in rats after either acute or chronic treatment with most SGA and some FGA drugs [64-66]. While fasting glucose levels are a useful initial indicator of hyperglycemia, confirmation of impaired glucose regulation is required with more accurate and reliable techniques. As discussed, the GTT is one such procedure that is commonly used in the clinic and has good translational capacity with rodents [67]. Due to the ease of the technique, it is useful in studies involving larger numbers of animals, such as dose and time dependent relationships [65, 66, 68]. A limitation of this technique is that the specific sites for major glucose metabolism, which include the liver, muscle and adipocytes, as well as the nature of APD-induced elevated glucose levels, i.e. insulin resistance versus increased HGO, cannot be determined unless complementary procedures are employed. These include the pyruvate and glycerol tolerance tests, whereby both of these compounds act as substrates for gluconeogenesis, permitting alterations in both fasting and non-fasting plasma glucose to be attributable to increases in HGO. Smith and colleagues employed this technique to demonstrate that acute treatment with haloperidol, quetiapine or clozapine resulted in hyperglycemia secondary to increased HGO [69]. Further experiments in clozapine treated rats noted that glucagon levels were also elevated, despite concurrent increases in glucose and insulin, which normally exert potent negative feedback to decrease glucagon levels. In a follow-up study by the same research group [70], both acute quetiapine and clozapine increased glucagon levels while concurrently decreasing levels of glucagon-like peptide-1 (GLP-1) - a peptide known to inhibit glucagon secretion.

Table 1.2: *Summary of in vivo antipsychotic drug-induced metabolic side effects in the rat*

CLO: Clozapine, **OLA**: Olanzapine, **RIS**: Risperidone, **HAL**: Haloperidol, **CHL**: Chlorpromazine, **SUL**: Sulpiride, **ZIP**: Ziprasidone, **ARI**: Aripiprazole, **QUE**: Quetiapine, **LOX**: Loxapine, **YOH**: Yohimbine, **HEX**: Hexamethonium, **PER:** Perphenazine**, Des**: Desmethyl-clozapine, **AD**: Antipsychotic drug, **D**: Days, **WAT**: White adipose tissue, **PU**: Periuterine fat pad, **FFAs**: Free Fatty Acids, **M**: Male, **BAT**: Brown adipose tissue **RP**: Retroperitoneal fat pad, **W**: Wistar Rat, **F**: Female, **PR**: Perirenal fat pad, **FAS**: Fatty acid synthase, **HW**: Han-Wistar, **NS**: Not specified, **PM**: Perimetrial fat pad, **E**: Epididymal fat pad, **SD**: Sprague-Dawley, **NE**: Not significant, **p.o**: Oral, **i.p**: Intraperitoneal, **s.c**: subcutaneaous, **OP**: osmotic pump, **HL**: Hooded-Lister **Mes**: Mesenteric fat pad, **IAF**: Intra-abdominal fat, **VF**: Visceral fat, **TG**: Triglycerides, **FC**: Fat Content, **g**: Glucose, **i**: insulin, **IRS**: Insulin receptor substrate, **HSL**: Hormone sensitive lipase**, HGO**: Hepatic glucose output, **GLP-1**: Glucagon-like peptide-1, **GLUT2**: Glucose Transporter 2, **HGC**: Hyperglycemic clamp, **Blank boxes**: Data not reported, **WT**: Wild-type, **NS**: Not specified, **SA**: Swiss Albino, **KE**: Ketone, F**Chol**: Free Cholesterol, **HDL**: High density lipoprotein, **NEFA**: Nonesterified fatty acid, **GIP**: Glucose-depedent insulinotropic polypeptide, **HIEC**: Hyperinsulinemic-euglycemic clamp, **HOMA-IR**: Homeostatic Assessment of Insulin Resistance

△ : increase unknown percentage   ▽ : decrease unknown percentage

▲ : increase ≤ 5 %   ▼ : decrease ≤ 5 %

▲ ▲ : increase 5 - 10 %   ▼ ▼ : decrease 5 – 10 %

▲ ▲ ▲ : increase > 10 %   ▼ ▼ ▼ : decrease > 10 %

Drug, Dose (mg/kg/day), Route	Strain, Gender	APD Duration	Weight Change	Glucose & Insulin Levels	Insulin Resistance	TGs; FFAs	Visceral Adiposity	Other	Ref.
Olanzapine									
(8), i.p	W, F	19 D	▲ ▲						a-a
(1, 2, 4, 8), i.p	HW, F	20 D	△	NE (g); △(i)	△HOMA – IR		▲PR & PM	NE leptin, △adiponectin	a-b
(2, 4, 8), i.p	HW, M	20 D	▽	NE (g & i)			▲ PR	▽ Gastro. Muscle, △adiponectin	a-c
(1, 2), i.p	SD, F	20 D	△	NE (g); △ (i)	△HOMA – IR	NE			a-j
(2, 7.5), OP	SD, F	28 D	NE	△ HGO; NE (i)	△ clamp		△		b-c
(10), p.o	W, F	14 D	△	▽ (g)		△ FFA, NE TG	△ FC	▽ lean, NE cholesterol, HDL, glycerol	c-a
(2), p.o	SD, F	56 D	▲ ▲ ▲				▲ ▲ ▲ WAT		c-g
Melatonin (0.4 µg/ml)			NE (Melatonin + Ola)				NE (Melatonin & Olanz)		
(2), p.o	SD, F	7 D	▲ ▲ ▲						c-m
(3), s.c	SD, M	100-310 min (HIEGC)		△ HGO (g); ▽ HGC (i)	△ clamp	NE		▽ C-peptide	d-g
		90 min (HGC)		NE (g & i)					
(29.17 mg/ml), OP	SD, F	28 D	△	△ (g)				△ cholesterol, △ I, Mes, RP	e-c
Liraglutide (50-100 ug/ml) OP & (0.1, 0.2 mg/kg), s.c			▽	▽ (g)				▽ Mes & RP	
Clozapine									
(6, 12), i.p	W, F	21 D	▽	NE (g & i)					a-d
(1-4), i.p		20 D		NE (g & i)			▲ PR	△adiponectin	
(0.25, 0.5), i.p		12 D	▽	NE (g & i)					
(5, 10, 20), i.p	SD, M	15, 30, 60 min		△ (g & i)				△ ghrelin	a-e
(20), p.o	SD	28 D	△	△ (g & i)		△ TG		△ leptin, GIP mRNA	c-n
(7.5), s.c	SD, M & F	60, 75, 90 min		△F (g & i)				△corticosterone	d-d
(15), s.c		3 D		△F (g)				△corticosterone	
(15), s.c		13 D		△F (g & i)		NE		▽leptin	

16

Drug, Dose (mg/kg/day), Route	Strain, Gender	APD Duration	Weight Change	Glucose & Insulin Levels	Insulin Resistance	TGs; FFAs	Visceral Adiposity	Other	Ref.
					Metabolic Symptoms Investigated				
Risperidone									
(0.01, 0.1, 1), s.c	SD, M	21 D	△ (0.01), ▽ (1)					△ leptin gene WAT (0.01, 1), △ Ucp1 BAT (1)	d-e
(0.01, 0.1), s.c	SD, M	21 D				▽ NEFA (0.1)		△ mRNA genes in WAT, muscle & liver	d-f
Sulpiride									
(20), i.p	W, F	10, 30 D	△	▽ (g); NE (i)					a-f
(20), i.p	W, F	30, 60, 90 min	NE	NE					a-g
		10-30 D	△	NE					
(20), i.p	W, M	21 D	NE	△ (g & i)		NE			a-h
Chlorpromazine									
(7.5), p.o	W, M	70 D	NE	△ (g & i)			△ VF	△cholesterol (+ sucrose), △ leptin (+ sucrose), △ TNF-α (+ sucrose)	c-c
(5, 50), p.o	SD, M	56 D	△	△ (g & i)	△ HOMA-IR (50)		△ E	▽ Glucokinase, NE GLUT2, ▽ IRS2	c-p
(5, 50), p.o	SD, M	56 D	△	△ HGO		△ TG (50)	△ E	△ leptin, ▽ glycogen, protein levels altered	c-q
Multiple drugs									
OLA (2), i.p	HL, F	22 D	▲						a-i
RIS (0.5), i.p				▽ (g), NE (i)		NE			
ZIP (2.5), i.p								▽ leptin	
OLA (4), i.p	HL, F	21 D	△				△ IAF		a-k
RIS (0.5), i.p			△				△ IAF		
SUL (10), i.p			△				△ IAF		
HAL (0.5), i.p			△				△ IAF		
ZIP (2.5), i.p									
RIS (0.1, 0.5, 1.0), i.p	HL, F	21 D	▲ ▲ ▲						a-l
HAL (0.1, 0.5, 1.0), i.p			▲ ▲ ▲						
OLA (2), i.p	HL, F	28 D	NE						a-m
RIS (0.5), i.p									
ZIP (2.5), i.p									
OLA (1.5, 15), i.p	SD, F	60, 180, 360 min		△ (g & i)	△ HOMA-IR				a-n
CLO (2, 20), i.p				△ (g & i)	△ HOMA-IR				
RIS (0.5, 2.5), i.p				△ (g & i)	△ HOMA-IR				
HAL (0.1, 1), i.p				△ (g & i)					
RIS (2.13), OP	SD, M	11-14 D, 28 D	▽	▽ (g)		NE		△ glucagon, ▼ Mes, ▼ pancreas	b-a
HAL (0.4), OP			NE	△ (g) vs Risp; △ (i)		NE			
OLA (5), OP	SD, M & F	11 D	△ F						b-b
CLO (10), OP			NE						
OLA(2, 4, 20), p.o	SD & W, F	7 D	△						c-a
ARI (4, 8, 16), p.o			△						
OLA (5, 20), p.o	HW, M & F	21 D	▽M						c-b
HAL (0.08, 0.3), p.o			△F						
OLA (1), p.o	SD, M	42 D					△ BAT, △ RP, ▽ kidney weight	△ leptin, △HDL & total cholesterol	c-d
HAL (1), p.o			NE	NE		▽ TG	△ BAT, △ RP, ▽ kidney weight	△ leptin	
ZIP (10), p.o							▽ liver weight & kidney		
OLA (1), p.o	SD, M	35 D	NE				△ WAT	△ mRNA FAS, ▽mRNA HSL	c-e
HAL (1), p.o									
ZIP (10), p.o									
OLA (0.01, 0.1, 0.5, 1, 2), p.o	SD, M	21 & 42 D	△				△ WAT (0.5, 2)		c-f
HAL (1), p.o		21 D	NE						
ZIP (10), p.o			NE						
OLA (4), p.o	SD, F	7 D	△						c-h
ZIP (4, 12, 20), p.o			△						
OLA (1), p.o	SD, M	42 D	NE	NE (g & i)		NE	NE	NE, (cholesterol & HDL)	c-i
HAL (1), p.o			NE	NE (g & i)		NE	NE	NE, (cholesterol & HDL)	

17

Drug, Dose (mg/kg/day), Route	Strain, Gender	APD Duration	Metabolic Symptoms Investigated						Ref.
			Weight Change	Glucose & Insulin Levels	Insulin Resistance	TGs; FFAs	Visceral Adiposity	Other	
OLA (2.5-40), p.o	SD, M	1 hr		△ (g)				△ corticosterone	c-j
CLO (2.5-40), p.o				△ (g)				△ corticosterone	
HAL (0.63-10), p.o				△ (g)				△ corticosterone	
RIS (0.63-40), p.o				△ (g)				△ corticosterone	
ZIP (2.5-40), p.o				NE				△ corticosterone	
ARI (0.63-40), p.o				△ (g)				△ corticosterone	
OLA (1.5), p.o	SD, F	7 & 84 D	△					▼ ▼ ▼ cannabinoid bind	c-k
HAL (0.3), p.o			NE						
ARI (2.25), p.o			NE						
OLA (1.5), p.o	SD, F	7 & 84 D	△				▲ ▲ ▲ WAT (7 D)	▽ mRNA H1 R	c-l
HAL (0.3), p.o			NE				NE		
ARI (2.25), p.o			NE				NE		
OLA (2), p.o	SD, M	26 & 42 D	NE	△ (46 D)		NE	△ E	NE cholesterol	c-o
HAL (1), p.o			NE			NE		NE cholesterol	
OLA (1, 3.2, 10)s.c (0.1-10 µM)	HW, M; SD, F	40-60 min, 5 D		△ HGO	△ clamp				d-a
CLO (1, 3.2, 10) s.c (0.1-10 µM)				△ HGO	△ clamp				
ZIP (3.2, 10, 32) s.c				NE					
RIS (2), s.c									
RIS (0.125, 0.5), s.c	W, M & F	12-15 D	△F					△corticosterone (M), NE leptin	d-b
SUL (20), s.c		12 D		△ (g)				△leptin	
RIS (0.5), s.c	W, F	12 D	△				NE		d-c
SUL (20), s.c			△				NE		
CLO (10), s.c	SD, M	1-2 hrs, 7 & 28 D	▽	△ HGO, △ (i)	NE		NE	△ glucagon	d-h
HAL (0.25), s.c			▽	△ HGO △ (i)	NE		NE		
QUE (10), s.c			NE	△ HGO △ (i)	NE		▲ ▲ FC		
OLA (3), s.c	SD, M	130 min		△ HGO ▽ HGC (i)	△ clamp			▽ C-peptide	d-i
CLO (10), s.c				△ HGO ▽ HGC (i)	△ clamp			▽ C-peptide	
RIS (1), s.c				△ HGC (i)	△ clamp				
HAL (0.25), s.c				NE					
ZIP (3), s.c				▽ HGO					
OLA (1.2), p.o	SD, F	180 min, 10 or 30 D	△					△ 5-HT	e-a
OLA (1.2), i.p									
HAL (0.04), p.o									
HAL (2), i.p			NE						
CLO (10), s.c	SD, M	60 min, 42 D	▽	△(60 min) (g); △(60 min) (i)	NE			△ glucagon, NE leptin, ▽ GLP-1	e-b
QUE (10), s.c			△	△(60 min) (g); △(60 min) (i)	NE			△ glucagon, NE leptin, ▽ GLP-1	
Sitagliptin (25), p.o		60 min		△ (i)				△ GLP-1	
Exendin-4 (5 ug), p.o				▽ (g); △ (i)				▽ glucagon, △ GLP-1	

Reference Guide:

a-a: Goudie et al., (2002). J Psychopharmacology. 16, 291-296; *a-b*: Cooper et al., (2005). J Psychopharmacology (Berl). 181, 80-89; *a-c*: Cooper et al., (2007). J Psychopharmacology. 21, 405-413; *a-d*: Cooper et al., (2008). Prog Neuropsychopharmacol Biol Psychiatry. 32, 428-436; *a-e*: Murashita et al., (2007). Psychoneuroendocrinology. 32, 777-784; *a-f*: Baptista et al., (1999). Prog Neuropsychopharmacol Biol Psych. 23: 277-287; *a-g*: Lacruz et al., (2000). Mol Psych. 5:70-76; *a-h*: Baptista et al., (2002). Eur J Pharmacol. 447:91-98; *a-i*: Fell et al., (2007). J Psychopharmacology (Berl). 194, 221-231; *a-j*: Patil et al., (2006). Eur J Pharmacol. 551, 112-115; *a-k*: Fell et al., (2005). J Psychopharmacology (Berl). 182, 499, 507; *a-l*: Fell et al., (2004). Eur Neuropsychopharmacol. 14(5): 385-392; *a-m*: Fell et al., (2008). J Psychopharmacology. 22(2): 182-186; *b-a*: Lin et al., (2006). Neuropharmacology. 51(7-8): 1129-1136; *b-b*: Choi et al., (2007). Life Sci. 81(12): 1024-1030; *b-c*: Chintoh et al., (2008). Schizophr Res. 104(1-3): 23-30; *c-a*: Kalinichev et al., (2005). Psychopharmacology (Berl). 182(2): 220-231; *c-b*: Pouzet et al., (2003). Pharmacol Biochem Behav. 75(1): 133-140; *c-c*: Amamoto et al., (2006). J Pharmacol Sci. 102(2): 213-219; *c-d*: Minet-Ringuet et al., (2006). Appetite. 46(3): 254-262; *c-e*: Minet-Ringuet et al., (2007). Mol Psychiatry. 12(6): 562-571; *c-f*: Minet-Ringuet

et al., (2006). Psychopharmacology (Berl). 187(4): 447-454; *c-g*: Raskind et al., (2007). Neuropsychopharmacol. 32(2): 284-288; *c-h*: Kalinichez et al., (2006). Behav Pharmaol. 17(3): 289-292; *c-i*: Minet-Ringuet et al., (2005). Behav Brain Res. 163(2): 204-211; *c-j*: Assie et al., (2008). Eur J Pharmacol. 592(1-3): 160-166; *c-k*: Weston-Green et al., (2008). Int J Neuropsychopharmacol. 11(6): 827-835; *c-l*: Han et al., (2008). Psychoneuroendocrinology. 33(5): 569-580; *c-m*: Davoodi et al., (2009). Psychopharmacology (Berl). 203(4): 693-702; *c-n*: Sondhi et al., (2006). Pharmacogenomics J. 6(2): 131-140; *c-o*: Victoriano et al., (2009). Psychopharmacology (Berl). 206(1): 167-176; *c-p*: Park et al., (2008). J Psychopharmacology. 22(5): 522-531; *c-q*: Park et al., (2007). Life Sci. 80(26): 2428-2435; *d-a*: Houseknecht et al., (2007). Neuropsychopharmacol. 32(2): 289-297; *d-b*: Baptista et al., (2002). Brain Res. 957(1): 144-151; *d-c*: Baptista et al., (2004). Pro Neuropsychopharmacol Biol Psych. 28(8): 1305-1311; *d-d*: Tulipano et al., (2007). Neuroendocrinology. 85(2): 61-70; *d-e*: Ota et al., (2002). Clin Exp Pharmaol Physiol. 29(11): 980-989; *d-f*: Ota et al., (2005). Clin Exp Pharmacol Physiol. 32(4): 279-287; *d-g*: Chintoh et al., (2008). J Clin Psychopharmacol. 28(5): 494-499; *d-h:* Smith et al., (2008). Diabetologia. 51(12): 2309-2317; *d-i*: Chintoh et al., (2009). Schizophr Res. 108(1-3): 127-133; *e-a*: Arjona et al., (2004). Behav Brain Res. 152(1): 121-127; *e-b*: Smith et al., (2009). Schizophr Res. 115(1): 30-40; *e-c*: Lykkegaard et al., (2008). Schizophr Res. 103(1-3): 94-103

Houseknecht and colleagues [71] confirmed that the liver is the primary site of increased glucose output. The two SGA drugs clozapine and olanzapine caused acute elevations of glucose production which was measured using the HIEC. With this technique, exogenous glucose tracers were administered to observe changes in endogenous hepatic glucose production (for more information, see below –*Insulin resistance*; Table **1.3**). These results are consistent with those found by Chintoh and colleagues [72], utilizing a similar tracer-clamp technique, who demonstrated that olanzapine acutely induces HGO. An in-depth follow-up study by the same group on acute clozapine, olanzapine, risperidone, haloperidol and ziprasidone on HGO was carried out which illustrated that SGA drugs differ in their propensities to impair glucose production, whereby clozapine and olanzapine increase HGO, whereas ziprasidone decreases HGO [73]. These combined results demonstrate that rodent paradigms of APD treatment can accurately model glucose disturbances and provide novel insights into the biological pathways involved.

Table 1.3: *Summary of techniques utilized to evaluate antipsychotic drug-induced metabolic disturbances*

[   ]: Concentration, **HIEC/HGC**: Hyperinsulinemic-euglycemic/Hyperglycemic clamp, **GTT**: Glucose tolerance test, **ITT**: Insulin tolerance test, **HGO**: Hepatic glucose output, **ELISA**: Enzyme-linked immunosorbent assay, **RIA**: Radioimmunoassay, **HOMA-IR**: Homeostatic assessment of insulin resistance, **ISI**: Insulin sensitivity index, **FFAs**: Free fatty acids, **DEXA**: Dual X-Ray Absorptiometry, **CCA**: Chemical Carcass Analysis

Metabolic Parameter Investigated	Location	Tech. Utilized	Explanation	E.g. Ref.
[Glucose] & Glucose Intolerance	Peripheral	HIEC/HGC	In vivo method to determine infusion, clearance rates and concentrations of glucose (and insulin). Allows determination of HGO and acute glucose metabolism. Disadvantage: invasive procedure.	*a-a*
		GTT	Approximates glucose clearance through administration of a glucose load, followed by blood glucose readings (glucometer or other biochemical determination). Exists as oral, intravenous, intraperitoneal or subcutaneous. Disadvantage: Must limit stress.	*a-b*
		Reducing Methods	Changes the state of a metal ion while glucose is oxidized to estimate [glucose]. Disadvantage: cross reaction with a strong reducing agent	*a-c*
		Enzymatic Methods	Glucose oxidase reacts with beta-D-glucose, water and oxygen to produce hydrogen peroxide & gluconic acid (pH and temp dependent). Hydrogen peroxide produced then oxidizes a chromogen to estimate [glucose]. Exists as a home-test strip method. Disadvantage: Reducing substances can react; limitations exist for urine-glucose analysis.	*a-d*
	Hepatic	Pyruvate/ Glycerol tolerance test	Approximates HGO through administration of pyruvate/glycerol load, by acting as substrates for gluconeogenesis. Disadvantage: Need in conjunction with other tests to analyze specific molecule.	*a-e*
		Isotope Dilution	Stable isotope-labeled glucose molecules (or radioactive) are infused alone or under a specific protocol utilizing glucose and/or insulin to calculate HGO suppression and hepatic insulin resistance. Disadvantage: Expense	*a-f*
[Insulin] & Insulin Resistance	Peripheral & Pancreatic	ELISA	Detects analytes [insulin]. Enzyme-linked antigen. Disadvantage: Cross-reactivity.	*b-a*
		RIA	Detects analytes. Antigen labeled with isotope of iodine attached to tyrosine. Disadvantage: Radioactivity	*b-b*
		HIEC/HGC	In vivo method to determine infusion, clearance rates and concentrations of insulin. Allows determination of insulin secretion and insulin resistance. Disadvantage: invasive procedure	*b-c*
	Peripheral	HIEC/HGC	See above	*b-d*
		ITT	Measures glucose clearance (rate of decay) after bolus injection insulin. Data is then logarithmically transformed and the slope of the line is used to rank insulin sensitivity. Disadvantage: Hypoglycemic counter-regulation.	*b-e*
		Isotope Dilution	See above.	*b-f*
		HOMA-IR	Basal measurements of near steady [glucose] and [insulin] levels are used in a mathematical function. High values indicate high degree of insulin resistance. Disadvantage: Estimate does not provide site of insulin resistance.	*b-g*

Metabolic Parameter Investigated	Location	Tech. Utilized	Explanation	E.g. Ref.
		ISI	Mathematical transformation for detection of insulin resistance in non-fasting individuals in conjunction with GTT. The lower the IR value, the greater the insulin resistance.	b-h
[Glucagon]	Peripheral, target	Biochemical, RIA, ELISA	See above	c-a
[Ghrelin], [leptin] & [adiponectin]	Peripheral, target	Biochemical, RIA, ELISA	See above	d-a
[Triglycerides] [FFAs] & [Cholesterol]	Peripheral, target	Biochemical, RIA, ELISA	See above.	e-a
Adipose Tissue	Subcutaneous, visceral	Fat Pad removal	Invasive procedure to dissect WAT and BAT in subcutaneous and visceral regions. Disadvantage: Incomplete detection of total amount.	f-a
Body composition	Total	DEXA	Utilizes low exposure of radiation and computes absorption of radiation to determine body density (ratio of fat to muscle mass). Disadvantages: Expense	g-a
		CCA	Eviscerated carcass put through a series of analyses (such as drying, extraction and combustion) to determine fat mass and fat-free dry mass. Total ash-content determined	g-b

Reference Guide:

a-a: Chintoh et al., (2008). J Clin Psychopharmacol. 28(5): 494-499; *a-b*: Smith et al., (2008). Diabetologia. 51(12): 2309-2317; *a-c*: Kalinichev et al., (2005). Psychopharmacology. 182:220-231; *a-d*: Amamoto et al., (2006). J Pharmacol Sci. 102:213-219; *a-e*: Smith et al., (2008). Diabetologia. 51(12): 2309-2317; *a-f*: Chintoh et al., (2009). Schizophr Res. 108(1-3): 127-133; *b-a*: Fell et al., (2007). J Psychopharmacology (Berl). 194, 221-231; *b-b*: Cooper et al., (2007). Psychopharmacology. 21:405-413; *b-c*: Houseknecht et al., (2007). Neuropsychopharmacology. 32:289-297; *b-d*: Chintoh et al., (2008). J Clin Psychopharmacol. 28(5): 494-499; *b-e*: Smith et al., (2008). Diabetologia. 51(12): 2309-2317; *b-f*: Chintoh et al., (2009). Schizophr Res. 108(1-3): 127-133; *b-g*: Patil et al., (2006). Eur J Pharmacology. 551:112-115; *b-h*: Matsuda et al., (1999). Diab Care. 22:1462-1470; *c-a*: Lin et al., (2006). Neuropharmacology. 51:1129-1136; *d-a*: Murashita et al., (2007). Psychoneuroendocrinology. 32, 777-784; *e-a*: Ota et al., (2005). Clin Exp Pharmacol Physiol. 32: 279-287; *f-a*: Minet-Ringuet et al., (2006). Appetite. 46: 254-262; *g-a*: Kalinichev et al., (2005). Psychopharmacology. 182:220-231; *g-b*: Cope et al., (2005). Int J Obs. 29: 607-614

1.6.2 Insulin resistance

Insulin, the most important hormone that regulates glucose metabolism, can be modulated by APDs. Peripheral insulin resistance in APD-treated patients occurs when drug treatment changes insulin levels and/or when normal peripheral insulin levels fail to activate the insulin receptor, i.e. blunted insulin-mediated glucose uptake into peripheral cells.

The simplest and most commonly reported effects of APDs on insulin involve the single measurement of fasting peripheral insulin levels in blood (Tables **1.2** & **1.3**). Both fasting and non-fasting levels of insulin typically show either increases or no change, but rarely decreases with APDs [68, 74-76]. When insulin measurement is concurrently performed with sampling of blood glucose, the HOMA-IR can be applied. Generally, HOMA-IR values indicate insulin resistance after APD administration in rats [68]. In order to monitor the acute effects of APDs on insulin in more detail, several studies have used the insulin tolerance test [70, 77], whereby a bolus of insulin is administered and glucose levels are monitored over time.

Currently, the HIEC is best technique to measure direct *in vivo* changes of peripheral insulin sensitivity [67]. This procedure involves controlled infusion of insulin while glucose is concurrently infused to maintain euglycemia. Different infusion rates can be observed and calculated, such as the glucose infusion rate (GIR; a high GIR is indicative of tissues that are insulin sensitive). Usually, stable glucose tracers are also employed to allow calculation and differentiation of insulin-stimulated glucose production by the liver as well as changes in glucose catabolism [71]. Additional informative rates on glucose turnover, known as the endogenous glucose appearance (R_a) and glucose disappearance rates (R_d) are determined

and applied mathematically to a steady state formula [73]. A variation of this clamp procedure, known as the hyperglycemic clamp (HGC) allows for the determination of pancreatic β-cell secretion by monitoring the response of insulin to a sustained hyperglycemic state. Consistent decreases in the GIR, reflecting insulin resistance, have been reported by multiple groups, with greatest effects for clozapine and olanzapine, but also noted with risperidone and chlorpromazine [71-74, 78]. High HOMA-IR values have been shown to correlate well with increased hepatic insulin resistance (decreased GIR) using clamp studies [72, 73, 79].

1.6.3 Lipid and hormonal alterations

Triglycerides, FFAs and cholesterol are measured both *in vivo* and *in vitro* after treatment with APDs (Table **1.3**). However, unlike the reliable increases in glucose levels reported in the literature, there are notable inconsistencies in lipid concentrations in APD-treated rats (Table **1.2**), which may reflect, to some degree, the greater variability in drug-specific effects in humans. Kalinichev and colleagues [80] reported that chronic olanzapine administration increased FFAs in female rats, with no change in triglycerides, HDL-cholesterol or glycerol content. More commonly, though, chronic olanzapine was found to have no effect on free lipids or cholesterol levels [75, 81, 82]. In contrast, multiple research groups have found changes in adipose tissue content, whereby subcutaneous and visceral adipose depots proliferate after chronic APD administration [74, 76, 83-86]. These findings are supported by *in vitro* analyses on both adipokines (leptin, adiponectin), and enzyme modulation (fatty acid synthase) [76, 87].

Adipose tissue itself generates multiple influential hormones, such as leptin (involved with satiety and metabolism) and adiponectin (glucose regulation and FFA catabolism). Antipsychotic effects on changes in leptin secretion were also inconsistent, with either increases [85, 88], decreases [66, 81] or no change in levels [70]. While increases in leptin are widely reported in patients on APDs, there is strong evidence that this may be related to drug-induced weight gain, rather than direct effects of the drug *per se* [89]. As many rat models do not show weight gain (see below), interpretation of these data is challenging. Ghrelin, the "counter" regulatory hormone to leptin, was measured by Murashita and colleagues [90] in a recent study where increases in ghrelin, glucose and insulin levels were found after clozapine administration. Regarding adiponectin, Cooper and colleagues [76, 84] reported increased levels after olanzapine and clozapine in both female and male rats. It is worth recalling that lipids and related hormones in humans are potently modified by diet, and therefore studies of these indices in rats under controlled conditions represents a promising area for future study, particularly when more "realistic", higher-calorie diets are included as part of the protocol [70, 85].

1.6.4 Weight gain

Weight gain is the most commonly reported side-effect of SGA drugs in humans. However, this seemingly straightforward phenomenon has proven to be surprisingly difficult to model in rodents, and there are numerous conflicting studies on APD drug-induced weight gain in rats (Table **1.2**). Raskind and colleagues [91] treated rats chronically with the high metabolic risk APD olanzapine and observed an 18% increase in body weight. Recent pharmacological

experiments by Kirk and colleagues indicate that olanzapine-induced weight gain may be mediated through 5-HT_{2C} receptor antagonism, in the presence of a D_2 receptor antagonist [92]. The majority of studies that report weight gain with olanzapine treatment involve the use of female rats [81, 93-96], which may be related to differences in hormones and drug pharmacokinetics [97]. Some studies have reported that SGA drugs can cause a decrease in weight gain over time [70, 76, 77, 84], while others note no effect of APD on body weight but a change in adiposity, i.e. fat as a proportion of body weight. Several studies have used more sophisticated *in vivo* techniques to measure longitudinal changes in body composition during chronic treatment with APDs, including dual energy x-ray absorptiometry [70, 77] (Table **1.3**). It is worth noting that several research groups have independently provided evidence that APD-induced glucose intolerance and insulin resistance can occur in the absence of weight gain [65, 77, 79, 85].

The nature of animals' diet can affect weight gain. Humans treated with SGA drugs report increased craving for palatable foods [98]. Rodents may be less motivated to consume increased amounts of standard chow than more palatable foods [99]. However, Smith and colleagues noted that while rats treated chronically with clozapine or quetiapine showed an increased preference for a high fat/high sugar diet, they did not exhibit greater weight gain than diet-matched controls [70]. The role of locomotor activity in APD-induced weight gain remains largely unexplored in rodent models: human patients treated with APDs exercise less than normals [100]. Future preclinical studies may benefit from including more active groups, as the sedating effects of APDs on weight gain [101] are likely under-evaluated using standard housing conditions.

1.6.5 Blood pressure

Cardiovascular adverse events observed in humans treated with SGA drugs include postural hypotension, ECG alterations, tachycardia, and drug-dependent hypertension [102]. There are few studies on the effects of APDs on blood pressure in rodents. Patil and colleagues [75] reported that olanzapine increased body weight, insulin resistance, as well as systolic blood pressure in female rats. More preclinical studies are needed to evaluate the effects of APDs on blood pressure.

1.7 Overview, objective and aims

Recognizing the high prevalence of SGA drug-induced metabolic side-effects and their impact on patient quality of life and medication adherence, numerous research groups are committed to using animal models to study the biological basis of such side-effects. The time is therefore appropriate for a critical evaluation of the metabolic side-effects of SGA drugs, and investigation of factors that cause impairments in glucose metabolism. Thus, the main objective of the current thesis will focus on evaluating the predictive validity of an established rodent paradigm and determine the effects of interventional therapy on SGA drug-induced glucose dysregulation and insulin resistance.

Aim 1 (Chapter 2): To validate the predictive validity of the rodent paradigm on antipsychotic drug-induced metabolic side-effects. We will assess the acute effects of APD-induced glucose dysregulation and insulin resistance with i) six different APDs at multiple

doses, ii) APD combinational therapy (antipsychotic polypharmacy), iii) and the chronic, longitudinal effects of olanzapine on multiple metabolic indices.

Aim 2 (Chapter 3): Using our animal model, we will determine the effects of pharmacological intervention (four different classes of antidiabetic drug treatments) on olanzapine-induced glucose dysregulation and insulin resistance.

Aim 3 (Chapter 4): To ascertain the effects of non-pharmacological intervention (routine exercise treatment) on olanzpine-induced metabolic side-effects.

1.8 Specific hypotheses

Chapter 2:

Specific hypothesis 1) Using standard *in vivo* techniques and an acute-based timeline, animals treated with the SGA drugs (specifically clozapine and olanzapine) will exhibit significant differences in metabolic indices, including hyperglycemia, hyperinsulinemia, glucose intolerance, and insulin resistance compared to vehicle treated animals. These effects will be substantially time- and dose-dependent. By contrast, animals treated with the APDs of 'lower metabolic risk' (risperidone, haloperidol) will exhibit milder metabolic changes than vehicle-treated animals.

Specific hypothesis 2) Predictive validity of the current animal paradigm will be tested with two novel SGA drugs, asenapine and iloperidone. Acute administration of asenapine will be

associated with negligible glucose intolerance and insulin resistance, compared to vehicle treated animals. By contrast, animals that receive acute iloperidone treatment will exhibit substantial glucose intolerance and insulin resistance, as measured by the IGTT and HIEC. A positive control (olanzapine) will be compared at the end of the study to confirm drug-induced effects in the animal model.

Specific hypothesis 3) The acute effects of antipsychotic drug combinations (risperidone and clozapine; haloperidol and clozapine) will be evaluated using the intraperitoneal glucose tolerance test and the homeostatic model assessment of insulin resistance in the present animal model. Administration of risperidone or haloperidol alone will be associated with minimal effects in glucose intolerance and insulin resistance in female rats, while clozapine treatment will exhibit significant impairments in glucose tolerance and insulin sensitivity compared to vehicle-treated animals. Concurrent administrations of either risperidone and clozapine, or haloperidol and clozapine, will cause greater impairments in metabolic indices than single treatment with any of the antipsychotic drug treatments.

Specific hypothesis 4) Chronic administration of olanzapine to female rats will cause glucose intolerance and insulin resistance that will remain stable throughout the course of 10 weeks. Animals that receive intermittent olanzapine treatment (once a week) will also exhibit significant glucose dysregulation, which will increase with the passage of time. Animals that receive olanzapine treatment will exhibit increased visceral adiposity but little overall body weight gain.

Chapter 3:

Specific hypothesis 1) Pretreatment with antidiabetic drugs (metformin and rosiglitazone) will improve peripheral glucose tolerance and insulin sensitivity associated with olanzapine, when compared to control animals. The antidiabetic drug glyburide will increase plasma insulin levels, however, no significant reductions in glucose will be observed during the glucose tolerance test.

Specific hypothesis 2) During an acute IGTT, the GLP-1 receptor agonist (exenatide) will cause a dose-response reduction in post-prandial glucose levels in olanzapine-treated rats, compared to controls.

Chapter 4:

Specific hypothesis 1) Non-pharmacological treatment (chronic exercise) will improve, but not fully reverse, olanzapine-induced glucose intolerance over the course of 10 weeks. Exercised animals will consume more rat chow when compared to sedentary rats, whereas there will be no significant difference in body weights of exercised vs sedentary olanzapine-treated or vehicle-treated animals.

Chapter 2: Evaluating the predictive validity of the rodent paradigm on antipsychotic drug-induced metabolic side-effects

Several versions of this chapter have been published as:

Boyda, H.N., Tse, L., Procyshyn, R.M., Wong, D., Wu, T.K., Pang, C.C., Barr, A.M. (2010). A parametric analysis of the acute effects of antipsychotic drugs on glucose sensitivity in an animal model. Prog Neuropsychopharmacol Biol Psychiatry. Aug 16; 34(6): 945-54.

Boyda, H.N., Procyshyn, R.M., Pang, C.C., Hawkes, E., Wong, D., Jin, C.H., Honer, W.G., Barr, A.M. (2013). Metabolic side-effects of the novel second-generation antipsychotic drugs asenapine and iloperidone: a comparison with olanzapine. PLoS One. 8(1): e53459.

Boyda, H.N., Procyshyn, R.M., Tse, L., Wong, D., Pang, C.C., Honer, W.G., Barr, A.M. (2012). Intermittent treatment with olanzapine causes sensitization of the metabolic side-effects in rats. Neuropharmacology. Mar;62(3): 1391-400.

2.1 Study 1: A parametric study of the acute effects of antipsychotic drugs on glucose sensitivity in an animal model

2.1.1 Overview

The purpose of the present series of experiments was to conduct a comprehensive study to date of the acute effects of antipsychotic drugs on glucose tolerance and insulin resistance in a rat model, using multiple supporting indices of both measures. We have directly compared the effects of two atypical drugs with a higher risk for metabolic side effects (clozapine and olanzapine), an atypical drug with lower risk of metabolic side-effects (risperidone) and a common typical antipsychotic drug with low risk of metabolic side effects (haloperidol). In order to verify the acute nature of metabolic effects, high or low doses of all drugs were administered to naïve animals, and effects on glucose tolerance and insulin resistance were assessed at three different time points (1, 3 or 6 hours) after drug administration.

We hypothesized that animals exposed to the higher metabolic risk APDs (clozapine and olanzapine) would exhibit substantial hyperglycemia, glucose intolerance and insulin resistance over the first 60 min, and these effects will become substantially less at 180 and 360 min, compared to vehicle treated animals. The lower risk metabolic APDs (risperidone and haloperidol) will show a mild increase in metabolic indices, compared to the control cohort.

2.1.2 Materials & methods

Animals

Adult, nulliparous female Sprague-Dawley rats (225-250 g) were purchased from an animal supplier (Charles River, Montreal, Canada) and allowed to habituate to the UBC colony for one week before experiments commenced. Rats were group-housed in groups of 3-4 and given *ad libitum* access to food and water; all animals were maintained on a 12-hour light-dark cycle (lights on at 0700 hours) in a temperature controlled colony at 22 ± 1ºC. Experimental procedures were conducted during the light cycle, and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Approval by the University of British Columbia's Animal Care and Use Committee was established for all methods.

Pharmaceutical agents and solutions

Doses of antipsychotic drugs were chosen to represent pharmacologically relevant levels *in vivo*, and therefore based on previously reported behavioral studies in rats, such as reversal of prepulse inhibition (PPI) deficits of the acoustic startle reflex. Two doses were chosen for each drug, with the higher dose representing the upper limit commonly seen in dosing for behavioral studies and the lower dose an order of five to tenfold lower (see Appendices 1 and 2 of reference [103] for a summary of drug doses used in PPI studies). Doses for the present study included clozapine (2 mg/kg; 20 mg/kg), olanzapine (1.5 mg/kg; 15 mg/kg), risperidone (0.5 mg/kg; 2.5 mg/kg) [purchased from Toronto Research Chemicals Inc, Toronto, ON, Canada] and haloperidol (0.1 mg/kg; 1.0 mg/kg) [purchased from Sigma

Aldrich, St. Louis, MO]; sedation effects were only evident in rats treated with the higher dose of haloperidol. Dosing solutions were prepared fresh daily: clozapine, olanzapine and risperidone were formulated in a vehicle composed of 50% polyethylene glycol 400, 40% distilled water and 10% ethanol. Haloperidol was formulated in a vehicle of 0.3% tartaric acid. All other chemicals were commercially available and of reagent grade. Each rat received a 1 ml/kg intraperitoneal (i.p.) injection of the vehicle control or antipsychotic formulation. The results of the studies indicated no differences between the two types of vehicle on glucose tolerance or insulin levels.

Intraperitoneal glucose tolerance test: (see Fig. **2.1** for representation of sequence of events) Animals were randomly assigned to one of the three treatment groups (vehicle, low dose or high dose antipsychotic [n=8 per group]): each animal was only tested once. Rats were fasted overnight for 16 ± 2 hours. On the morning of testing, rats were transferred to the laboratory, weighed and allowed to rest for approximately 15-20 min. A baseline blood glucose level measurement was then taken from the hind leg using a 25-guage needle to procure a drop of venous blood, which was measured by a glucometer (One Touch Ultra 2). For all blood draws, animals were wrapped in a towel and the hind leg was exposed; this technique minimizes stress to the animals and so no anesthesia is required [104].

The antipsychotic drug or vehicle was then administered acutely as a single i.p. injection, and separate cohorts of rats were tested either 60, 180 or 360 min after injection. At the appropriate time after injection, a second measurement of glucose levels was taken, to assess the effects of drug treatment on fasting glucose levels. Immediately afterwards, a saphenous blood draw (200 µl) using heparinized collecting tubes, was performed to obtain

plasma for measurement of insulin levels; blood samples were centrifuged (10,000 RPM, 10 Min, 4ºC) and plasma samples were stored at -80ºC for future analysis. The intraperitoneal glucose tolerance test (IGTT) commenced approximately 5 min after saphenous blood draws, as all rats were given a challenge i.p injection of 1 g/ml/kg of glucose. Glucose levels were then measured every 15 min for 120 min duration. Each animal handler was blinded to respected drug treatment. Analysis of results for haloperidol-treated animals indicated that there was no effect of the drug by 180 minutes on the IGTT, and so the 360 min group was not run.

In order to measure changes in insulin levels and insulin resistance throughout the entire duration of the IGTT, a separate cohort of two groups of rats (n = 8 per group) was treated with either vehicle or the high dose of olanzapine (15 mg/kg, i.p.); olanzapine was chosen as a representative drug due to its large effects in the first series of experiments and its widespread use clinically. One hour after drug treatment, an initial saphenous blood draw was carried out, and then every 30 min from the challenge glucose injection throughout the 120 min IGTT. Plasma samples were collected and stored in -80℃ for future analysis of insulin levels.
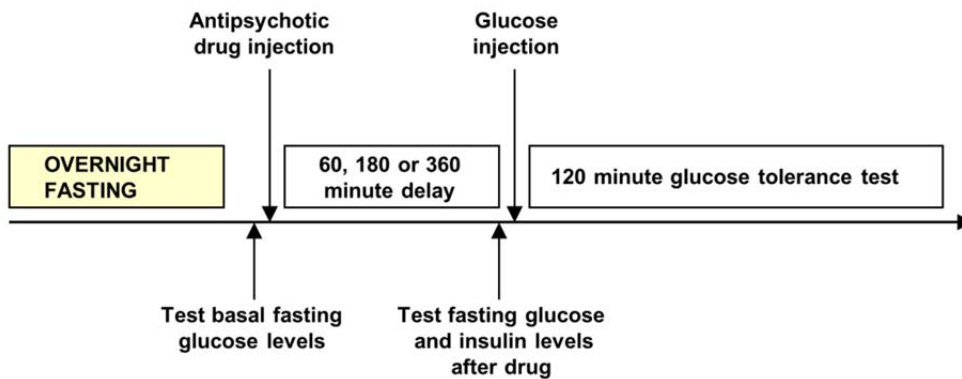
Fig. 2.1: *Experimental protocol describing the intraperitoneal glucose tolerance test with acute antipsychotic drug treatment.*

Insulin measurement

Plasma levels of insulin were measured using Enzyme-Linked ImmunoSorbent Assay (ELISA). Plasma samples were thawed and assayed for insulin using the Rat Insulin 96-well ELISA kit (Mercodia, Uppsala, Sweden). Briefly, 50 µl plasma samples were added and analyzed, in duplicate, on each plate according to the specific time points studied. Samples were incubated at room temperature for two hours followed by repeated washes. Substrate was added for 15 min and absorbance was measured at 450 nm. Calibrators provided with the kit were prepared and used to generate a calibration curve to interpolate sample data values. In addition, a reference (non-fasted) animal's plasma was added to all plates to serve as a reference standard; this confirmed a high intra-plate reliability, with the mean run-to-run correlation of 0.95 (range 0.92-0.99).

Insulin resistance

To determine whether acute treatment with antipsychotic drugs created a state of insulin resistance, the homeostatic model assessment of insulin resistance (HOMA-IR) was utilized for fasting measurements. This technique has been previously validated as a measure of insulin resistance in pregnant female Sprague-Dawley rats [105]. The product of the converted fasting insulin values (expressed as µU/mL) and glucose (mmol/L) are divided by a constant of 22.5 as shown in equation (1). The larger the calculated HOMA-IR value, the greater the insulin resistance.

(1)

$$(I_0 \times G_0) \div 22.5$$

where G_0 and I_0 are fasting glycemia and insulinemia.

For non-fasting measurement of insulin resistance (i.e., after injection of glucose during the IGTT), insulin resistance was calculated according to the Insulin Sensitivity Index (ISI) method as devised by [106], as shown in equation (2). This index has been validated for use in glucose tolerance tests with 30 min interval measurements of glucose and insulin: the lower the IR value, the greater the insulin resistance.

(2)

$$\left(10^4\right) \div \left(\left(G_0 \times I_0 \times G_m \times I_m\right)^{1/2}\right)$$

where G_0 and I_0 are fasting glycemia and insulinemia, and G_m and I_m. are mean glycemia and insulinemia taken during an IGTT.

Statistical analysis

Group differences between glucose and insulin levels taken after drug, but before glucose injection, were analyzed by one-way ANOVA, with alpha value set at $p < 0.05$. To measure changes in glucose levels throughout the IGTT, as well as insulin levels in the small, separate cohort, data were analyzed by repeated measures ANOVA, with group treatment as the between subject factor and time as the within subject factor. All significant main or interactive effects were analyzed with *post hoc* LSD tests (SPSS software, Chicago, IL).

2.1.3 Results

Post-drug basal glucose levels:

By measuring glucose levels in fasting rats at either 60, 180 or 360 minafter they had received the antipsychotic drug, but before the injection of glucose for the IGTT, it was possible to determine whether the antipsychotic could affect fasting basal levels of glucose. Analysis of the data for clozapine indicated that there was a significant main effect of drug treatment on basal glucose levels at 60 [$F_{(2,21)} = 3.86$; $p < 0.05$], 120 [$F_{(2,21)} = 8.32$; $p = 0.001$] and 360 min [$F_{(2,21)} = 6.30$; $p = 0.005$] after drug treatment. Glucose levels were significantly higher in the low-dose clozapine group than vehicle-treated animals ($p < 0.05$) at the 60 min time point, whereas glucose levels were higher in both the high-dose ($p < 0.001$) and low-dose clozapine groups ($p < 0.05$) compared to vehicle at the 180 min time point (Fig. (**2.2**); Table **2.1**); at 360 min, glucose levels were only greater than vehicle-treated animals in the high-dose clozapine group ($p = 0.001$).
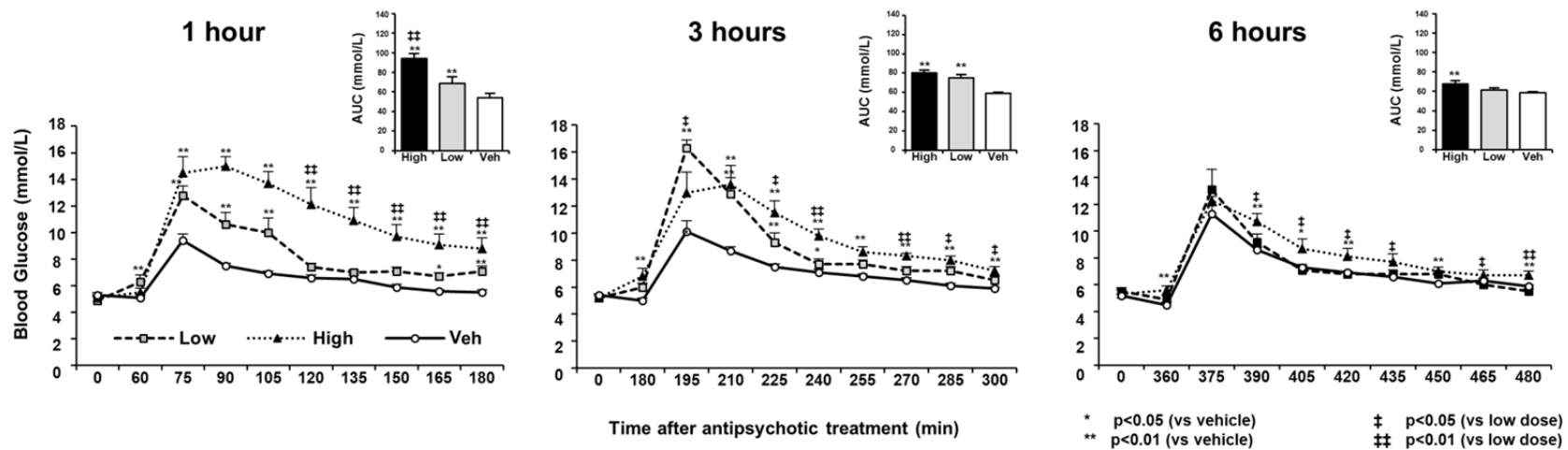
Fig. 2.2: *The acute effects of the atypical antipsychotic drug clozapine on glucose levels in adult female rats.* Separate cohorts of animals (n=8 per group) were treated with vehicle, low (2 mg/kg) or high (20 mg/kg) doses of clozapine. Glucose levels were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then either 60, 180 or 360 min following drug administration (x-axis on all graphs). Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2 hours. Total cumulative glucose levels for each treatment group are summed as the "area under the curve" during the glucose tolerance test by graph insets (top right of each graph). Values represent group mean ± SEM.

Treatment		I_0 (μU/ml)	G_0 (mmol/L)	HOMA-IR
Clozapine				
1 hour	vehicle	6.4±0.3	5.1±0.2	1.5±0.1
	low	9.3±1.2	6.3±0.5 **	2.6±0.3
	high	13.3±3.6 **	5.5±0.3	3.5±1.1 **
3 hour	vehicle	7.8±0.3	5.0±0.2	1.7±0.1
	low	8.6±1.3	6.0±0.3 **	2.3±0.4
	high	10.2±1.2 *	6.2±0.2 **	2.9±0.4 **
6 hour	vehicle	7.7±0.3	4.5±0.2	1.5±0.1
	low	9.2±1.6	5.1±0.3	2.1±0.4
	high	11.4±1.6 **	5.6±0.3 **	2.8±0.4 **
Olanzapine				
1 hour	vehicle	6.9±0.5	4.8±0.2	1.6±0.2
	low	7.3±0.4 ‡	4.5±0.1 ‡‡	1.4±0.1 ‡
	high	19.2±5.8*	6.8±1.0 *	6.4±2.4 *
3 hour	vehicle	9.5±1.9	4.4±0.1	1.8±0.4
	low	8.6±1.0	5.9±0.4 *	2.2±0.2
	high	11.5±1.7	6.4±0.6 **	3.5±0.8 *
6 hour	vehicle	5.7±0.5	6.0±0.7	1.5±0.2
	low	7.0±1.2	7.0±1.7	2.3±0.9
	high	10.5±2.1 *	7.6±1.4	3.4±0.6 *
Risperidone				
1 hour	vehicle	8.0±0.3	4.8±0.4	1.9±0.1
	low	20.6±5.3 *	5.2±0.3	4.8±1.4 *
	high	18.3±2.7 *	6.1±0.4 *	5.1±1.0 *
3 hour	vehicle	8.2±1.1	4.3±0.2	1.6±0.3
	low	9.4±0.5 ‡‡	6.0±0.6 **	2.5±0.2 ‡‡
	high	17.4±2.4 **	5.4±0.3	4.3±0.7 **
6 hour	vehicle	5.3±0.3	4.7±0.2	1.1±0.0
	low	6.8±0.7 ‡‡	5.9±0.4 **	1.8±0.2 ‡‡
	high	13.6±2.0 **	5.3±0.1	3.2±0.5 **
Haloperidol				
1 hour	vehicle	8.5±1.3	5.2±0.1	2.2±0.3
	low	8.8±0.7	7.0±0.4 **‡‡	2.7±0.2 *
	high	9.7±0.7	4.7±0.1	2.0±0.2
3 hour	vehicle	7.9±0.5	5.4±0.4	1.9±0.2
	low	8.8±1.0	4.8±0.2	1.8±0.2
	high	11.0±1.2 *	5.0±0.2	2.5±0.3

Table 2.1: *Fasting insulin, glucose levels and HOMA-IR values in drug treated rats.*

I_0 = fasting insulin levels; G_0 = fasting glucose levels; HOMA-IR = homeostasis model assessment of insulin resistance. Rats were treated with vehicle, low or high dose drug: clozapine (2 mg/kg; 20 mg/kg), olanzapine (1.5 mg/kg; 15 mg/kg), risperidone (0.5 mg/kg; 2.5 mg/kg) and haloperidol (0.1 mg/kg; 1.0 mg/kg).
Values represented as means ± SEM.
   * $p < 0.05$ (vs vehicle)
   ** $p < 0.01$ (vs vehicle)
   ‡ $p < 0.05$ (vs high dose drug)
   ‡‡ $p < 0.01$ (vs high dose drug)

For olanzapine-treated rats, there was a significant main effect of drug treatment at 60 min [$F_{(2,21)} = 4.94$; $p < 0.05$], and 180 min [$F_{(2,21)} = 6.42$; $p < 0.01$] after injection, but not at the 360 min time point [$F_{(2,21)} = 0.43$; NS]. Post-hoc analysis revealed that glucose levels were significantly higher in the high-dose olanzapine group compared to both the low-dose ($p < 0.01$) and vehicle-treated rats ($p < 0.05$) at 60 min (Fig. (**2.3**); Table **2.1**). At 120 min after treatment, glucose levels were higher in both the high-dose ($p < 0.005$) and low-dose ($p < 0.05$) olanzapine groups compared to vehicle-treated rats.
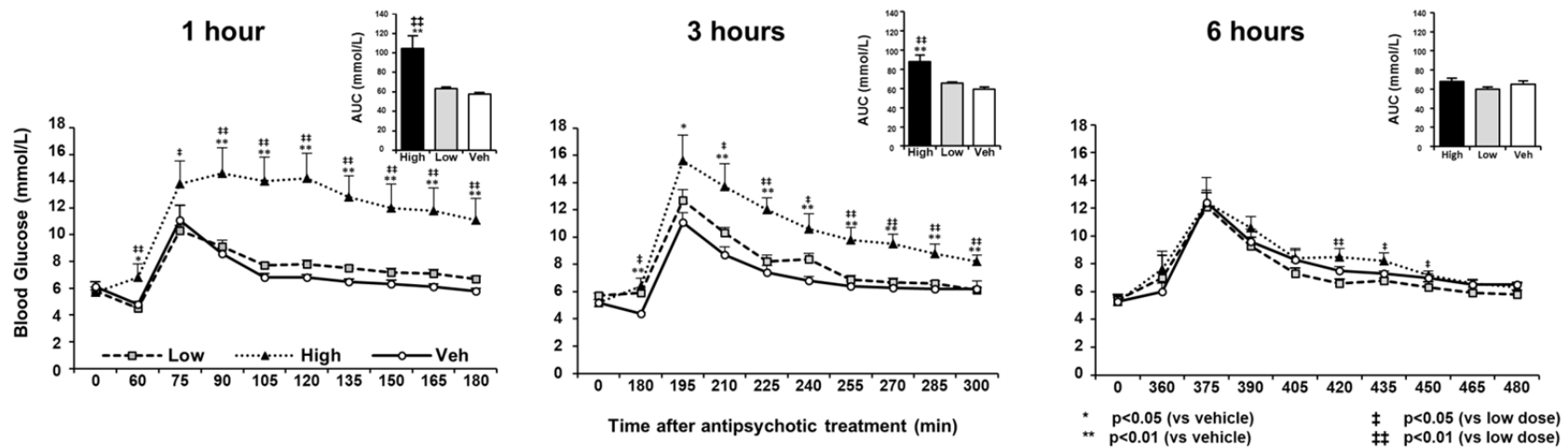
Fig. 2.3: *The acute effects of the atypical antipsychotic drug olanzapine on glucose levels in adult female rats.*
Separate cohorts of animals (n=8 per group) were treated with vehicle, low (1.5 mg/kg) or high (15 mg/kg) doses of olanzapine. Glucose levels were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then either 60, 180 or 360 min following drug administration (x-axis on all graphs). Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2 hours. Total cumulative glucose levels for each treatment group are summed as the "area under the curve" during the glucose tolerance test by graph insets (top right of each graph). Values represent group mean ± SEM.

Analysis of the effects of risperidone indicated that there was a significant main effect of drug treatment on basal glucose levels at 60 [$F_{(2,21)}$ = 4.95; $p < 0.05$], 120 [$F_{(2,21)}$ = 4.49; $p < 0.05$] and 360 min [$F_{(2,21)}$ = 6.01; $p < 0.01$] after drug treatment. At the earliest time point of 60 minafter injection, glucose levels were higher in the high-dose risperidone group than either the low-dose ($p < 0.05$) or vehicle ($p < 0.01$) groups (Fig. (**2.4**); Table **2.1**). By 180 min, the low-dose group had higher glucose levels than vehicle-treated animals ($p < 0.01$) but the difference between high-dose risperidone and vehicle groups was only marginally significant ($p = 0.07$); it should be noted, though, that pre-drug glucose levels were significantly higher in the low-dose drug group compared to the other two groups. At 360 min, a similar pattern of results occurred as with the 180 mincohort, with significantly greater glucose levels in the low-dose drug group compared to vehicle ($p < 0.005$) but only a marginal increase in the high-dose drug group ($p = 0.10$).
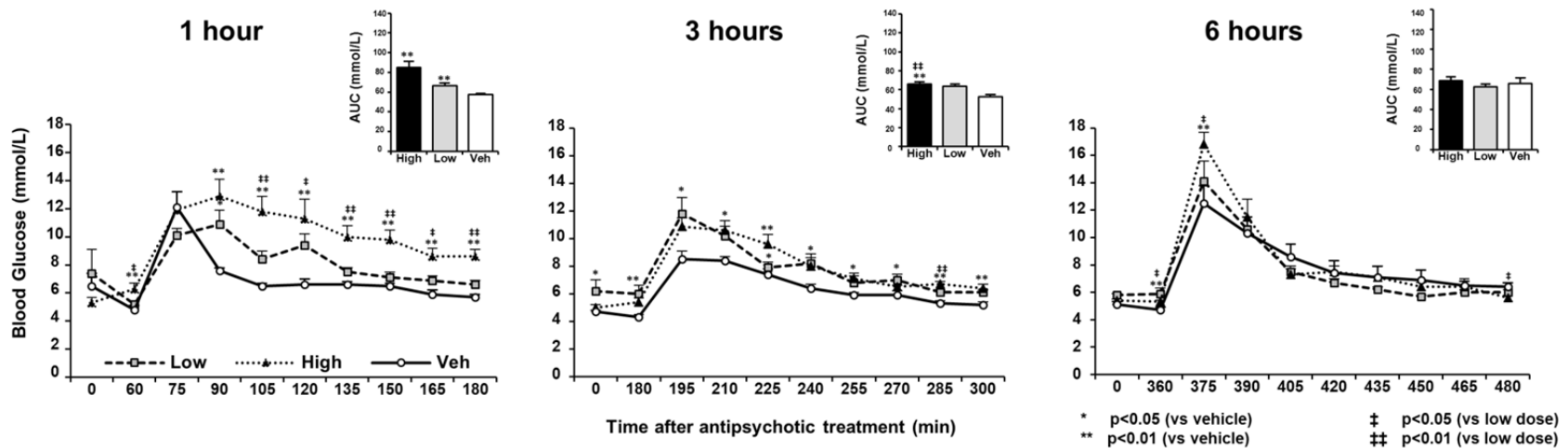
Fig. 2.4: *The acute effects of the atypical antipsychotic drug risperidone on glucose levels in adult female rats.*
Separate cohorts of animals (n=8 per group) were treated with vehicle, low (0.5 mg/kg) or high (2.5 mg/kg) doses of risperidone. Glucose levels were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then either 60, 180 or 360 min following drug administration (x-axis on all graphs). Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2 hours. Total cumulative glucose levels for each treatment group are summed as the "area under the curve" during the glucose tolerance test by graph insets (top right of each graph). Values represent group mean ± SEM

Interestingly, there was a strong main effect of drug treatment on basal glucose levels in haloperidol-treated animals [$F_{(2,21)}$ = 28.01; $p < 0.0001$] at 60 minutes, although no effect by 180 minutes [$F_{(2,21)}$ = 1.34; NS]. Post-hoc analysis revealed that treatment with the low-dose of haloperidol caused a large increase in basal glucose levels compared to both other groups ($p < 0.001$) (Fig. (**2.5**); Table **2.1**).
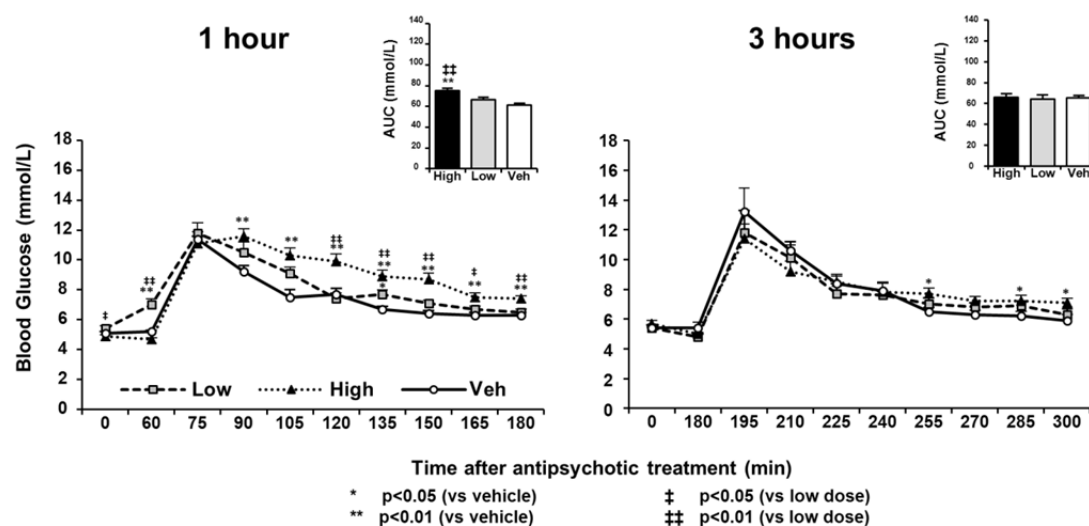
Fig. 2.5: *The acute effects of the typical antipsychotic drug haloperidol on glucose levels in adult female rats.*
Separate cohorts of animals (n=8 per group) were treated with vehicle, low (0.1 mg/kg) or high (1.0 mg/kg) doses of haloperidol. Glucose levels were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then either 60, 180 or 360 min following drug administration (x-axis on all graphs). Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2 hours. Total cumulative glucose levels for each treatment group are summed as the "area under the curve" during the glucose tolerance test by graph insets (top right of each graph). Values represent group mean ± SEM.

Post-drug insulin levels:

As described above, plasma samples were obtained immediately after the glucose tests performed 60, 180 or 360 min following injection (but before glucose injection), and insulin levels were measured by ELISA.

Clozapine treatment significantly increased fasting insulin levels at 60 [$F_{(2,21)} = 5.10$; $p < 0.05$] and 360 min [$F_{(2,21)} = 4.23$; $p < 0.05$] after injection, with a non-significant trend at 180 min [$F_{(2,21)} = 2.73$; $p = 0.08$]. At all time points, insulin levels were significantly higher in the high-dose clozapine group than the vehicle group ($0.001 < p < 0.05$) (Table **2.1**). Treatment with olanzapine was also associated with a main effect of drug treatment at 60 [$F_{(2,21)} = 4.90$; $p < 0.05$] and 360 min [$F_{(2,21)} = 3.60$; $p < 0.05$], but no effect at 180 min [$F_{(2,21)} = 0.98$; NS]. Post-hoc analysis indicated that at the two significant time points, insulin levels were higher in the high-dose olanzapine group than the vehicle group ($p < 0.05$), and also higher than the low-dose olanzapine group at 60 min ($p < 0.05$) (Table **2.1**). Analysis of the effects of risperidone revealed a significant main effect of drug treatment at 60 [$F_{(2,21)} = 4.19$; $p < 0.05$], 180 [$F_{(2,21)} = 12.26$; $p < 0.0001$] and 360 min [$F_{(2,21)} = 14.26$; $p < 0.0001$]. At 60 min, both doses of risperidone were associated with higher insulin levels than vehicle ($p < 0.05$), while at 180 and 360 min, the high-dose risperidone group had substantially greater levels of insulin than either of the two other groups ($p < 0.001$) (Table **2.1**). There was no significant main effect of haloperidol on insulin levels at either 60 [$F_{(2,21)} = 0.47$; NS] or 180 min [$F_{(2,21)} = 3.40$; $p = 0.06$], although there was a strong trend at the latter time, reflecting higher insulin levels in the high-dose group compared to the vehicle group ($p < 0.05$) (Table **2.1**).

HOMA-IR values:

Insulin resistance was calculated using the HOMA-IR equation, as described above. The values were calculated from the glucose and insulin levels described above, i.e. at either 60, 180 or 360 min after antipsychotic drug treatment, but before glucose injection with the IGTT.

There was a significant main effect of clozapine treatment on HOMA-IR values at 60 [$F_{(2,21)} = 4.98$; $p < 0.05$], 180 [$F_{(2,21)} = 6.05$; $p < 0.01$] and 360 min [$F_{(2,21)} = 8.23$; $p < 0.005$]. Post-hoc analysis revealed that at all time points, HOMA-IR values were greater in the high-dose clozapine group compared to vehicle-treated animals ($0.001 < p < 0.005$) (Table **2.1**). Analysis of the effects of olanzapine on insulin resistance indicated a significant main effect of drug treatment at 60 min [$F_{(2,21)} = 4.56$; $p < 0.05$], with a non-significant trend at 180 minutes [$F_{(2,21)} = 3.00$; $p = 0.07$], and no effect at 360 min [$F_{(2,21)} = 2.34$; NS]; the high dose olanzapine group exhibited higher HOMA-IR values compared to both vehicle and low-dose olanzapine treated rats at 60 min ($p < 0.05$), while high-dose treated rats had higher values than vehicle treated animals ($p < 0.05$) at 180 min (Table **2.1**). For the risperidone treated rats, the ANOVA indicated a significant main effect of drug treatment at 60 [$F_{(2,21)} = 3.89$; $p < 0.05$], 180 [$F_{(2,21)} = 10.15$; $p < 0.001$] and 360 min [$F_{(2,21)} = 13.04$; $p < 0.0001$]. By the 60 mintime point, both doses of risperidone resulted in higher HOMA-IR values than vehicle ($p < 0.05$). At both 180 minutes and 360 min post treatment, the high dose risperidone group had greater HOMA-IR values than either of the other two groups ($0.0001 < p < 0.005$) (Table **2.1**). Finally, there was no significant main effect of drug treatment with haloperidol on HOMA-IR values at either 60 [$F_{(2,21)} = 3.00$; $p = 0.07$] or 180 min [$F_{(2,21)} = 2.51$; NS].

Intraperitoneal glucose tolerance test:

Analysis of data from the IGTT was performed by repeated-measures ANOVA, starting with the first measurement of glucose levels after the glucose injection, through to the end of the test (Figs. (**2.2-2.5**)). Due to the pattern of glucose levels in the IGTT, which shows a consistent rapid increase followed by a delayed decrease, the main effect of time was highly significant in all analyses ($p < 0.0001$), and has therefore not been included for brevity. To clarify the main effect of drug treatment across the entire IGTT, the total area under the curve has also been presented (Figs. (**2.2-2.5**), *inset figures, top right of each graph*).

For clozapine, there were significant main effects of drug treatment [$F_{(2,21)} = 52.29$; $p < 0.0001$] and a drug × time interaction [$F_{(14,147)} = 4.49$; $p < 0.0001$] on glucose levels at 60 min. Similarly strong effects of drug treatment [$F_{(2,21)} = 24.66$; $p < 0.0001$] and drug × time interaction [$F_{(14,147)} = 7.14$; $p < 0.0001$] were observed 180 min post-drug. At 360 min post-drug, the main effect of drug treatment remained significant [$F_{(2,21)} = 5.06$; $p < 0.05$], but the drug × time interaction was only marginally significant [$F_{(14,147)} = 7.14$; $p = 0.058$]. Post-hoc analysis indicated that glucose levels in the high-dose clozapine group remained higher than vehicle subjects at virtually all time points during the IGTT, and at all three periods after drug administration (Fig. (**2.2**)). By contrast, glucose levels from the low-dose clozapine group were higher than in vehicle rats, but only at 60 and 180 min after drug administration. Glucose levels were also generally higher in the high-dose compared to the low-dose groups at both 60 and 180 min post-drug.

There was a strong main effect of olanzapine drug treatment on glucose levels at 60 min [$F_{(2,21)} = 10.58$; $p = 0.001$] and a drug × time interaction [$F_{(14,147)} = 3.30$; $p < 0.0001$]. By 180 min, drug [$F_{(2,21)} = 13.90$; $p < 0.0001$] effects remained significant, but the interaction

between drug and time was not [$F_{(14,147)} = 0.88$; NS]. At 360 min after drug treatment, the effect of drug treatment was no longer significant [$F_{(2,21)} = 1.80$; NS], nor was there a drug × time interaction [$F_{(14,147)} = 0.34$; NS]. Post-hoc analysis revealed that glucose levels were significantly higher at most time points for the high dose olanzapine group compared to both other groups at the 60 and 180 min periods (Fig. (**2.3**)).

Analysis of the data in risperidone-treated animals indicated a significant main effect of drug treatment [$F_{(2,21)} = 12.42$; $p < 0.0001$] and a drug × time interaction [$F_{(14,147)} = 3.43$; $p < 0.0001$]. There was also a strongly significant main effect of drug treatment at 180 min post treatment [$F_{(2,21)} = 11.45$; $p = 0.001$], although the interaction with time was not significant [$F_{(14,147)} = 1.43$; NS]. By 360 min, the drug effect was no longer significant [$F_{(2,21)} = 0.47$; NS], although there was a drug × time interaction [$F_{(14,147)} = 2.44$; $p < 0.005$]. The post-hoc analysis revealed that the high-dose risperidone rats exhibited significantly higher levels of glucose during the IGTT than vehicle animals in both 60 and 180 min treatment groups, and higher levels than the low-dose group at 60 min (Fig. (**2.4**)).

Of particular interest, treatment with haloperidol resulted in a significant main effect of drug treatment on glucose levels at 60 min post-injection [$F_{(2,21)} = 11.55$; $p < 0.0001$] as well as a drug × time interaction [$F_{(14,147)} = 2.93$; $p < 0.005$]. Both the drug [$F_{(2,21)} = 0.09$; NS] and interaction [$F_{(14,147)} = 1.24$; NS] were no longer significant at 180 min. The effect at 60 min was due to greater glucose levels during the IGTT in the high-dose group, compared to both other groups (Fig. (**2.5**)).

Repeated insulin sampling during IGTT and insulin resistance:

In order to determine the relationship between insulin resistance both before and after the glucose injection in the IGTT, we performed the IGTT and repeatedly sampled insulin levels every 30 min in two separate groups of rats. As noted above, animals were treated with either the high dose of olanzapine (15 mg/kg) or vehicle. Analysis confirmed that, as before, there was a strong main effect of olanzapine on increased fasting glucose levels [$t_{(1,14)} = 4.69$; $p < 0.001$] as well as during the IGTT [$F_{(1,14)} = 15.05$; $p < 0.005$] (Fig. (**2.6**)). Insulin levels were also significantly increased by olanzapine both during the fasting state [$t_{(1,14)} = 3.05$; $p < 0.01$] and during every half hour throughout the entire IGTT [$F_{(1,14)} = 8.40$; $p < 0.05$] (Fig. (**2.6**)). Insulin resistance, calculated by HOMA-IR during the fasting state, was greater in the olanzapine group [$t_{(1,14)} = 4.14$; $p < 0.005$]; in the same rats, insulin resistance in the post-glucose (non-fasting) state during the IGTT, calculated by the ISI, was also significantly increased by olanzapine treatment [$F_{(2,21)} = 13.90$; $p < 0.0001$].
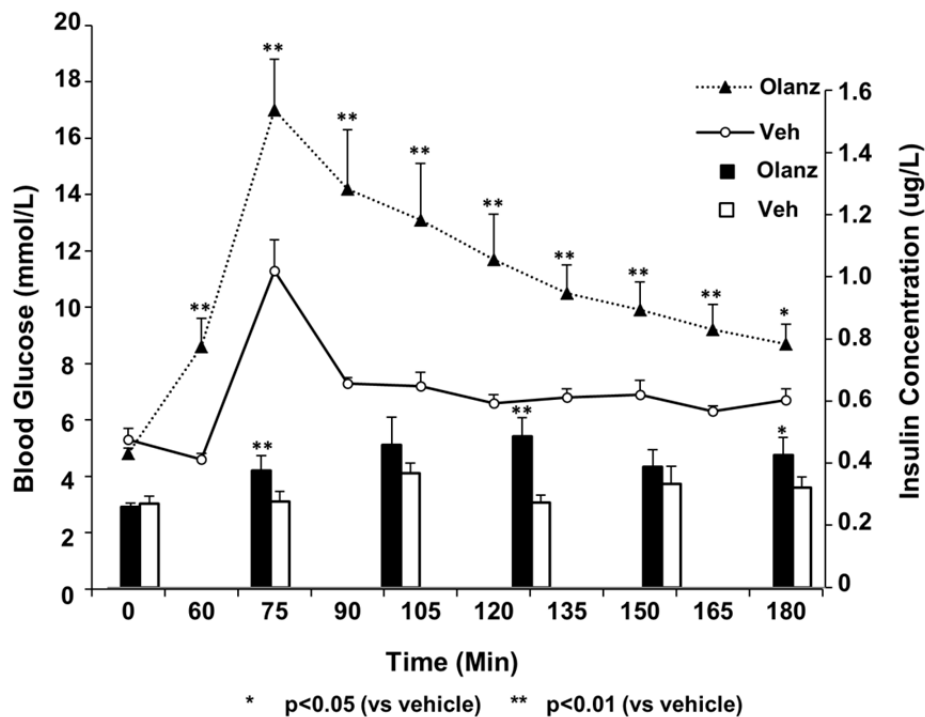
Fig. 2.6: *The acute effects of the atypical antipsychotic drug olanzapine on glucose and insulin levels in adult female rats.* Animals (n=8 per group) received an intraperitoneal injection of either olanzapine (15 mg/kg) or vehicle. Glucose levels (lines) were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then 60min following drug administration. Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2 hours. Insulin levels (columns) were recorded prior to drug and every 30min after drug administration.

In order to determine the relationship between fasting and non-fasting insulin levels, as well as insulin resistance, regression analysis was used to compare pre with post glucose measures for both variables: area under the curve was used to create a single value for the post-glucose repeated measures of insulin levels and insulin resistance. Analysis indicated that fasting levels of insulin were strongly correlated with post-glucose insulin levels ($r^2 = 0.74$, $p < 0.001$). Similarly, fasting insulin resistance HOMA-IR values were strongly

associated with post-glucose insulin resistance values measured by ISI ($r^2 = 0.80$, p < 0.0001).

2.1.4 Discussion

The present series of experiments evaluated the effects of two atypical antipsychotic drugs with high metabolic risk, and one atypical and one typical drug with lower metabolic risk on a number of different indices related to glucose metabolism and insulin sensitivity in adult female rats. We determined the acute effects of both low and high doses of clozapine, olanzapine, risperidone and haloperidol on fasting glucose and insulin levels, as well as insulin resistance, which were measured by HOMA-IR values and glucose clearance in the IGTT. Broadly, we found that the atypical antipsychotics produced effects on most metabolic indices. We also report, for the first time in the literature, that the effects of these three atypical drugs are clearly dose and time dependent, as greater effects were observed with the higher dose of drug, and closer in time to the administration of the antipsychotic drug. There was also a modest effect on metabolic indices by haloperidol.

In the fasting state, the general effect of antipsychotic drugs was to increase either glucose or insulin levels, but not necessarily both at the same time. The greatest effects were observed at 60 min after antipsychotic drug treatment, compared to 180 or 360 min after treatment. The effects on fasting levels of glucose and insulin were stronger, on average, with the higher dose of drug. However, these effects could be evident as increases in either glucose *or* insulin levels, and significant increases in only one of these indices occurred almost twice as often as both concurrently. In contrast, the fasting measure of insulin

52

resistance, obtained from the HOMA-IR equation, showed much greater reliability than either of the two individual values. The increase in HOMA-IR scores was universally greater for the high dose of each atypical drug, at all time points, than either the low dose of drug or vehicle. This suggests that a combined measure of insulin resistance that indexes both fasting glucose and insulin levels may be more reliable than simply reporting individual levels of the two, and should be used in future animal studies. For the only typical antipsychotic drug tested, haloperidol, there was no evidence that any one of HOMA-IR value, glucose or insulin levels provided a more consistent way to detect or measure metabolic side-effects.

A complementary evaluation of the effects of antipsychotic drugs on metabolic function was obtained using the IGTT. This technique assesses glucose "intolerance" by measuring the capacity of the fasted subject to restore glucose levels to the normal range over time after a glucose challenge; this procedure is widely used in both clinical and preclinical studies of Type II DM [107]. The glucose tolerance test has been viewed positively for its physiological relevance and practicality for measuring metabolic side-effects in large numbers of animals or humans [67]; however, it is worth noting that confirmation of insulin resistance should ideally be supported by additional studies with techniques that directly control insulin levels, such as the euglycemic clamp [79]. The results of the IGTTs showed clearly that the high doses of all antipsychotics produced glucose intolerance one hour after administration. The effect size was greatest with clozapine and olanzapine, whereby the "area under the curve" of total glucose levels during the 120 min test nearly doubled compared to vehicle, while risperidone showed an approximately 70% increase and haloperidol the smallest increase at 60%. By three hours after drug administration, glucose levels were still greater than controls in the high-dose groups of the three atypical drugs, and at six hours,

only the high dose of clozapine remained significant. Direct comparison of the magnitude of side-effects between atypical drugs is complicated by dose, as equivalency dosing cannot be based on factors that are commonly used to compare first generation drugs, such as dopamine D_2 receptor occupancy [108]. In humans, standardized doses are often based on clinical efficacy; we have attempted to model this in rats by choosing doses from the high end of behavioral studies, with a lower dose that is five to tenfold lower to provide a broad range of drug concentration.

Although insulin levels were not measured directly during the IGTT for the majority of experiments, as this is not a standard part of the test, we repeatedly sampled insulin levels during an IGTT in a separate cohort of rats that were treated with the high dose of olanzapine or vehicle. The results of this study indicated that there was a very strong relationship between fasting insulin resistance measured by HOMA-IR, and insulin resistance during the IGTT measured by the ISI equation (r = 0.89), indicating that the increased fasting HOMA-IR values after antipsychotic drug treatment for the main study are likely predictive of insulin resistance following the glucose challenge, and consistent with the results of the IGTT.

With the increased recent interest in understanding the biological basis of the metabolic side-effects of atypical antipsychotic drugs, studies have used a number of different techniques to measure homologous effects in rodents. The majority of studies have examined the effects of chronic antipsychotic drug treatment, although a smaller number have examined the acute effects of antipsychotic drugs. In both types of study, it has been observed that female rats provide a better model for metabolic side-effects than males. This is evident both in terms of glucose intolerance, where female, but not male, rats show hyperglycemia after acute treatment with antipsychotic drugs [66], as well as weight gain,

where more consistent increases are noted in females [84]. For these reasons, we conducted the present study with female rats only. An important novel finding that we demonstrate, for the first time ever in the same study, is that the acute effects of atypical and typical antipsychotic drugs are both dose and time dependent. While the time dependency of the effect is not surprising, it provides important and strong evidence that antipsychotic drugs can produce effects on glucose tolerance that are related to the presence of the drug, and do not remain after the compound is cleared. The half life for all four drugs in rats ranges from 1 to 2.5 hours [109-112], and so our results indicate for the first time that higher doses of most drugs can cause glucose intolerance for at least one half life, with only clozapine having any effect by the end of the second half life. Whether similar effects occur in humans remains to be determined, but if so, these results highlight the importance of knowing plasma levels of antipsychotic drugs when testing for glucose tolerance in patients, which are almost never reported in the literature.

Our evidence of increased fasting glucose levels in acute antipsychotic drug treated rats is consistent with findings by Assie and colleagues [113] who demonstrated that the same antipsychotic drugs we presently studied could all increase fasting glucose levels in rats, although no form of tolerance test was conducted in their study. Due to differences in route of administration between the two studies, i.e. intraperitoneal versus oral, direct comparison of dose effects is complex, and insulin levels were not measured in the Assie study, thus precluding any comparison of HOMA-IR values. Dose-dependent increases in plasma glucose levels were clearly evident in clozapine-treated rats 30 min after injection in another study [90], with significant increases for doses between 5-20 mg/kg, although only the highest dose of 20 mg/kg increased insulin levels, consistent with our findings; HOMA-

IR values were not calculated. Smith and colleagues [69] recorded the effects of a single dose each of haloperidol (0.25 mg/kg), quetiapine (10 mg/kg) and clozapine (10 mg/kg) on glucose clearance with the IGTT. Similar to our results, and within the drug range of our experiments, both haloperidol and clozapine increased basal glucose levels and total glucose levels during the IGTT, 60 min after drug injection.

Two separate research groups have also determined the acute effects of antipsychotic drugs on insulin resistance using the hyperinsulinemic euglycemic clamp. In the first study of insulin resistance [71], acute doses of clozapine and olanzapine (3.2 - 10 mg/kg) both reduced glucose infusion rates within 40-60 min of injection, indicating that the drugs caused insulin resistance. There was no effect of either a single dose of risperidone (2 mg/kg) or quetiapine (3.2 – 32 mg/kg) on insulin resistance, and importantly, the effects of typical antipsychotics were not tested. More recently, two studies have confirmed that a single dose of olanzapine [72] and olanzapine (3 mg/kg), clozapine (1 mg/kg) and risperidone (1 mg/kg), but not haloperidol (0.25 mg/kg) or ziprasidone (3 mg/kg), increased glucose infusion rates [73]. The latter study only assessed the effects of a single dose of each drug, and none of the above studies examined insulin resistance at different durations after drug treatment. While the effects of clozapine and olanzapine concur between these two research groups, it is interesting that a lower dose of risperidone induced insulin resistance in one study while a higher dose had no effect in the other report. Our current results are consistent with both studies regarding clozapine and olanzapine, but fit better with the findings of Chintoh et al. (2009) for risperidone, as we observed a significant effect of a dose as low as 0.5 mg/kg on glucose intolerance in the IGTT. While we differ from the Chintoh et al. (2009) study with regard to the effects of haloperidol, it is possible that this is due to dose differences, as our

effects in the IGTT were only apparent at a dose of 1mg/kg. This latter point reinforces the advantages of concurrently testing multiple doses of antipsychotic drugs, as done presently, because accumulating evidence in animal models strongly suggests that the effect of these drugs on metabolic indices is dose-dependent, and even typical antipsychotics can have effects if dosed high enough.

The present experiments provide no additional information about the molecular or pharmacological mechanisms that can account for acute effects of antipsychotic drugs on glucose regulation, although they do provide additional support for the use of acute models to study the direct actions of these compounds. A number of studies have demonstrated *in vitro* that acute treatment with atypical antipsychotic drugs can directly modify insulin release from rat pancreatic islets [114, 115] while we have recently noted that both peripheral nitric oxide and plasma catecholamines play a permissive role in drug-induced glucose dysregulation (Boyda et al., in preparation). Likely there are a number of different pathways involved in the effects of antipsychotic drugs on glucose and insulin regulation, and acute models are needed to dissociate these effects without the interference of the effects of chronic drug treatment.

When viewed from the clinical perspective, the results of our rodent studies correlate well with established metabolic side-effects of antipsychotic drug treatment in patients. Our fasting HOMA-IR values were increased most by the high dose of clozapine, followed by high dose olanzapine and risperidone, with a moderate effect of low dose haloperidol. Similarly, the data from the IGTTs clearly show that the greatest effect on glucose intolerance was caused by clozapine, followed by olanzapine, then risperidone, and finally haloperidol. This order matches that observed in humans, as metabolic sequelae, such as

weight gain and hyperlipidemia, typically reflect this pattern [15]. As noted above, this may be dependent to some degree on the doses of drugs chosen for the present study, although these effects reflect robust and consistent findings over multiple doses and at three different time points after treatment. Full dose response curves will need to be calculated in future studies to definitively compare effect sizes between different drugs.

We have only examined the acute effects of antipsychotic drugs, whereas the vast majority of reported metabolic side-effects in humans are described following chronic drug treatment, although see [45]. Several preclinical studies have addressed this issue, e.g. [71], and generally found that the acute effects resemble those found with chronic treatment, although some tolerance to the metabolic effects of the drugs has been observed [69]. The advantage of studying the acute effects of antipsychotic drugs includes the ability to exclude abnormalities of glucose regulation that may be secondary to sequelae of chronic antipsychotic drug treatment, such as weight gain and hyperlipidemia. This, in turn, allows the development of a simpler and less confounded model to examine specifically and independently the biological basis of how antipsychotic drugs directly affect glucose and insulin metabolism. The results of the current study confirm that, at least in drug naïve animals, the atypical antipsychotic drugs produce potent effects on glucose regulation and insulin sensitivity, which represent a model for better understanding the biochemical basis of similar effects in humans.

2.2 Study 2: Metabolic side-effects of the novel second-generation antipsychotic drugs asenapine and iloperidone: a comparison with olanzapine

2.2.1 Overview

Acute and chronic studies of SGA drug-induced insulin resistance and glucose intolerance in laboratory animals have largely replicated clinical findings. Furthermore, the animal models are strongly homologous with clinical data, as drugs with greater metabolic liability in humans exert stronger metabolic effects in the preclinical paradigms [64, 71, 73, 77, 116-121]. These models are therefore useful not only in helping to understand the biological basis of metabolic side-effects, but also in predicting such effects in novel antipsychotic drugs.

The two novel SGA drugs asenapine and iloperidone were both approved by the US Food and Drug Administration in 2009 for the treatment of schizophrenia [122]. While the clinical efficacy of these new drugs compared to placebo has been confirmed in registration trials, little is known about the metabolic side-effects of these compounds [123]. Some data are published regarding weight gain, but to our knowledge, there has been no reported documentation of glucose intolerance and insulin resistance with these drugs, which are the core symptoms of Type II DM. Given the utility of preclinical models in predicting metabolic dysregulation caused by SGAs, we conducted a study of both glucose tolerance and insulin resistance with asenapine and iloperidone, using established procedures, across a wide range of doses. For reference, we concurrently measured metabolic dysregulation in animals treated with olanzapine, as this drug is known to reliably cause metabolic dysregulation.

We hypothesized that animals exposed to acute asenapine treatment would not demonstrate the significant glucose intolerance and insulin resistance, as demonstrated with acute olanzapine treatment. Furthermore, animals that received acute iloperidone treatment would exhibit significant glucose intolerance and insulin resistance, when compared to controls.

2.2.2 Materials & methods

Animals

Adult female Sprague-Dawley rats (250 - 275 g) from Charles River (Montreal, Canada) were habituated to the UBC colony for one week. Females are the preferred sex for rodent models of antipsychotic drug-induced metabolic dysregulation because they exhibit more consistent metabolic abnormalities than males [97, 124, 125]. Rats were pair-housed and maintained on a 12-hour light-dark cycle as previously described (Section 2.1.2). Approval by the UBC Animal Care and Use Committee was established for all procedures; animals were treated in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

Pharmacological agents and solutions

Antipsychotic drugs included asenapine [Sigma-Aldrich Inc., St. Louis, MO,], iloperidone and olanzapine [Toronto Research Chemicals Inc., Toronto, ON]. All dosing solutions were prepared daily. Asenapine was formulated in a vehicle composed of 0.9% saline with the addition of 10 µL 1 M hydrochloric acid; iloperidone and olanzapine were formulated in

50% polyethylene glycol 400, 40% distilled water and 10% ethanol (PEG solution) [126].

Antipsychotics were dissolved in a volume of 1 ml/kg. For clamp experiments, recombinant

human insulin (Humulin R) [Eli Lilly, Indianapolis, IN) and dextrose (50%) were formulated

in 0.9% w/v saline. All other chemicals were of reagent grade.

Intraperitoneal glucose tolerance test (IGTT) (see Fig. **2.6** for sequence of events)

All rats were given a baseline IGTT prior to drug administration, as described previously

[64]. Briefly, animals were fasted overnight for 16 ± 2 hours; the following morning animals

were wrapped in a towel to minimize stress, and a drop of saphenous venous blood was

procured with a 25-gauge needle for baseline blood glucose measurement. Subsequently,

animals received a glucose challenge (1 g/kg/ml, i.p.) followed by repeated sampling of

blood glucose readings every 15 min for two hours. Glucose measurements were determined

by handheld glucometer (One Touch Ultra). Based on total glucose levels, rats were rank

ordered and randomly matched to one of six treatment groups (vehicle or five different doses

of asenapine [$n = 8 - 9$ per group]) the following week. There was always one week

minimum duration between subsequent IGTTs.

A
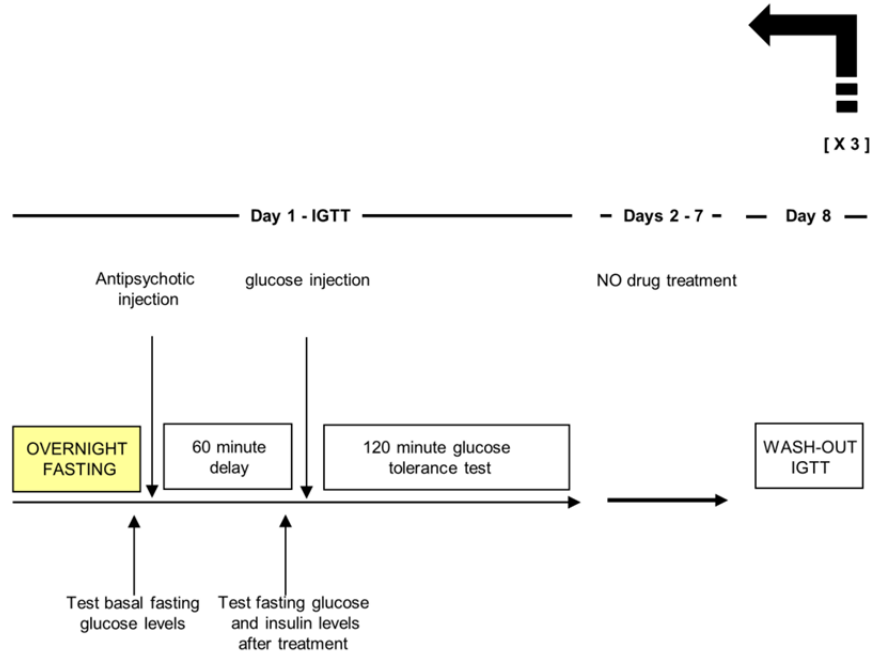
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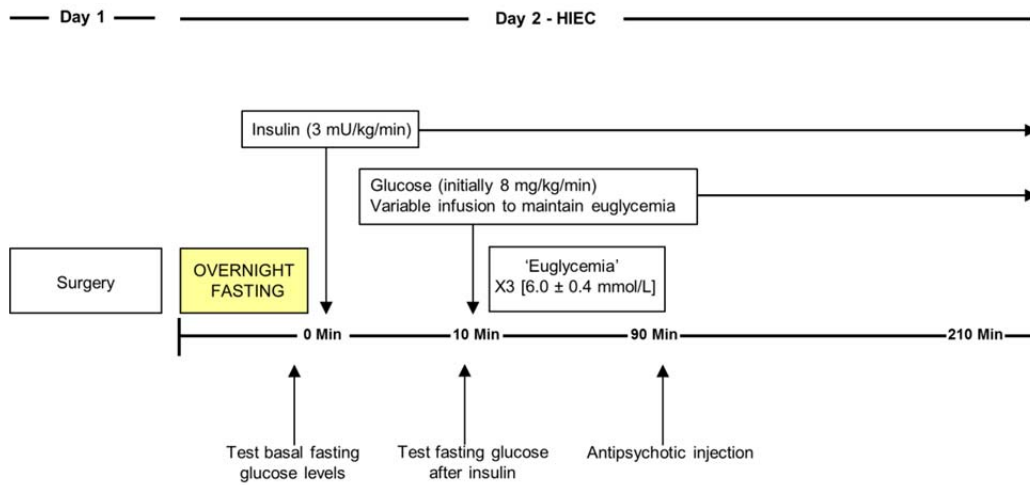
Fig. 2.7: *Experimental protocol describing (**A**) the intraperitoneal glucose tolerance test and (**B**) the hyperinsulinemic-euglycemic clamp with acute antipsychotic drug treatment.*

For the asenapine IGTT, rats were fasted as above. Baseline blood glucose levels were measured, and then a single dose of asenapine (0.01, 0.05, 0.1, 0.5 or 1.0 mg/kg) or vehicle was administered by s.c. injection; each rat received only one dose. A second measurement of blood glucose was taken 30 min later, to assess drug treatment on fasting glucose levels. Subsequently, a saphenous blood draw using heparinized collecting tubes was performed to obtain plasma samples for analysis of insulin levels; extracted blood samples were centrifuged (10,000 RPM, 10 Min, 4ºC) and samples stored at -80ºC. Immediately afterwards, the IGTT commenced, whereby animals received a challenge injection of 1 g/ml/kg of glucose. Glucose levels were monitored and recorded every 15 min for two hours duration. Animal handlers were blinded to drug treatment. One week after the asenapine IGTT, rats received another baseline IGTT, for re-randomization for treatment with iloperidone the following week. Rats received either vehicle or iloperidone (0.03, 0.5, 1.0, 5.0 or 10.0 mg/kg). Following another baseline IGTT and re-randomization, rats received the final antipsychotic treatment with vehicle or olanzapine (0.1, 0.5, 1.5, 5.0 or 10.0 mg/kg).

Surgical preparations for hyperinsulinemic-euglycemic clamp (HIEC)

Rats were prepared for surgery under isoflurane anesthesia and pre-operative ketoprofen (5 mg/kg, s.c). Heparinized saline-filled polyethylene cannulae (PE50) were inserted into the right common carotid artery and both exterior jugular veins. The arterial cannula was used to sample blood for measurement of glucose levels and venous cannulae were used for the infusion of insulin and dextrose. All cannulae were tunneled subcutaneously to the nape of neck and exteriorized. Animals recovered for 24 hours prior to the HIEC: two animals did not make a full recovery and were excluded.

HIEC procedures (see Fig. (**2.6**) for sequence of events)

Overnight fasted rats (16 ± 2 hours) were habituated to the cage prior. The two venous cannulae were connected to auxiliary heparinized saline-filled PE50 tubing, directly attached to infusion-only pumps (Harvard Apparatus, Holliston, MA). After a baseline blood glucose reading from the arterial cannula, insulin infusion (3 mU/kg/min) was initiated (t = 0 min) and kept running at a constant rate for the entire experiment. Dextrose (50% w/v) infusion commenced at 8 mg/kg/min (0.96 ml/kg/hour) at t = 10 min and the glucose infusion rate (GIR) was adjusted as needed, every 10 min, to maintain glucose concentrations at 6.0 mmol/L. Euglycemia was determined when three consecutive blood glucose measurements presented 6.0 ± 0.4 mmol/L at the same GIR. Animals then randomly received a single s.c injection of either vehicle or asenapine (0.1, 1.0 mg/kg), iloperidone (1.0, 10.0 mg/kg) or olanzapine (1.5, 15.0 mg/kg) [n = 5-7 per group], and the clamp was continued for 120 min duration. The sample size of n = 5 -7 animals per group is consistent with previously published studies [71, 73]. Handlers were blinded to drug treatment.

Plasma insulin measurement by ELISA

Insulin levels were measured using the ultra-sensitive rat insulin ELISA kit (Crystal Chem Inc., IL, USA) as previously described in Section 2.1.2.

Insulin resistance

Determination of insulin resistance in rats was accomplished using the homeostatic model

assessment of insulin resistance (HOMA-IR) equation, as first presented in Section 2.1.2.

Statistical analysis

Metabolic indices during the IGTT were analyzed by one-way analysis of variance

(ANOVA), with drug dose as the between group factor. For the IGTT, glucose data were

summed as the area-under-the-curve throughout the 120 min procedure [126]. For the HIEC

data, all drugs were included in the overall ANOVA, as the same vehicle group was used for

all between-drug comparisons. Alpha value was set at $p < 0.05$. LSD post-hoc tests were

conducted when a main effect or interaction between main effects was significant. Data were

analyzed with SPSS software, Chicago, IL, version 20.

2.2.3 Results

IGTT

Analysis of the IGTT with asenapine revealed no significant effect of drug treatment on

fasting glucose levels or glucose intolerance following glucose challenge (Fig. **2.7**).

Interestingly, asenapine affected fasting insulin levels [$F_{(5,43)} = 3.03$, p < 0.05], whereby the

three lower doses of the drug *decreased* insulin levels compared to controls, although this

was only significant for the lowest asenapine dose (p < 0.05) (Table 1). Similarly, asenapine

significantly affected insulin resistance [$F_{(5,43)} = 2.59$, p < 0.05], measured by HOMA-IR, as

insulin resistance was significantly reduced with the lowest asenapine dose (0.01 mg/kg) and

a strong trend was observed with the next two doses (0.05 and 0.1 mg/kg) (Table **2.2**).
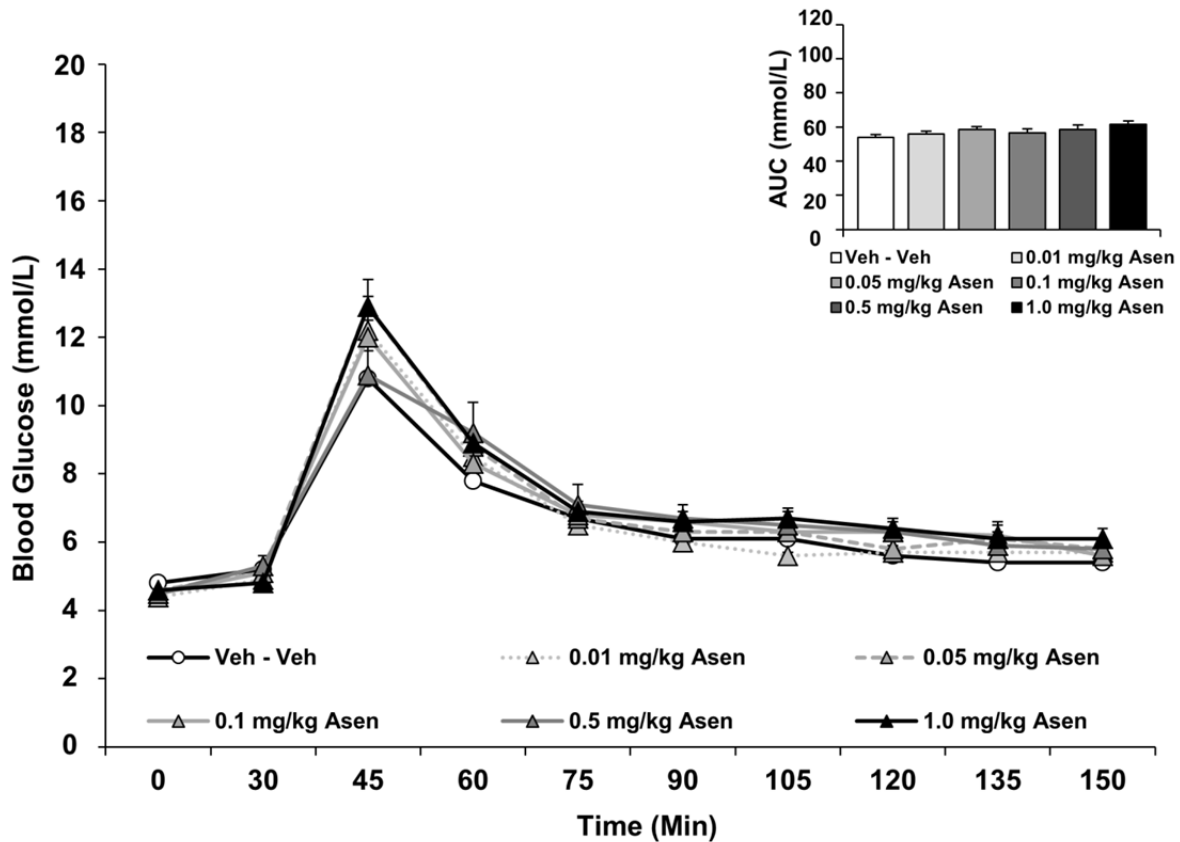
Fig. 2.8: *Acute effects of the antipsychotic drug asenapine on glucose levels in adult female rats.* Animals (n = 8 - 10 per group) received a single injection of vehicle or asenapine (0.01, 0.05, 0.1, 0.5, 1 mg/kg, s.c). Glucose levels were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then 30 min following drug administration (*x-axis*). Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next two hours. Total cumulative glucose levels for each treatment group are summed as the "area under the curve" during the glucose tolerance test by graph inset (*top right*). Values represent group means ± SEM. Values represent group means ± SEM.

Antipsychotic Drug	Measure	Treatment Dose (mg/kg)					
Asenapine		**0**	**0.01**	**0.05**	**0.1**	**0.5**	**1.0**
	G_0	5.1 ± 0.3	4.9 ± 0.3	5.1 ± 0.2	5.0 ± 0.1	5.4 ± 0.4	4.8 ± 0.2
	I_0	24.6 ± 5.7	16.3 ± 2.2*	21.7 ± 3.0	20.4 ± 3.1	29.7 ± 4.4	33.3 ± 3.5
	HOMA-IR	5.7 ± 1.5	3.4 ± 0.4*	4.9 ± 0.7	4.5 ± 0.7	7.2 ± 1.3	7.2 ± 1.0
Iloperidone		**0**	**0.03**	**0.5**	**1.0**	**5.0**	**10.0**
	G_0	4.6 ± 0.2	4.9 ± 0.2	4.6 ± 0.2	4.9 ± 0.2	5.1 ± 0.2	5.2 ± 0.3
	I_0	21.6 ± 3.6	21.6 ± 2.6	28.2 ± 5.0	40.1 ± 4.9*	44.9 ± 3.6*	58.8 ± 7.7*
	HOMA-IR	4.6 ± 0.8	4.7 ± 0.5	5.9 ± 1.2	8.8 ± 1.2*	10.2 ± 0.8*	13.8 ± 2.2*
Olanzapine		**0**	**0.1**	**0.5**	**1.5**	**5.0**	**15.0**
	G_0	5.0 ± 0.2	5.3 ± 0.2	5.3 ± 0.3	5.7 ± 0.3	5.2 ± 0.2	5.0 ± 0.2
	I_0	19.7 ± 1.7	32.5 ± 6.0	35.9 ± 5.7	36.3 ± 2.7	41.4 ± 6.6*	47.8 ± 5.8*
	HOMA-IR	4.5 ± 0.5	7.8 ± 1.5	8.7 ± 1.6	9.2 ± 1.1	9.6 ± 1.5	10.8 ± 1.5

Table 2.2: *Mean concentration of fasting glucose, insulin and HOMA-IR scores in antipsychotic drug-treated rats.*

I_0 = fasting insulin levels (µU/ml); G_0 = fasting glucose levels (mmol/L); HOMA-IR = homeostasis model assessment of insulin resistance (µU·mmol) / (ml·L).
Rats were treated with five separate doses of asenapine, iloperidone, olanzapine or vehicle. Values represented as means ± SEM at t = 30 min during the IGTT. * indicates different from vehicle-treated animals, $p < 0.05$.

By contrast, while iloperidone had no effect on fasting glucose levels, it exhibited a strongly significant effect on glucose tolerance during the IGTT [$F_{(5,43)} = 13.06$, $p < 0.0001$] (Fig. **2.8**). Post-hoc analysis revealed a dose-dependent effect whereby the three highest doses of iloperidone (1.0, 5.0 and 10.0 mg/kg) increasingly elevated glucose intolerance compared to controls ($p < 0.05$), with the two highest doses causing increased glucose intolerance compared to both controls and the three lower doses of the drug ($p < 0.001$). This was manifest as a striking increase of 88% and 91% in glucose levels with the two highest doses of iloperidone compared to vehicle-treated rats. Regarding insulin, the ANOVA indicated a main effect of drug on fasting insulin levels [$F_{(5,43)} = 8.47$, $p < 0.0001$] (Table **2.2**), reflecting a dose-dependent elevation of insulin levels. Insulin was significantly higher than controls with the three highest doses of iloperidone ($p < 0.05$). Similarly, HOMA-IR values also significantly increased by drug treatment [$F_{(5,43)} = 7.90$, $p < 0.0001$] (Table **2.2**), as the three highest doses of iloperidone induced greater insulin resistance compared to controls ($p < 0.01$).
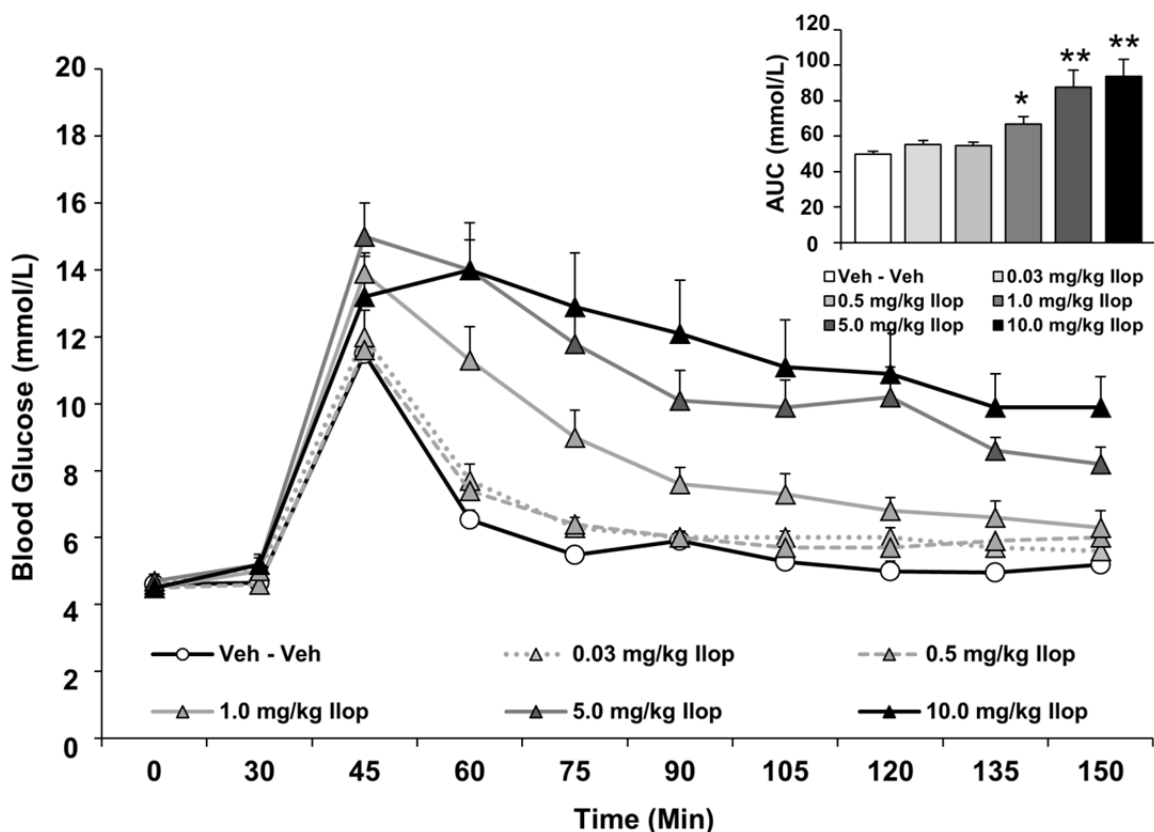
Fig. **2.9**: *Acute effects of the atypical antipsychotic drug iloperidone on glucose levels in adult female rats*. Animals (n = 8 - 10 per group) received a single injection of vehicle or iloperidone (0.03, 0.5, 1.0, 5.0, 10.0 mg/kg, s.c). Glucose levels were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then 30 min following drug administration (*x-axis*). Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next two hours. Total cumulative glucose levels for each treatment group are summed as the "area under the curve" during the glucose tolerance test by graph inset (*top right*). Values represent group means ± SEM. * indicates different from vehicle-treated animals, $p < 0.05$; ** indicates different from vehicle and 0.03 – 1.0 mg/kg iloperidone-treated animals, $p < 0.01$

The effects of olanzapine on glucose metabolism were consistent with our previous findings [64, 126]. There was no effect of olanzapine treatment on fasting glucose levels, but a strong effect on glucose tolerance in the IGTT [$F_{(5,43)} = 8.47$, $p < 0.0001$] (Fig. **2.9**). There was a dose-dependent effect of olanzapine, whereby the three higher doses increased glucose levels versus controls. This was highly significant for the 5 and 15 mg/kg doses ($p < 0.001$),

69

which increased glucose levels by 24% and 69% compared to controls. Olanzapine also

significantly increased insulin levels [$F_{(5,43)}$ = 2.90, p < 0.05] (Table **2.2**). All doses increased

insulin levels: this was a trend for the 0.5 and 1.5 mg/kg doses (p = 0.06), while the two

highest doses (5 and 10 mg/kg) caused larger increases (p < 0.01). The effect of olanzapine

on HOMA-IR values was a non-significant trend [$F_{(5,43)}$ = 2.05, p = 0.09] to increase values.

Importantly, values on the washout IGTT given the week after olanzapine treatment did not

differ from the baseline IGTT values prior to SGA treatment, indicating that glucose

tolerance did not change during the course of the study.
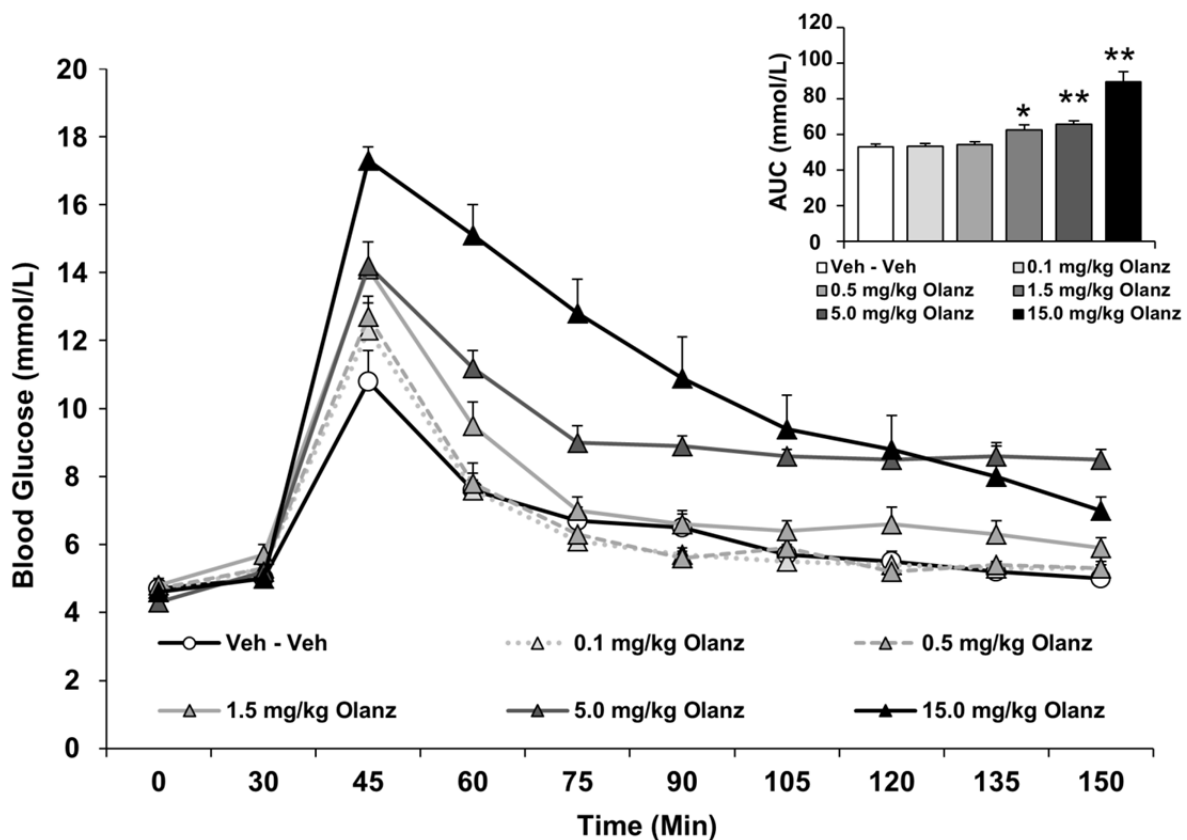
Fig. **2.10**: *Acute effects of the atypical antipsychotic drug olanzapine on glucose levels in adult female rats*. Animals (n = 8 - 10 per group) received a single injection of vehicle or olanzapine (0.1, 0.5, 1.5, 5.0, 15.0 mg/kg, s.c). Glucose levels were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then 30 minutes following drug

administration (*x-axis*). Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 minutes for the next two hours. Total cumulative glucose levels for each treatment group are summed as the "area under the curve" during the glucose tolerance test by graph insets (*top right*). Values represent group means ± SEM. * indicates different from vehicle-treated animals, p < 0.05; ** indicates different from vehicle-treated animals, p < 0.01

HIEC

Average basal glucose levels were similar for all groups prior to euglycemia and

administration of antipsychotic drugs. Insulin resistance during the HIEC is indicated by a

reduction in the GIR, and therefore the primary analysis compared the effects of

antipsychotics on GIR.

For the overall ANOVA, specific antipsychotic drug (asenapine, iloperidone,

olanzapine or vehicle) and dose (vehicle, lower or higher dose) were represented by between-

subjects factors. The results indicated significant main effects of both drug [$F_{(2,34)}$ = 12.76, p

< 0.0001], dose [$F_{(2,34)}$ = 20.78, p < 0.0001] and a drug × dose interaction [$F_{(2,34)}$ = 2.93, p <

0.05]. Drug effects were evident as asenapine treatment, regardless of dose, had no effect on

the GIR compared to vehicle-treated rats (Figure **2.10**). By contrast, both iloperidone and

olanzapine, regardless of dose, significantly decreased the GIR compared to vehicle-treated

rats (p < 0.01) (Figure **2.11** & **2.12**). Furthermore, both iloperidone (p < 0.01) and olanzapine

(p < 0.05) decreased GIR significantly more than asenapine. For iloperidone, both doses (1.0

and 10.0 mg/kg) caused significant decreases in the GIR. In both iloperidone dose groups this

was evident by 30 mins post treatment, and by 40 mins the higher-dose group had a

significantly lower GIR than the lower-dose group. In the olanzapine group, only the higher

dose of the drug (15 mg/kg) significantly decreased infusion rate throughout the duration of

the HIEC (p < 0.01). The higher dose olanzapine group showed significantly decreased GIR

compared to both controls and lower-dose animals within 30 mins of treatment. While the

magnitude of the decrease in GIR was greater with iloperidone than olanzapine, this effect

did not quite achieve statistical significance.
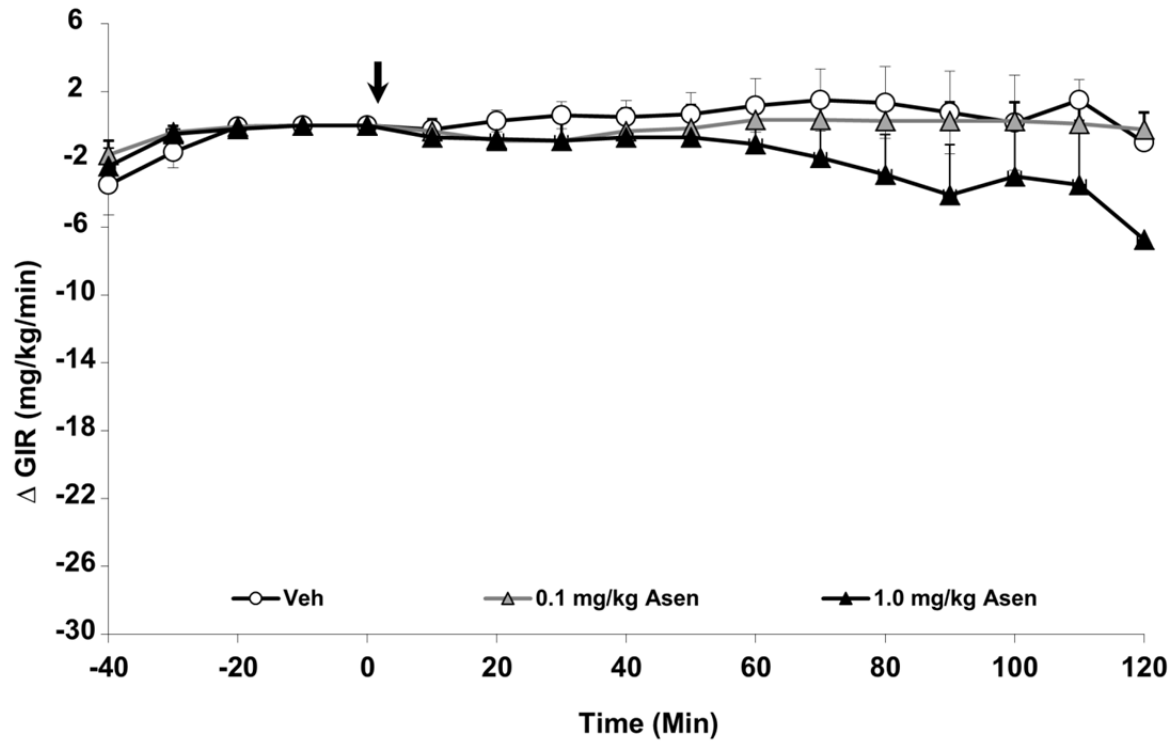
Fig. **2.11:** *The acute effects of the antipsychotic drug asenapine on insulin resistance in adult female rats.* A separate cohort of animals (n = 6-8 per group) were fasted overnight and subjected to the hyperinsulinemic-euglycemic clamp. After animals reached euglycemia (three consecutive blood glucose readings of 6.0 ± 0.4 mmol/L), rats were treated with vehicle, low (0.1 mg/kg) or high dose (1.0 mg/kg) asenapine (arrow at t = 0 min). Glucose levels were recorded every 10 min and the glucose infusion rate was adjusted as needed. Glucose infusion rates for each treatment group are presented as change in glucose infusion rate from euglycemia. Values represent group means ± SEM.
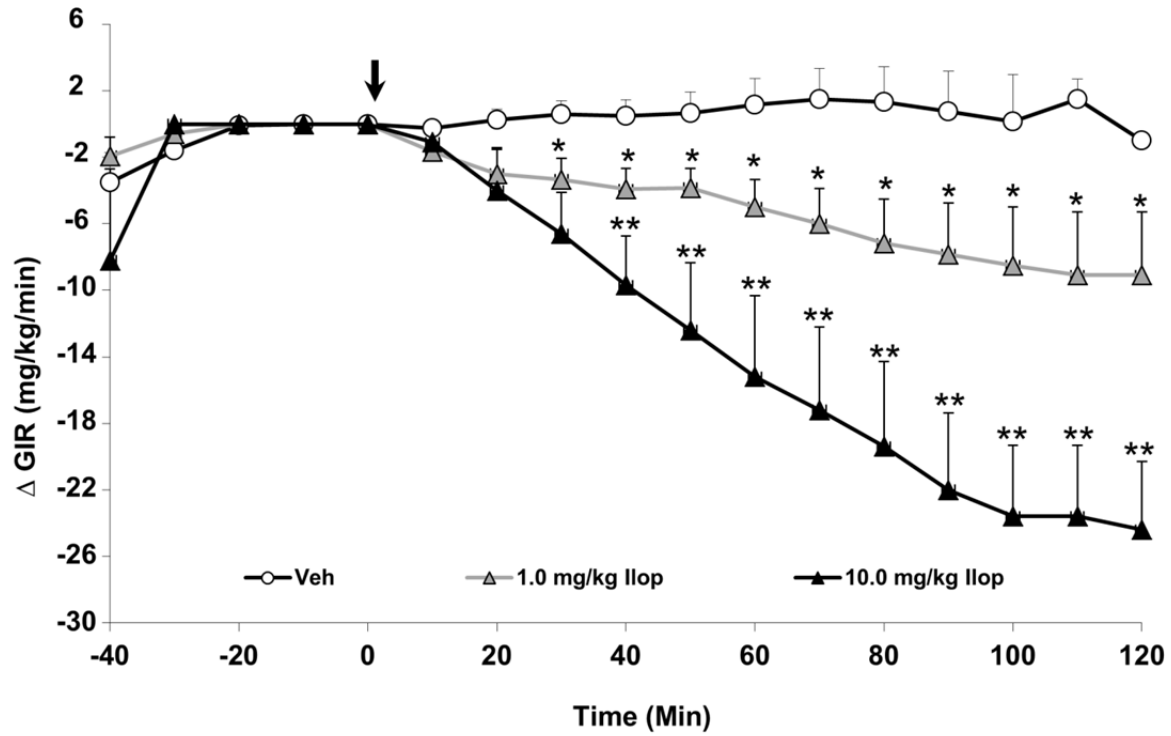
Fig. **2.12**: *The acute effects of the antipsychotic drug iloperidone on insulin resistance in adult female rats*. A separate cohort of animals (n = 6-8 per group) were fasted overnight and subjected to the hyperinsulinemic-euglycemic clamp. After animals reached euglycemia (three consecutive blood glucose readings of 6.0 ± 0.4 mmol/L), rats were treated with vehicle, low (1.0 mg/kg) or high dose (10.0 mg/kg) iloperidone (arrow at t = 0 min). Glucose levels were recorded every 10 min and the glucose infusion rate was adjusted as needed. Glucose infusion rates for each treatment group are presented as change in glucose infusion rate from euglycemia Values represent group means ± SEM. * indicates different from vehicle-treated animals, $p < 0.05$; ** indicates different from vehicle and 1.0 mg/kg iloperidone-treated animals, $p < 0.05$.
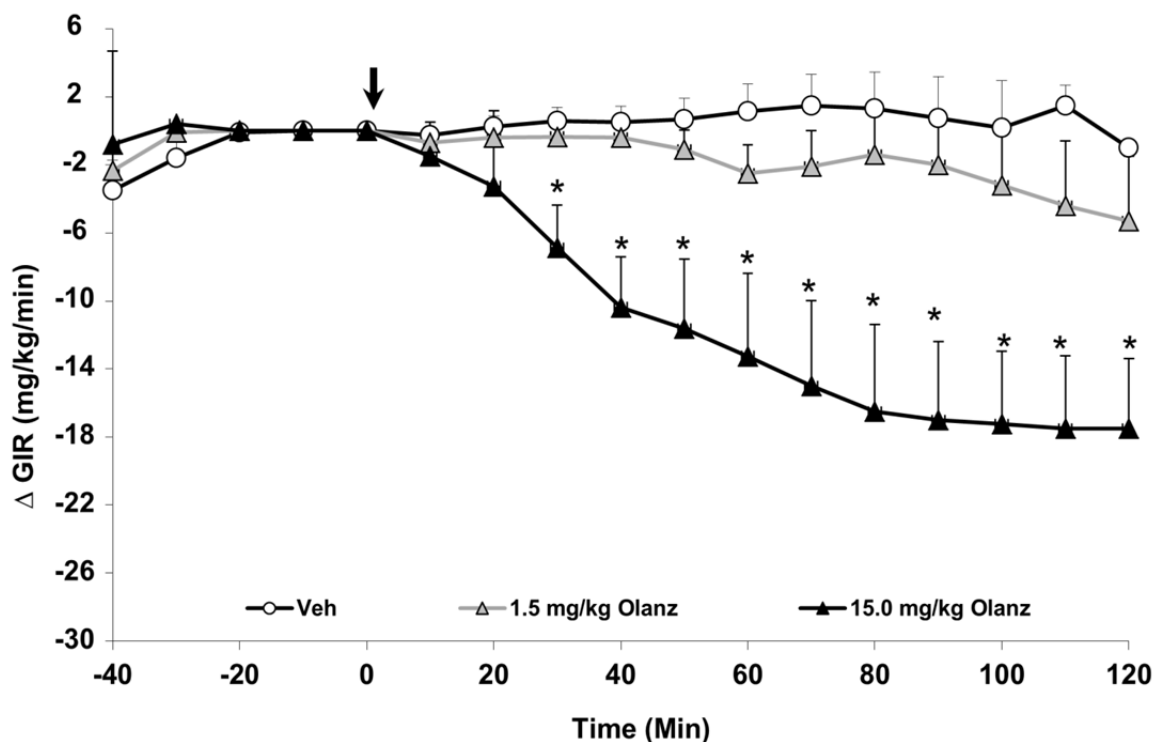
Fig. 2.13: *The acute effects of the antipsychotic drug olanzapine on insulin resistance in adult female rats.* A separate cohort of animals (n = 6-8 per group) were fasted over-night and subjected to the hyperinsulinemic-euglycemic clamp. After animals reached euglycemia (three consecutive blood glucose readings of 6.0 ± 0.4 mmol/L), rats were treated with vehicle, low (1.5 mg/kg) or high dose (15.0 mg/kg) olanzapine (arrow at t = 0 min). Glucose levels were recorded every 10 min and the glucose infusion rate was adjusted as needed. Glucose infusion rates for each treatment group are presented as change in glucose infusion rate from euglycemia. Values represent group means ± SEM. * indicates different from vehicle and low-dose olanzapine-treated animals, $p < 0.05$.

2.2.4 Discussion

In the present study, we assessed the metabolic side-effects of the novel SGA drugs asenapine and iloperidone for the first time, as well as olanzapine for reference. Two separate techniques were used to measure metabolic dysregulation, including both the glucose tolerance test and the hyperinsulinemic-euglycemic clamp. Consistent with previous preclinical studies, olanzapine caused dose-dependent glucose intolerance in the IGTT and

insulin resistance in the clamp [64, 65, 71, 72, 75, 83, 127-130]. By contrast, the novel SGA drug asenapine was largely devoid of metabolic effects at the doses tested, causing only a slight reduction in insulin levels and HOMA-IR values with the lowest dose. In comparison, the novel SGA drug iloperidone showed potent dose-dependent effects on metabolic function. The three highest doses in the IGTT substantially increased glucose intolerance, to a greater degree than that observed with olanzapine. Similarly, both low- and high-dose iloperidone increased insulin resistance in the euglycemic clamp.

These overall findings reconfirm the powerful effect of the SGA olanzapine in animal models of glucose control and uptake, and demonstrate for the first time that similar-to-greater magnitude effects were observed with iloperidone, while asenapine shows minimal metabolic liability. The glucose tolerance test assesses glucose "intolerance" by measuring the capacity of the fasted subject to restore glucose levels to the normal range over time after a glucose challenge; this procedure is widely used in both clinical and preclinical studies of prediabetes and Type II DM [107]. The glucose tolerance test has been viewed positively for its physiological relevance and practicality in measuring metabolic side-effects in animals or humans, as it reflects how glucose will be controlled after a meal [67]. However, as both glucose and insulin levels are free to vary in this test, it is necessary to use "clamp" procedures, such as the hyperinsulinemic-euglycemic clamp, to confirm the presence of whole-body insulin resistance, which provides a specific measure of cell-mediated glucose uptake via the action of insulin. These procedures should be considered as complementary, and the high degree of correlation between them with the current results provides converging evidence for drug-specific metabolic liability. The only exception to this was in rats treated with the 1.5 mg/kg dose of olanzapine, where animals in the IGTT displayed significant

glucose intolerance, but the same dose did not cause significant insulin resistance in the clamp. It is therefore possible that the IGTT is more sensitive at detecting SGA-induced metabolic dysregulation.

As noted above, numerous studies have previously reported metabolic dysregulation following both acute and chronic treatment with olanzapine. We recently reported that rats treated daily with olanzapine for ten weeks showed no change in the magnitude of glucose intolerance in the IGTT compared to their first challenge with the drug [126], indicating that acute treatment with SGAs can model chronic drug-treatment effects. However, to our knowledge, there are no studies of glucose intolerance or insulin resistance with the SGAs asenapine and iloperidone, in either animals or humans. Clinically, in three short-term phase 3 trials of iloperidone for schizophrenia, non-fasting glucose levels in all three dose ranges were significantly increased compared to placebo-treated subjects, whereas the risperidone comparator group did not differ from placebo [131]. Both risperidone- and iloperidone-treated subjects exhibited significant weight-gain versus placebo. A separate 28 day clinical trial of iloperidone in head-to-head comparison with ziprasidone noted greater weight gain in the iloperidone arm [132], with 21% of iloperidone subjects (versus 7% of ziprasidone and 3% of placebo) displaying clinically significant weight gain. Changes in glucose levels, which were not specified with regard to fasting status, were 7.9 mg/dL for iloperidone versus 4.7 mg/dL for ziprasidone. An analysis of three long-term safety trials of iloperidone, with haloperidol as comparator, noted both greater weight gain and increases in glucose levels at six weeks and 52 weeks following treatment in the iloperidone group [133]. Thus, clinical data suggest that both fasting and non-fasting glucose levels are increased by iloperidone, compared to other antipsychotic drugs including risperidone, ziprasidone and haloperidol,

none of which may be considered high metabolic risk. However, data from preclinical studies with SGAs indicate that fasting levels of glucose in the absence of a glucose challenge may underestimate loss of glycemic control. For example, in the present study, fasting glucose levels were not increased by any of the SGAs, yet for two drugs severe glucose intolerance was observed when the glucose challenge was applied.

There are a larger number of studies that have reported the effects of asenapine on weight gain and fasting glucose levels, in both schizophrenia and bipolar disorder, although a full summary of these findings is beyond the scope of the present discussion. Asenapine causes less weight gain and glucose elevation than olanzapine [134-136]. When compared head-to-head against haloperidol, neither drug caused significant weight gain versus placebo, and fasting glucose abnormalities were actually marginally lower in the two doses of asenapine than in the haloperidol-treated group [137]. A short term study of asenapine only, with no comparator, for psychosis in the elderly reported a non-significant decrease in the number of subjects meeting criteria for metabolic syndrome compared to baseline [138]. Clinical data therefore indicate minimal effects of asenapine on weight gain and fasting glucose. Similarly, preclinical evidence indicates that the antipsychotic drug sulpiride may also have negligible effects on glycemic control and may even improve glucose clearance in female rats. Compared to both control and risperidone-treated animals, sulpiride administration was associated with a 13% reduction in the area under the curve for the GTT, despite increased body weight gain [124].

A potential issue regarding the current findings is the choice of SGA doses, as metabolic effects can be dose-dependent [139]. Clinical studies can compare metabolic side-effects between antipsychotic drugs at doses of equivalent clinical efficacy, using

standardized measures such as chlorpromazine equivalency [140-142]. This is not possible in animal models, and so "head-to-head" comparison between drugs represents a theoretical challenge, despite its common practice. It has been suggested that dosing based on dopamine D_2 receptor occupancy may represent one strategy, but issues remain regarding route of administration and inconsistent effects for all SGAs [143]. We chose our current dosing based on the behavioral effects of drugs in preclinical screens and models of schizophrenia. Behavioral effects in the latter share some homology with clinical symptomatology in humans [144]. Previous behavioral paradigms with asenapine in rats have shown potent antipsychotic-like effects below a dose of 0.2 mg/kg. Franberg and colleagues reported improvement in the conditioned avoidance response task with doses from 0.05 - 0.2 mg/kg [145] while doses from 0.01 - 0.075 mg/kg reversed phencyclidine (PCP)-induced deficits in a novel object recognition task [146]. Marston and colleagues demonstrated that 0.03 mg/kg asenapine reversed low-dose amphetamine hyperactivity, while 0.1 mg/kg reversed high-dose amphetamine hyperactivity [147]. In the same study, 0.03 - 0.1 mg/kg asenapine reversed apomorphine-induced deficits in prepulse inhibition (PPI) of the acoustic startle reflex. Thus, the 100-fold dose range in the current study more than encompasses the dosing required to produce behavioral effects, and the total absence of metabolic effects at doses as high as 0.5 and 1.0 mg/kg strongly indicates the metabolic liability of asenapine is low at behaviorally relevant doses. Regarding iloperidone, an initial report observed behavioral effects at doses between 0.7 – 5.2 mg/kg [148] while Barr and colleagues reported that both 1.0 and 3.0 mg/kg iloperidone reversed apomorphine-induced PPI deficits [149]. However, 3.0 mg/kg of iloperidone did not reverse the effects on PPI of 1.5 mg/kg of PCP, which is a dose commonly used to screen for antipsychotics, indicating that higher doses of iloperidone

would have been needed to demonstrate antipsychotic efficacy. By comparison, 10 mg/kg olanzapine robustly reversed PPI deficits caused by 1.5 mg/kg PCP [150], implying that both iloperidone and olanzapine may be reasonably well-matched dose-wise in the current study. The clinically approved daily dose of iloperidone for psychosis is 12 – 24 mg [122], while olanzapine is 10 – 20 mg [151] and therefore very similar. Thus, the greater glucose intolerance and insulin resistance caused by iloperidone versus olanzapine at similar doses (e.g. 5 mg/kg) indicates that iloperidone may have acute metabolic effects at least as strong as olanzapine.

The results of the current study strongly suggest that metabolic testing of patients treated with novel SGAs is warranted. Although, like numerous preclinical studies, the current results were based on acute treatment without weight gain, there is an increasing body of evidence demonstrating weight-independent and drug-specific effects on glucose intolerance and insulin resistance. Results from the Clinical Antipsychotic Trial of Intervention Effectiveness (CATIE) study observed that 42.7% of patients treated with SGAs had metabolic dysregulation [152]. When controlling for Body Mass Index, CATIE men were 85% and CATIE women 137% more likely to have metabolic syndrome than the normal population. Newcomer and colleagues used the glucose tolerance test in adiposity-matched patients to show that those treated with SGAs had greater glucose elevations than patients treated with first generation drugs [31]. Importantly, in the first double-blind, placebo-controlled crossover trial, it was recently demonstrated that acute 3-day treatment of normals with 10 mg of olanzapine caused significant impairments in the glucose tolerance test [51]. The glucose area-under-the-curve increased by 42%, which is highly consistent with our present findings in rats. Future clinical evaluation of glucose intolerance and insulin

resistance in novel SGA-treated patients should therefore remain a priority, using techniques

specifically designed to challenge metabolic regulation, despite the greater difficulty of using

such protocols, given the long-term health implications for loss of glycemic control.

2.3 Study 3: Antipsychotic polypharmacy increases metabolic dysregulation in female rats

2.3.1 Overview

Currently, all clinically effective antipsychotic drugs alleviate idiopathic psychosis through a common pharmacological mechanism, which principally involves the antagonism of D_2 receptors in the brain [153]. Additional receptor affinities differentiate the antipsychotic drugs, with many compounds having varying affinities for serotonergic, cholinergic, histaminic and noradrenergic receptors; it is these affinities for non-D_2 receptors that largely determine the side-effects of the drugs [154]. Given that central D_2 receptor antagonism is the sole known mechanism for controlling psychosis, there is not a strong pharmacological rationale for combining different antipsychotic drugs to increase D_2 receptor blockade in non-responsive patients, versus alternate techniques, such as by increasing monotherapy drug dose. Yet despite this caveat, antipsychotic polypharmacy (i.e., concurrent administration of two or more antipsychotic drugs in the same individual over an extended period) remains a common practice. For example, we recently reported that in an urban outpatient population in Vancouver, Canada approximately 26% of the study population was being treated with persistent antipsychotic polypharmacy [10]. This figure is consistent with a recent meta-analysis reporting worldwide rates of antipsychotic polypharmacy of 20% [155].

There is now considerable clinical evidence that despite inconsistent benefits in terms of clinical efficacy, antipsychotic polypharmacy is frequently associated with an increased prevalence of metabolic side-effects [123, 156] [60, 157, 158], and metabolic dysregulation

appears to be greater in patients on antipsychotic polypharmacy, even after accounting for lifestyle differences [159]. These side-effects include weight gain, hyperlipidemia, hyperglycemia and insulin resistance, all of which have been faithfully modeled in rodent paradigms using individual antipsychotic drugs [125]. As a general consensus, females are the preferred sex for rodent models of antipsychotic drug-induced metabolic dysregulation because they exhibit more reliable metabolic alterations when compared with males, and this parallels the observed trend in humans [97, 124]. However, to our knowledge, rodent paradigms have never been used to model metabolic effects with antipsychotic polypharmacy.

Despite a large number of possible drug-drug combinations, antipsychotic polypharmacy is frequently characterized by combining the SGA clozapine with another drug [160]. This is in part because clozapine represents the optimal drug for treating refractory psychosis, and polypharmacy is often a strategy to deal with refractory illness or as a "last resort" [161]. Commonly, clozapine is combined with a more D_2 "selective" compound, to increase binding of central D_2 receptors. In the present study, our goal was therefore to examine the metabolic side-effects of clozapine when combined with the SGA drug risperidone or the first generation drug haloperidol, and compare these effects to those noted by the drugs on their own. We assessed glucose intolerance and insulin resistance using techniques that are well established with rats to study the metabolic effects of antipsychotic drugs [65, 66, 93, 113, 118, 124, 127, 129].

2.3.2 Materials & methods

Animals

Female, Sprague-Dawley rats (Charles River, Montreal, Canada) weighing 250 to 275 g were pair-housed and maintained on a 12-hour light-dark cycle. Rats were allowed to habituate to the UBC colony for one week prior to experimental testing. Food and water were available *ad libitum*. Animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Approval by the University of British Columbia's Animal Care and Use Committee was obtained.

Drugs

Clozapine (5 mg/kg) and risperidone (1 mg/kg) were purchased from Toronto Research Chemical Inc (Toronto, ON, Canada). Haloperidol (0.1 mg/kg) was purchased from Sigma Aldrich (St. Louis, MO). Clozapine and risperidone were both formulated in a vehicle solution composed of 50% polyethylene glycol 400, 40% distilled water and 10% ethanol. Haloperidol was brought into solution with a vehicle consisting of 0.3% tartaric acid. All drugs were administered via the i.p route in a volume of 1 ml/kg. Dosing solutions were prepared fresh daily. Doses were based on pilot studies with separate animals, where we identified "threshold" doses that were the highest individual doses possible to administer to animals that did not quite induce significant metabolic dysregulation; theoretically, this would permit us to observe combined effects in the absence of single drug effects.

Intraperitoneal glucose tolerance test (see Fig. **2.14** for representation of sequence of events)

One week prior to Experiments 1 and 2, all rats were subjected to a baseline intraperitoneal

glucose tolerance test in which no drug was administered. These values were used to rank

order subjects, in order to create pseudo-random groups matched on mean glucose tolerance.
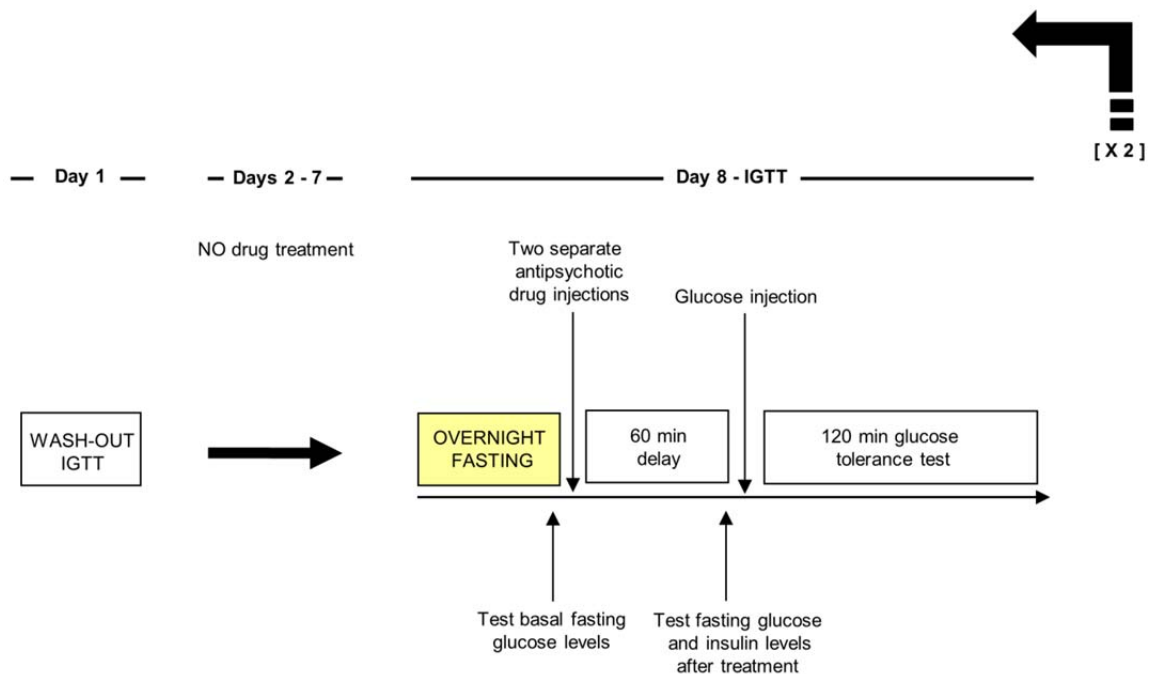
Fig. **2.14**: *Experimental protocol describing acute polypharmacy treatment with clozapine (5.0 mg/kg, i.p), risperidone (1.0 mg/kg, i.p), haloperidol (0.1 mg/kg, i.p) and drug-drug combinations or vehicle with the intraperitoneal glucose tolerance test (IGTT).*

Experiment 1: Rats were pseudo-randomized to receive one of four different treatments

(vehicle, clozapine, risperidone, or clozapine + risperidone) [n = 15-16 animals per group].

Each animal was only tested once and naïve to any experimental treatment prior to this study.

Rats were fasted overnight for 16 ± 2 hours. On the morning of testing, rats were transferred

to the laboratory, weighed and allowed to rest for approximately 20-30 min. A baseline blood

glucose level measurement was then taken from the hind leg using a 25-guage needle to

procure a drop of venous blood, which was measured by a glucometer [64] (One Touch Ultra 2). For all blood draws, animals were wrapped in a towel and the hind leg was exposed; this technique minimizes stress to the animals and so no anesthesia is required [104].

Subsequently, individual drug treatments (clozapine or risperidone) were administered as a single i.p injection along with a second additional i.p injection of vehicle. The polypharmacy group received separate injections of clozapine and risperidone, while the vehicle group received two i.p. injections of vehicle. Thus, each rat received a total of two i.p. injections, which occurred immediately after each other. After one hour following injection of antipsychotic drug treatment, all rats were subjected to a blood glucose measurement and a 200 μL blood extraction procedure with a heparinized capillary tube. This sample of blood was centrifuged for plasma (10 000 RPM, 10 min, 4°C) and stored at -80°C for subsequent analysis of insulin levels. From this point on, blood glucose levels were measured every 15 min for a 120 min time span.

Experiment 2: This was an almost identical procedure to Experiment 1. However, a separate cohort of rats was randomized into four different groups using haloperidol instead of risperidone (vehicle, clozapine, haloperidol, or clozapine + haloperidol) [n = 11-12 animals per group]. All rats in Experiment 2 were treatment naïve.

Insulin measurement

Individual plasma samples were thawed and analyzed for insulin content using Enzyme-Linked ImmunoSorbent Assay (ELISA), as described previously [64, 126, 130] and in Section 2.1.2 of this thesis.

Insulin resistance

Determination of insulin resistance in rats was accomplished using the homeostatic model assessment of insulin resistance (HOMA-IR) equation, as previously reported in Section 2.1.2.

Statistical analysis

Data from Experiments 1 and 2 were analyzed separately. Group differences between glucose and insulin levels and HOMA-IR measured after antipsychotic drug treatment, but before glucose injection, were analyzed by one-way ANOVA, with alpha value set at $p < 0.05$. To measure changes in glucose levels throughout the IGTT, individual glucose measurements at the eight time points during the IGTT were integrated to generate a single area under the curve value, which was subjected to a one way ANOVA to measure group effects. When appropriate, LSD post-hoc tests were conducted. Data were analyzed with SPSS software (version 20), Chicago, IL.

2.3.3 Results

Experiment 1

Pre-drug fasting glucose levels did not differ between groups. By measuring glucose levels in fasting rats at 60 min after they had received the antipsychotic drug, but before the injection of glucose for the IGTT, it was possible to determine how the antipsychotics affected fasting levels of glucose. Analysis of the data indicated a significant main effect of drug treatment on

basal glucose levels [$F_{(3,63)} = 8.89$; $p < 0.0001$]. Post-hoc analysis revealed that while risperidone-treated rats did not differ from vehicle-treated controls, both clozapine- and the polypharmacy-treated rats had significantly higher fasting glucose levels (Table **2.3**). The polypharmacy-treated rats had higher fasting glucose levels than all other groups ($p \leq 0.001$). There was also a main effect of treatment on fasting insulin levels [$F_{(3,63)} = 5.33$; $p < 0.005$]. Post-hoc analysis indicated that the polypharmacy-treated rats had significantly higher insulin levels than all other groups ($p < 0.05$), but no other groups differed significantly (Table **2.3**). For the HOMA-IR index of insulin resistance, the ANOVA indicated a strong significant main effect of drug treatment [$F_{(3,63)} = 7.84$; $p < 0.0001$]. In a manner similar to that observed with insulin values, neither risperidone nor clozapine on their own increased HOMA-IR values compared to vehicle-treated rats (Table **2.3**). However, polypharmacy-treated rats displayed a large increase in insulin resistance compared to all other groups ($p \leq 0.001$). With regards to the IGTT, glucose intolerance (measured as the integrated value for all eight time points) demonstrated a significant main effect of drug treatment [$F_{(3,63)} = 8.76$; $p < 0.0001$]. The post-hoc analysis indicated that both clozapine- and polypharmacy-treated rats had higher glucose intolerance values than vehicle-treated animals (Figure **2.15**). There was a strong trend ($p = 0.057$) for the polypharmacy-treated rats to exhibit higher glucose intolerance than the clozapine-treated animals.

Treatment	I_0 (µU/ml)	G_0 (mmol/L)	HOMA-IR (µU·mmol)/(ml·L)
Vehicle	14.0 ± 3.8	4.7 ± 0.1	2.9 ± 0.8
Clozapine	24.6 ± 6.7	$5.4 \pm 0.2*$	6.0 ± 1.7
Risperidone	32.0 ± 8.4	5.0 ± 0.2	6.8 ± 1.8
Clozapine + Risperidone	$66.2 \pm 15.9^{\#}$	$6.3 \pm 0.3**$	$19.5 \pm 4.7**$
Vehicle	14.2 ± 2.5	4.8 ± 0.3	3.2 ± 0.7
Clozapine	19.7 ± 7.1	5.6 ± 0.3	5.8 ± 2.7
Haloperidol	12.7 ± 2.9	4.7 ± 0.2	2.8 ± 0.7
Clozapine + Haloperidol	$43.2 \pm 13.1**$	6.3 ± 0.8	$15.0 \pm 6.5*$

Table 2.3: *Fasting insulin, glucose levels and HOMA-IR scores in antipsychotic drug treated rats.*

I_0 = fasting insulin levels; G_0 = fasting glucose levels; HOMA-IR = homeostasis model assessment of insulin resistance. Rats were treated with vehicle, clozapine (5 mg/kg), risperidone (1 mg/kg), haloperidol (0.1 mg/kg) or drug-drug combination. Values represented as means ± SEM.

\# $p < 0.05$ vs. all other groups
* $p < 0.05$ vs. vehicle
** $p < 0.01$ vs. all other groups
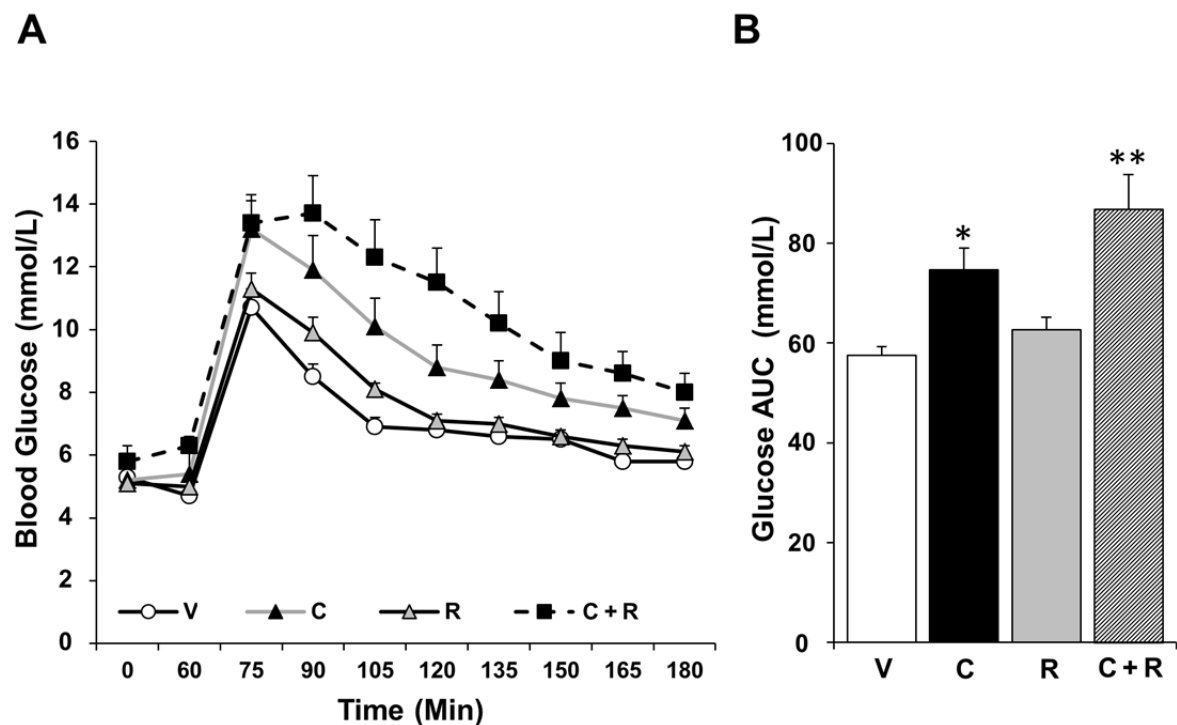
Fig. **2.15**: *Experiment 1: The acute effects of clozapine and risperidone combination treatment on glucose levels in adult female rats*. (**A**) Animals (n = 15-16 per group) received a two separate i.p injections of either: vehicle-vehicle [V], vehicle-clozapine (5 mg/kg) [C], vehicle-risperidone (1.0 mg/kg) [R] or clozapine + risperidone [C + R]. Glucose levels were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then 60 min following drug administration (*x-axis*). Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2 hours. (**B**) Total cumulative glucose levels for each treatment group are summed as area-under-the-curve. Values represent group means ± SEM. * $p < 0.05$ vs. [V]; ** $p < 0.01$ vs. [V] and strong trend ($p = 0.057$) vs [C].

Experiment 2

Pre-drug fasting glucose levels did not differ between groups. Analysis of fasting glucose levels 60 min after antipsychotic drug treatment revealed increased glucose levels in the polypharmacy group but the overall main effect of drug treatment was non-significant ($p = 0.06$) (Table **2.3**). However, there was a main effect of drug treatment on fasting insulin levels [$F_{(3,44)} = 3.54$; $p = 0.05$]. In the post-hoc analysis, the polypharmacy-treated group had

higher insulin levels than all other groups ($p < 0.01$) while no other groups differed from each other (Table **2.3**). The ANOVA indicated a main effect of drug treatment on HOMA-IR values [$F_{(3,44)} = 2.80$; $p = 0.05$], whereby the polypharmacy-treated group displayed significantly greater insulin resistance than all other groups ($p \leq 0.05$) (Table **2.3**). With regard to the IGTT, glucose intolerance displayed a strong main effect of antipsychotic drug treatment [$F_{(3,44)} = 11.15$; $p = 0.001$]. This was entirely due to a large increase in glucose intolerance by the polypharmacy-treated group compared to all other groups ($p < 0.001$) (Figure **2.16**).
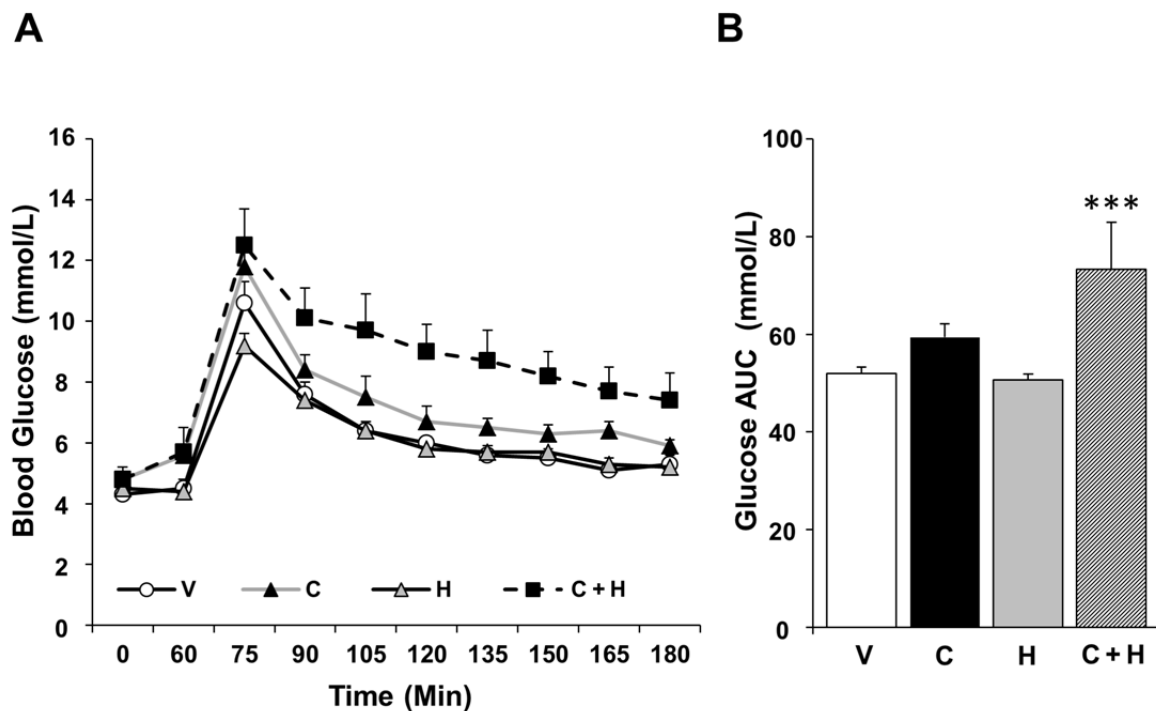
Fig. **2.16**: *Experiment 2: The acute effects of clozapine and haloperidol combination treatment on glucose levels in adult female rats.* (**A**) Animals (n = 11-12 per group) received a two separate i.p injections of either: vehicle-vehicle [V], vehicle-clozapine (5 mg/kg) [C], vehicle-haloperidol (0.1 mg/kg) [H] or clozapine + haloperidol [C + H]. Glucose levels were recorded prior to drug treatment in overnight-fasted rats at Time 0, and then 60 min following drug administration (*x-axis*). Immediately following this glucose measurement, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for

the next 2 hours. (**B**) Total cumulative glucose levels for each treatment group are summed as "area under the curve." Values represent group means ± SEM. ******* $p < 0.05$ vs all other groups.

2.3.4 Discussion

In the present study, the acute metabolic effects of antipsychotic drugs were assessed in adult female rats using the glucose tolerance test. This technique provided multiple measures of metabolic function following antipsychotic drug administration, including fasting glucose levels, fasting insulin levels, insulin resistance (measured by HOMA-IR) and tolerance to a glucose challenge. Compared to vehicle-treated control animals, the current doses of risperidone and haloperidol had no effect on any of the metabolic indices when administered on their own. In the first experiment, the clozapine-only treated rats exhibited mild but significant increases in fasting glucose and glucose intolerance with the IGTT. However, addition to clozapine of the same dose of risperidone that had no effect by itself significantly increased fasting glucose, fasting insulin and insulin resistance compared to the clozapine-only group, with a strong trend towards greater glucose intolerance in the IGTT. In the second experiment, with a separate cohort of rats, clozapine-treated rats did not differ from vehicle-treated controls on any metabolic index. Addition of a dose of haloperidol, at a dose that had no effect by itself, to clozapine significantly increased fasting insulin levels, insulin resistance and glucose intolerance.

These results therefore indicate that it is possible to demonstrate synergistic metabolic side-effects of antipsychotic polypharmacy in a rodent model. Numerous previous studies have confirmed the potent acute effects of antipsychotic drugs on metabolic dysregulation in female rats using antipsychotic monotherapy [125]. With regards to the drugs used in the

91

present study, clozapine has been shown to induce consistent metabolic dysregulation using a number of different protocols [66, 71, 73, 77, 127]. Metabolic effects of risperidone have been less consistent. For example, two groups who used the glucose tolerance test in female rats did not observe glucose intolerance with a dose of 0.5 mg/kg of risperidone [124, 162]. However, we noted previously that metabolic effects of antipsychotic drugs, including glucose intolerance, are dose-dependent, and could be observed with risperidone at higher doses [64]. In the present study, we used a dose of 1 mg/kg of risperidone, which in our experience represents a dose on the "cusp" of being able to induce glucose intolerance. Similar to risperidone, haloperidol's effects on glucose intolerance are less consistent in the literature than with clozapine. Smith and colleagues noted a modest but significant effect of 0.25 mg/kg haloperidol on glucose tolerance [77], while Victoriano and colleagues observed greater hyperglycemia only during the earlier stages of the glucose tolerance test in rats treated with 1 mg/kg haloperidol [65]. We also noted dose-dependent effects of haloperidol on glucose intolerance [64], with significant increases in glucose levels with a dose of 1 mg/kg but not 0.1 mg/kg, consistent with the present findings. As a whole, our current results with single drug treatments are therefore largely congruent with the field.

The major novel finding of this study is that when a second antipsychotic drug is added to clozapine, at a dose that has no effect by itself, the resulting effect on metabolic dysregulation is greater than with either drug alone. This was noted for both risperidone and haloperidol. To our knowledge, this effect has never been demonstrated previously in a preclinical model. Nevertheless, there is a growing body of research in the clinical literature which describes greater metabolic dysregulation in patients treated with antipsychotic polypharmacy than monotherapy [156]. The majority of studies that have prospectively

compared metabolic dysregulation in monotherapy- versus polypharmacy-treated patients have observed greater metabolic dysregulation in the latter [163, 164] [but see [165]]. The issue has been raised that this may simply reflect differences between patient populations [166], as many studies of polypharmacy use a naturalistic intake of subjects rather than randomization. However, even when lifestyle factors are controlled for statistically, patients on polypharmacy continue to exhibit greater metabolic dysregulation [159]. In a randomized clinical trial of antipsychotic polypharmacy, where we compared patients on clozapine alone to those on clozapine plus risperidone, we noted significantly higher fasting glucose levels in the polypharmacy patients [167], similar to the results of the current study with rodents.

At present, the biological basis of greater metabolic dysregulation in patients treated by antipsychotic polypharmacy remains unknown. Indeed, even for antipsychotic monotherapy, the physiological substrates of metabolic dysfunction, including insulin resistance and glucose tolerance, continue to be debated and are far from determined [157, 168]. Preclinical studies have identified a range of molecular targets, including diverse receptors and signaling pathways, that may contribute to metabolic dysregulation after treatment with antipsychotic drugs [reviewed in [125]]. It is possible that by using two distinct antipsychotic drugs with differing affinities for receptors, additional signaling pathways are activated - compared to those that would be with only a single drug - resulting in a greater activation of pathways that contribute to metabolic dysregulation. Alternatively, it is possible that addition of a second antipsychotic drug increases levels of the first. For instance, while clozapine is primarily metabolized by cytochrome P450 1A2, a significant proportion of the drug is also metabolized by the high-affinity, low capacity enzyme 2D6 [169]. As 2D6 is the primary metabolizing enzyme of risperidone, it is possible that

polypharmacy treatment with risperidone would result in competition for 2D6, and lead to a slower metabolism of clozapine, resulting in higher levels of the drug and – potentially – greater metabolic dysregulation. These and other possibilities clearly require future study, highlighting the need for valid animal models of antipsychotic polypharmacy.

There are a number of limitations associated with the present study. The first of these is that we only used a single dose of each drug. This was done for strategic reasons, as the evaluation of antipsychotic polypharmacy with two or more different drugs would represent a significant challenge to resources if multiple doses of each drug were studied, because it would result in a rapidly expanding number of permutations. The current doses of drugs were chosen to maximize our sensitivity to seeing increases in metabolic dysregulation. It is possible that other combinations of doses would be less sensitive to polypharmacy-induced increases in metabolic dysregulation, although this remains to be determined empirically. However, the doses of antipsychotics we used were well within the normal range of those used by other research groups to study metabolic side-effects [125], and so the results are highly relevant to previous studies in the literature. Another limitation of the present study was that we only examined two different polypharmacy combinations. In theory, many hundreds of different combinations are possible, given the number of existing antipsychotics, yet in clinical practice the number of drug-drug combinations used is much less than this [155, 161, 170]. We focused on clozapine polypharmacy, as this remains one of the best characterized combination strategies [171]. Nevertheless, it will be important for future studies to identify other drug-drug antipsychotic combinations commonly used in clinical practice to evaluate their potential liability for increased metabolic side-effects. An additional weakness to the current study involves the use of a surrogate index of insulin resistance

(HOMA-IR) in contrast to a more direct technique, such as the insulin tolerance test (ITT). One preclinical study that utilized the ITT in male rats demonstrated that clozapine monotherapy does not decrease insulin sensitivity, despite inducing derangements in glucose tolerance [69]. Evidence obtained from the same research group was further supported by the opposing changes in glucoregulatory hormones: in the face of hyperinsulinemia, increased glucagon and decreased glucagon-like peptide-1 (GLP-1) levels resulted from clozapine monotherapy [70]. Therefore, in line with this previous data, and as a result of feasibility issues that preclude the use of direct measurement, we speculate that the increased HOMA-IR scores after polypharmacy treatment may only approximate insulin resistance, and the impact of direct techniques such as the ITT needs further investigation.

In summary, the present article demonstrates the feasibility of utilizing an antipsychotic drug polypharmacy treatment approach to studying the metabolic side-effects of these drugs in rodents. The results with rats are largely consistent with the human clinical data, and thus offer a potential model to better understand the physiological basis of such effects, as well as predictive power to identify combinations that may be particularly harmful in the clinic.

2.4 Study 4: Intermittent treatment with olanzapine causes sensitization of the metabolic side-effects in rats

2.4.1 Overview

We recently demonstrated that the metabolic effects of olanzapine are both dose and time dependent, indicating that the drug can acutely affect both glucose regulation and insulin sensitivity [64]. However, what remains unknown is whether the acute metabolic effects of olanzapine show any adaptation over time. This is an important issue, as clinical studies typically assess metabolic indices only after chronic treatment [172], and it would be important to know if changes can occur over time: it has previously been shown that some properties of antipsychotic drugs can exhibit tolerance in rodents with repeated treatment, e.g. [173]. Studies using the euglycemic clamp in rodents have only ever tested for insulin resistance at a single time point after drug treatment [78, 79], due to technical issues, and so it has not been possible to measure putative changes in the metabolic effects of antipsychotic drugs over time. The goal of the present study was therefore to address this issue in the first longitudinal study of glucose intolerance and insulin resistance with an atypical antipsychotic drug, using the glucose tolerance test in animals treated chronically with olanzapine for 10 weeks.

2.4.2 Materials & methods

Animals

Female, Sprague-Dawley rats (Charles River, Montreal, Canada) weighing 250 to 275 g were group housed and maintained on a 12-hour light-dark cycle (lights on at 07:00 hours) in a temperature controlled colony at 22 ± 1ºC. Females are used because they exhibit much more consistent metabolic dysregulation than male rats (e.g. [94, 124]), and so are routinely the preferred sex in rodent models of antipsychotic drug-induced metabolic dysregulation; previous studies have indicated that there are no significant effects of olanzapine on uterine weights and estrous cycle synchronicity with chronic treatment [174, 175]. Rats were allowed to habituate to the UBC colony for one week prior to experimental testing. Food and water were available *ad libitum*. Animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Approval by the University of British Columbia's Animal Care and Use Committee was established for all methods.

Drug treatment and follow-up

The dose of olanzapine (15 mg/kg, i.p.) [Toronto Research Chemicals Inc, Toronto, ON, Canada] was carefully chosen, based on previously reported studies [64, 103], to represent the higher end of pharmacological relevant levels *in vivo*, so that potential tolerance effects could be observed. Doses as high as 20-40 mg/kg are commonly used in the literature to examine the metabolic side-effects of olanzapine [80, 113, 127]. The vehicle solution consisted of 50% polyethylene glycol 400, 40% distilled water and 10% ethanol. All

solutions were compounded fresh daily and administered via the intraperitoneal route in a volume of 1 ml/kg. Individual rat weights were monitored and recorded on a daily basis throughout the study. Standard rat chow consumption was recorded once a week at 16 ± 2 hours prior to the glucose tolerance test and was measured on a cage-by-cage basis (n = 3 per cage).

Weekly intraperitoneal glucose tolerance test (IGTT) (see Fig. **2.16** for representation of sequence of events)

In the current protocol, the effects of olanzapine were monitored once per week for a 10 week period. Two separate groups of rats [n = 18 per group] were randomly assigned to receive chronic treatment during this period with either olanzapine or vehicle. As we wished to compare changes in the acute response to an antipsychotic drug challenge over time, rats were weekly administered olanzapine (or vehicle) for five days continuously, followed by a two day "washout" period. This was so that when rats were re-challenged with olanzapine at the end of the washout period, over 10 drug half-lives had passed, and there was no residual drug from the previous five days of treatment. The drug challenge consisted of a single treatment with either the olanzapine dose (15 mg/kg, i.p.) or vehicle.
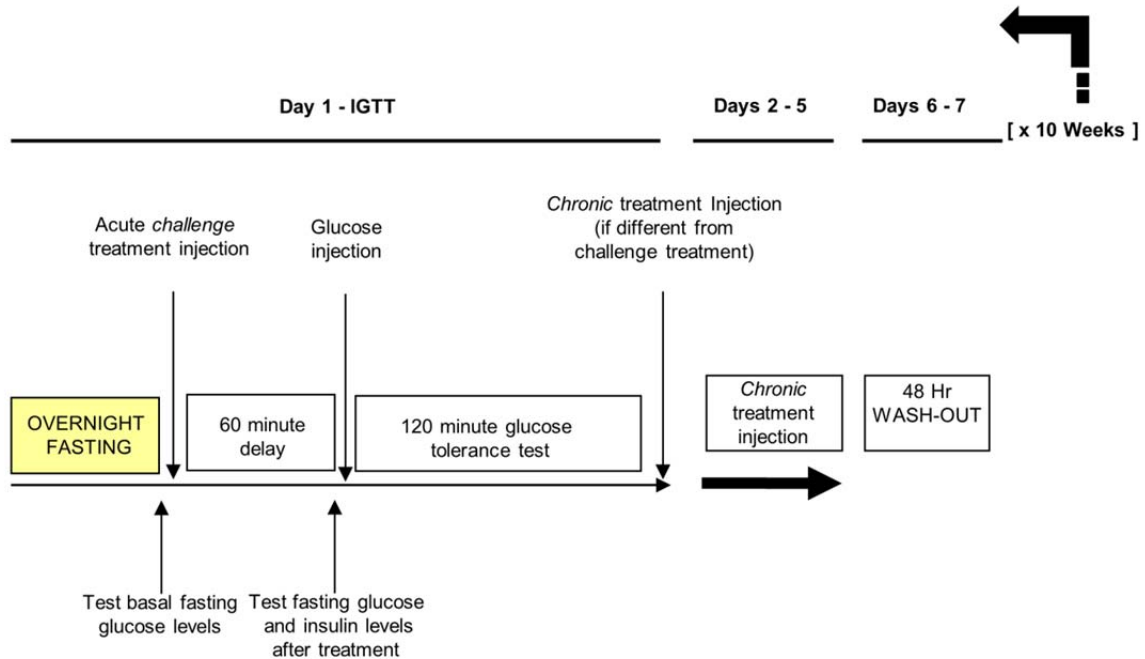
Fig**. 2.17**: *Experimental protocol describing the weekly treatment regimen with chronic olanzapine (15 mg/kg, i.p) and the repeated measures intraperitoneal glucose tolerance test (IGTT).*

This resulted in a matrix of four different treatment groups [n = 9 per group]: one group treated for five days per week with olanzapine and challenged following the washout period two days later with olanzapine [O-O], one group treated for five days per week with olanzapine and challenged with vehicle [O-V], one group treated for five days per week with vehicle and challenged with olanzapine [V-O], and one group treated and challenged with only vehicle [V-V]. The experiment began with an acute challenge with olanzapine or vehicle for the appropriate groups, to allow subsequent comparison over time to the initial effects of the antipsychotic drug on glucose tolerance and insulin sensitivity: this represented the "baseline" response to olanzapine against which subsequent challenges over the next 10 weeks were compared.

Rats were fasted overnight for 16 ± 2 hours. On the morning of testing, rats were transferred to the laboratory, weighed and allowed to rest for approximately 15-20 min. A baseline blood glucose level measurement was then taken from the hind leg using a 25-gauge needle to procure a drop of venous blood, which was measured by a glucometer (One Touch Ultra 2). For all blood draws, animals were wrapped in a towel and the hind leg was exposed; this technique minimizes stress to the animals and so no anesthesia is required [104]. Olanzapine or vehicle was then administered acutely as a single i.p. injection. Sixty min after olanzapine treatment, a second measurement of glucose levels was taken, to assess the effects of drug treatment on fasting glucose levels. Immediately afterwards, a saphenous blood draw (200 μl) using heparinized collecting tubes, was performed to obtain plasma for measurement of insulin levels; blood samples were centrifuged (10,000 RPM, 10 Min, 4ºC) and plasma samples were stored at -80ºC for analysis. The IGTT commenced approximately 5 min after saphenous blood draws, as all rats were given a challenge i.p injection of 1 g/ml/kg of glucose. Glucose levels were then measured every 15 minutes for 120 min duration: combined "area-under-the-curve" glucose values for this period are used for analysis [64]. Each animal handler was blinded to respected drug treatment. This procedure was repeated identically for all subsequent weeks.

Insulin measurement

Individual plasma samples extracted during each IGTT were analyzed for insulin content using Enzyme-Linked ImmunoSorbent Assay (ELISA), using techniques similar to previously [176, 177] and in Section 2.1.2 of this thesis.

Insulin resistance

Determination of insulin resistance in rats was accomplished using the homeostatic model assessment of insulin resistance (HOMA-IR) equation, as first presented in Section 2.1.2.

Tissue extraction

After the last IGTT, animals received their final corresponding chronic treatment injection and after 48 hours, animals were sacrificed. A lethal overdose of sodium pentobarbital (130 mg/kg) was administered i.p, and the upper left lobe of the liver, kidneys, adrenal glands, whole heart, left sole gastrocnemius muscle, intra-abdominal adipose tissue (periovarian, retroperitoneal and inguinal fat pads) were extracted and weighed.

Statistical analysis

All variables that were repeatedly tested from baseline were analyzed by repeated measures Analysis of Variance (ANOVA), with weekly drug treatment and drug challenge as the between-subjects factors, and time as the within-subject factor, with alpha value set at $p <$ 0.05. These variables included: fasting levels of glucose prior to and 60 min after the drug challenge treatment, the area under the curve (AUC) for the glucose tolerance test, fasting post-drug insulin, HOMA-IR values, body weights and food consumption. When appropriate, LSD post-hoc tests were conducted: this particular post-hoc test is routinely used in the literature, and is consistent with our prior studies in the field. At the end of the experiment, tissue weights were analyzed by two factor ANOVA; this was performed both on absolute tissue weights and also corrected tissue weights due to differences in body weights between the groups (see below, Results). For corrected weights, values were divided by mean body

weights for the relevant group. Associations between body weight and metabolic parameters were tested using Pearson's correlation. Data were analyzed with SPSS software, Chicago, IL.

2.4.3 Results

Weekly fasting glucose levels:

The repeated measures ANOVA indicated that there was a significant main effect of time (i.e., week of testing) on fasting, pre-drug challenge glucose levels [$F_{(10,320)}$ = 8.50, p < 0.001] which reflected a global general reduction in glucose levels over time (Table **2.4**). This did not vary systematically with treatment group. By contrast, the analysis of post-drug challenge fasting glucose levels revealed a significant three way interaction between time, weekly drug treatment and acute drug challenge treatment groups [$F_{(10,320)}$ = 8.10, p < 0.005] (Table **2.4**). Further analysis of this interaction revealed that during the first week of baseline testing, this was due to a trend for greater fasting glucose levels in the two groups that were treated with an olanzapine challenge compared to vehicle challenge rats. However, by the second week of testing, the group of rats that was treated weekly with vehicle but challenged with olanzapine [V-O] after the washout exhibited significantly greater fasting glucose levels than all other groups. This continued to be the case for all but the fourth, fifth and sixth weeks of testing, when the [V-O] group did not differ from the [O-O] group.

Tx Group	Measure	Time (weeks)										
		1	2	3	4	5	6	7	8	9	10	11
[O-O]	G_0	4.9± 0.1	6.0±0.4	4.9± 0.3	6.0± 0.2	5.0± 0.2	4.9± 0.3	5.6± 0.3	4.8± 0.3	5.4± 0.4	4.3± 0.1	4.2± 0.2
	$G_0^{\#}$	6.4*± 1.0	7.8*± 0.5	5.8± 0.4	6.6*± 0.6	9.0*± 2.0	6.2± 0.3	5.7*± 0.4	5.6*±0.4	4.8± 0.5	5.5± 0.5	5.7*± 0.3
	I_0	27.9*± 7.0	20.1*± 1.8	26.9*± 5.8	32.0*± 5.7	22.1*± 1.8	19.3*± 2.2	18.7± 4.7	19.5* ±2.2	26.0*± 4.8	19.5*± 1.7	25.4* ±3.5
[O-V]	G_0	5.1 ±0.1	5.2± 0.8	4.8± 0.3	5.6± 0.4	4.5± 0.2	4.8± 0.2	6.5±0.8	5.2±0.5	4.2±0.1	5.1± 0.5	4.1± 0.2
	$G_0^{\#}$	4.7 ±0.2	3.8± 0.2	4.0± 0.2	3.3± 0.1	3.6± 0.2	3.7± 0.1	3.9± 0.3	3.9± 0.1	3.4± 0.1	3.4± 0.2	3.7± 0.1
	I_0	11.7± 1.1	7.2± 0.9	8.7 ±1.8	16.1± 5.2	7.8± 1.0	9.3± 0.7	9.5 ±0.9	8.1±0.5	6.5± 0.5	7.9± 0.6	6.7±0.7
[V-O]	G_0	5.7± 0.1	7.0± 0.5	5.5± 0.3	6.0± 0.2	5.2±0.1	4.8±0.1	4.6±0.2	5.9±0.3	5.3± 0.2	4.7± 0.1	4.6± 0.3
	$G_0^{\#}$	6.3*± 0.8	11.6†± 1.0	9.5†±0.9	7.7*±0.6	9.9*±0.6	8.2*±0.9	9.5†±0.9	8.0†± 0.4	9.2†± 0.9	9.7†± 0.6	9.8†± 0.6
	I_0	17.1*± 3.6	25.4*± 4.0	26.2*± 5.3	36.6*± 5.4	46.3†± 8.0	33.0† ±4.9	27.8* ±4.4	30.9†± 5.5	25.1*± 2.0	30.9†± 4.2	40.9†± 4.2
[V-V]	G_0	4.8± 0.2	6.5±0.8	5.3±0.3	5.4±0.3	5.2±0.3	5.6±0.4	6.0±0.5	5.2± 0.4	5.4± 0.3	5.0± 0.2	4.8± 0.2
	$G_0^{\#}$	5.2±0.4	5.0±0.3	5.7±0.6	4.5±0.2	7.1± 1.4	5.7± 1.1	4.3±0.2	4.4 ±0.2	5.0± 0.3	5.4 ±0.6	4.6± 0.2
	I_0	10.6± 0.5	7.6± 0.5	8.9± 1.2	14.4± 2.5	12.4± 2.2	10.6± 1.6	15.0 ±1.8	10.3± 0.9	12.0±1.5	11.1± 0.7	8.7 ±0.7

Table 2.4: *Mean concentration of fasting glucose and insulin for olanzapine treated rats.*
Tx = Treatment; [O – O] = chronic olanzapine, challenge olanzapine; [O – V] = chronic olanzapine, challenge vehicle; [V – O] = chronic vehicle, challenge olanzapine; [V – V] = chronic vehicle, challenge vehicle; G_0 = fasting glucose levels (mmol/L); $G_0^{\#}$= fasting glucose levels post-challenge treatment; I_0 = fasting insulin levels (μU/ml);. Rats were treated with vehicle, or olanzapine (15 mg/kg).Values represented as means ± SEM. *p < 0.05 vs. [V - V] and [O - V] groups† p < 0.05 vs. all other groups

Comparing all four treatment groups to their own baseline post-drug challenge glucose levels by examining main effects of time for each group, both the [V-V] and [O-O] showed no consistent changes over time in glucose levels. In comparison, the [O-V] group showed a consistent decrease in glucose levels compared to baseline [$F_{(10,80)} = 6.39$, $p < 0.001$]. Most importantly, the animals treated weekly with vehicle and challenged with olanzapine [V-O] showed a major effect of time [$F_{(10,80)} = 3.89$, $p < 0.001$], in which post-challenge drug glucose levels were significantly higher than baseline values at all time points other than week four.

Weekly glucose tolerance test values:

As previously [64], the eight individual postprandial glucose values (t= 75 to 180 min) during the weekly IGTTs were integrated to generate a single area under the curve (AUC) value which was analyzed (Fig **2.18** & **2.19**, inset figures, top right of each graph). The repeated measures ANOVA indicated a significant interaction between time, weekly drug treatment and challenge drug treatment [$F_{(10,320)} = 2.82$, $p < 0.005$]. Further analysis of the results with post-hoc tests yielded a pattern of results similar to those for post-drug challenge fasting glucose levels. At baseline testing, both of the olanzapine challenged groups showed significantly greater AUCs than the vehicle challenged rats. The rats treated weekly with olanzapine and challenged with olanzapine [O-O] continued to display greater glucose AUCs than both of the vehicle challenged groups at all time points throughout the experiment. However, a major (but unexpected) finding was that the animals treated during the week with vehicle but challenged with olanzapine [V-O] displayed glucose AUCs greater than not only

both of the vehicle challenged groups, but also greater than the [O-O] group (Fig **2.20**). This effect was significant by the second week ($p < 0.001$), was a strong trend in weeks three and four, and remained highly significant thereafter until the end of the experiment ($p < 0.001$ each week). Hence, AUCs collapsed across all time points indicated that values were much greater in the [V-O] than the [O-O] group for the entire experiment ($p < 0.001$). To determine if this was due to a decrease in AUC values by the [O-O] group, an increase in values by the [V-O] group, or a combination of both, we compared the main effects of time for each treatment group. The [O-O] group showed no significant effect of time, and thus at no time point did the AUC glucose levels differ significantly from baseline values. By contrast, there was a large main effect of time in the [V-O] group [$F_{(10,80)} = 2.94$, $p < 0.005$]; post-hoc tests indicated that was because glucose AUC levels were increased compared to baseline values, commencing with the second weekly test (effects at weeks three and four were not significant, $p < 0.15$).
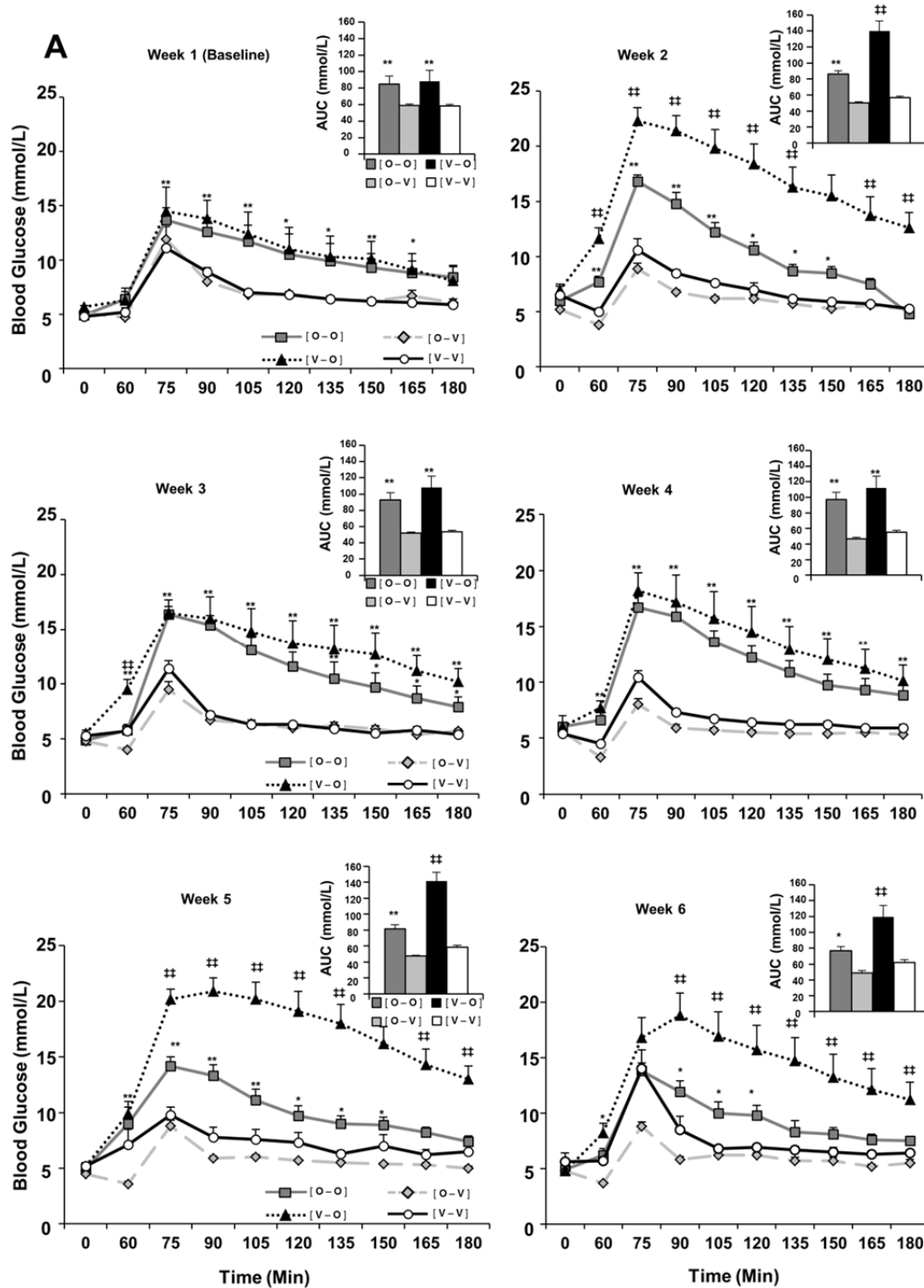
Fig. **2.18**: *The effects of chronic olanzapine on glucose tolerance for 10 consecutive weeks (A) weeks 1 - 6*. Adult female rats (n = 9 per group) received chronic administration of either olanzapine (15 mg/kg, i.p.) or vehicle for 5 consecutive days followed by a 48 hour "washout" period. Subsequently, fasted animals then received a challenge intraperitoneal injection of either olanzapine or vehicle. Glucose levels were recorded prior to treatment (Time 0) and then at 60 min. All rats were subjected to a glucose tolerance test (1 g/ml/kg of

glucose, i.p.), and blood glucose levels were measured every 15 min for a 2 hour duration. This procedure was repeated for 10 weeks. Total cumulative glucose levels for each treatment group are summed as "area under the curve" by graph insets (top right of each graph). Values represent group means ± SEM. *$p < 0.05$ vs. both [V-V] & [O-V];**$p < 0.01$ vs. both [V-V] & [O-V]; # $p < 0.05$ vs. all other groups; ## $p < 0.01$ vs. all other groups.

Fig. **2.19**: *The effects of chronic olanzapine on glucose tolerance for 10 consecutive weeks (B) weeks 7 - 11.* Adult female rats (n = 9 per group) received chronic administration of either olanzapine (15 mg/kg, i.p.) or vehicle for 5 consecutive days followed by a 48 hour

"washout" period. Subsequently, fasted animals then received a challenge intraperitoneal injection of either olanzapine or vehicle. Glucose levels were recorded prior to treatment (Time 0) and then at 60 min. All rats were subjected to a glucose tolerance test (1 g/ml/kg of glucose, i.p.), and blood glucose levels were measured every 15 min for a 2 hour duration. This procedure was repeated for 10 weeks. Total cumulative glucose levels for each treatment group are summed as "area under the curve" by graph insets (top right of each graph). Values represent group means ± SEM. *p < 0.05 vs. both [V-V] & [O-V];**p < 0.01 vs. both [V-V] & [O-V]; # p < 0.05 vs. all other groups; ## p < 0.01 vs. all other groups.

Fig. **2.20**: *The effects of chronic olanzapine on glucose intolerance for 10 consecutive weeks.* Total cumulative glucose levels for each treatment group are summed as "area under the curve" from week 1 (baseline) through to week 11. Values represent group means ± SEM. *p < 0.05 vs. both [V-V] & [O-V]; **p < 0.01 vs. both [V-V] & [O-V]; #p < 0.05 vs. all other groups; ##p < 0.01vs. all other groups.

Post-drug challenge insulin levels:

Rat plasma samples were obtained at the 60 min time point following challenge antipsychotic drug treatment injection (but before glucose injection) and were analyzed by ELISA methods. The repeated measures ANOVA indicated a significant interaction between time, weekly drug treatment and challenge drug treatment [$F_{(10,320)} = 1.97$, $p < 0.05$] (Table **2.4**). Post-hoc tests revealed that treatment effects were evident as greater insulin levels in both of the olanzapine challenged groups compared to both of the vehicle challenged groups. The increases in insulin were greater in the [V-O] than the [O-O] group but somewhat intermittent, attaining significance only on weeks five, six, eight, ten and eleven.

HOMA-IR values:

To calculate whole-body insulin resistance, equation (1) was utilized to obtain HOMA-IR scores. The values were calculated based from the fasting post-drug challenge glucose and insulin levels described above, i.e. at the 60 min time point after challenge treatment but before glucose injection for the IGTT. Analysis with the repeated measures ANOVA indicated a significant main effect of time [$F_{(10,320)} = 3.00$, $p = 0.001$], a significant time by weekly drug treatment [$F_{(10,320)} = 2.08$, $p < 0.05$] and a significant time by drug challenge interaction [$F_{(10,80)} = 2.68$, $p < 0.005$]. Post hoc tests revealed a similar pattern of results as in the IGTT (Fig. **2.21**), whereby the [O-O] group displayed values consistently higher than both of the vehicle-challenged groups, with the exception of week seven. The [V-O] group displayed significantly greater insulin resistance than all other groups by the second week of testing, with the exception of week four, when it was only marginally greater than the [O-O] group; by the fifth week of testing HOMA-IR values were reliably 2-3 times greater in the

109

[V-O] than the [O-O] group. Again, the increased effects in the [V-O] group was due to an increase in HOMA-IR values compared to baseline over time.
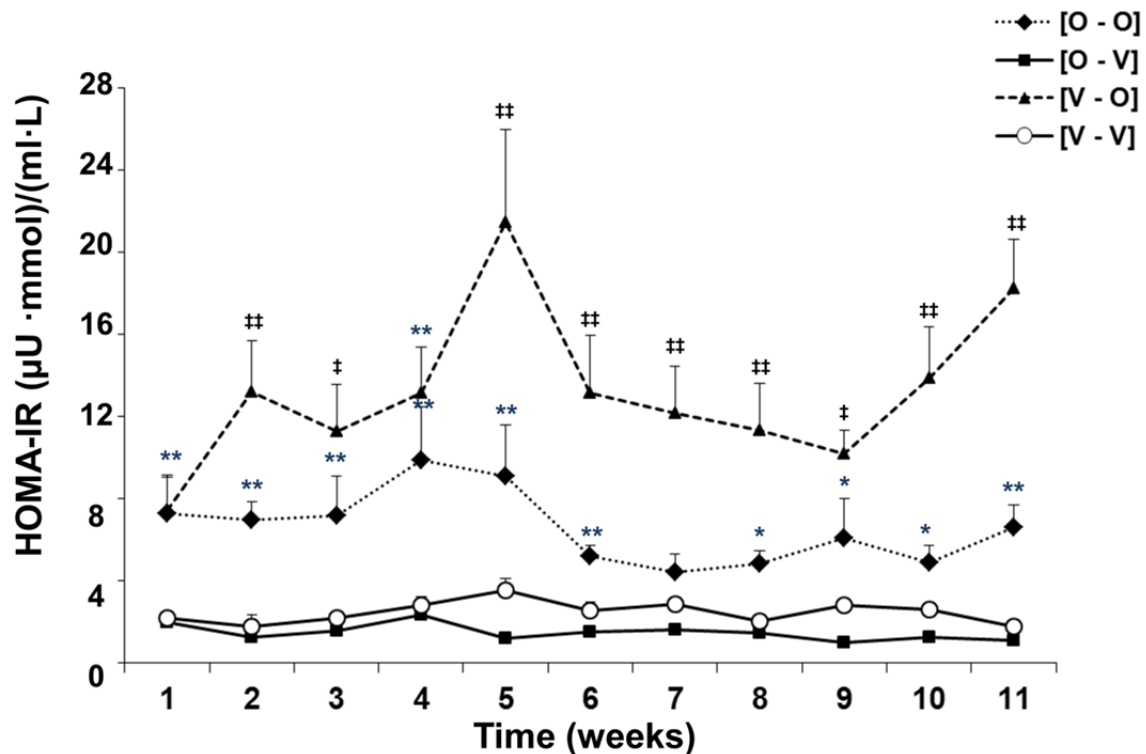
Fig. **2.21**: *The effects of chronic olanzapine on insulin resistance for 10 consecutive weeks.* Average HOMA-IR scores for each treatment group are calculated from week 1 through to week 11. Values represent group means ± SEM. *$p < 0.05$ vs. both [V-V] & [O-V]; **$p < 0.01$ vs. both [V-V] & [O-V]; #$p < 0.05$ vs. all other groups; ##$p < 0.01$ vs. all other groups.

Food consumption and rat body weights:

Food consumption was monitored on a weekly basis. The repeated measures ANOVA revealed that there was a main effect of time [$F_{(10,80)} = 30.39$, $p < 0.0001$], in which overall food consumption increased with time as the rats gained weight, but this did not differ systematically between groups (Table **2.5**), indicating that the repeated treatment with olanzapine did not cause any motor sedation that interfered with this behavior. Rat body weights were recorded on a daily basis from baseline to the end of the experiment, and mean weights over the entire week were used for analysis; as we are interested specifically in

weight gain, percentage weight gain from baseline values was used for analysis (Fig **2.22**).

The repeated measures ANOVA showed a clear effect of time as all rats gained weight

$[F_{(10,320)} = 122.24, p < 0.0001]$, as well as an interaction between time and weekly drug

treatment $[F_{(10,320)} = 18.95, p < 0.001]$ but no effect of challenge treatment: this was reflected

as a greater weight gain in both groups of the vehicle ([V-V] and [V-O]), compared to

olanzapine treated animals ([O-O] and [O-V]). There was no significant correlation between

weight gain and fasting glucose values, IGTT glucose AUCs or insulin resistance at either

the beginning or end of the study.

Mean rat chow consumption (g)				
Time (weeks)	Treatment			
	[O – O]	[O – V]	[V – O]	[V – V]
1	108 ± 7	106 ± 7	112 ± 7	105 ± 8
2	129 ± 5	126 ± 3	136 ± 2	132 ± 7
3	112 ± 4	113 ± 4	114 ± 4	103 ± 2
4	123 ± 7	122 ± 4	142 ± 9	125 ± 7
5	152 ± 1	157 ± 6	136 ± 2	163 ± 3
6	133 ± 2	132 ± 1	137 ± 2	136 ± 1
7	134 ± 1	138 ± 2	132 ± 1	134 ± 1
8	133 ± 2	134 ± 3	132 ± 3	137 ± 2
9	126 ± 3	120 ± 7	147 ± 5	129 ± 2
10	134 ± 2	133 ± 2	134 ± 1	133 ± 1
11	141 ± 3	135 ± 2	133 ± 2	132 ± 1
12	142 ± 4	136 ± 2	134 ± 2	135 ± 1

Table **2.5**: *Mean rat chow consumption in olanzapine drug treated rats.*

[O – O] = chronic olanzapine, challenge olanzapine; [O – V] = chronic olanzapine, challenge
vehicle; [V – O] = chronic vehicle, challenge olanzapine; [V – V] = chronic vehicle,
challenge vehicle. Rats were chronically treated with olanzapine (15 mg/kg-daily i.p) or
vehicle for 5 consecutive days. Once a week, all rats were subjected to an IGTT where each
rat was challenged with either olanzapine or vehicle. Rat food consumption was weighed on
a weekly basis per cage (n = 3) from week 1 (baseline) through to week 12, prior to sacrifice.
Values represent group means / 3 rats ± SEM.
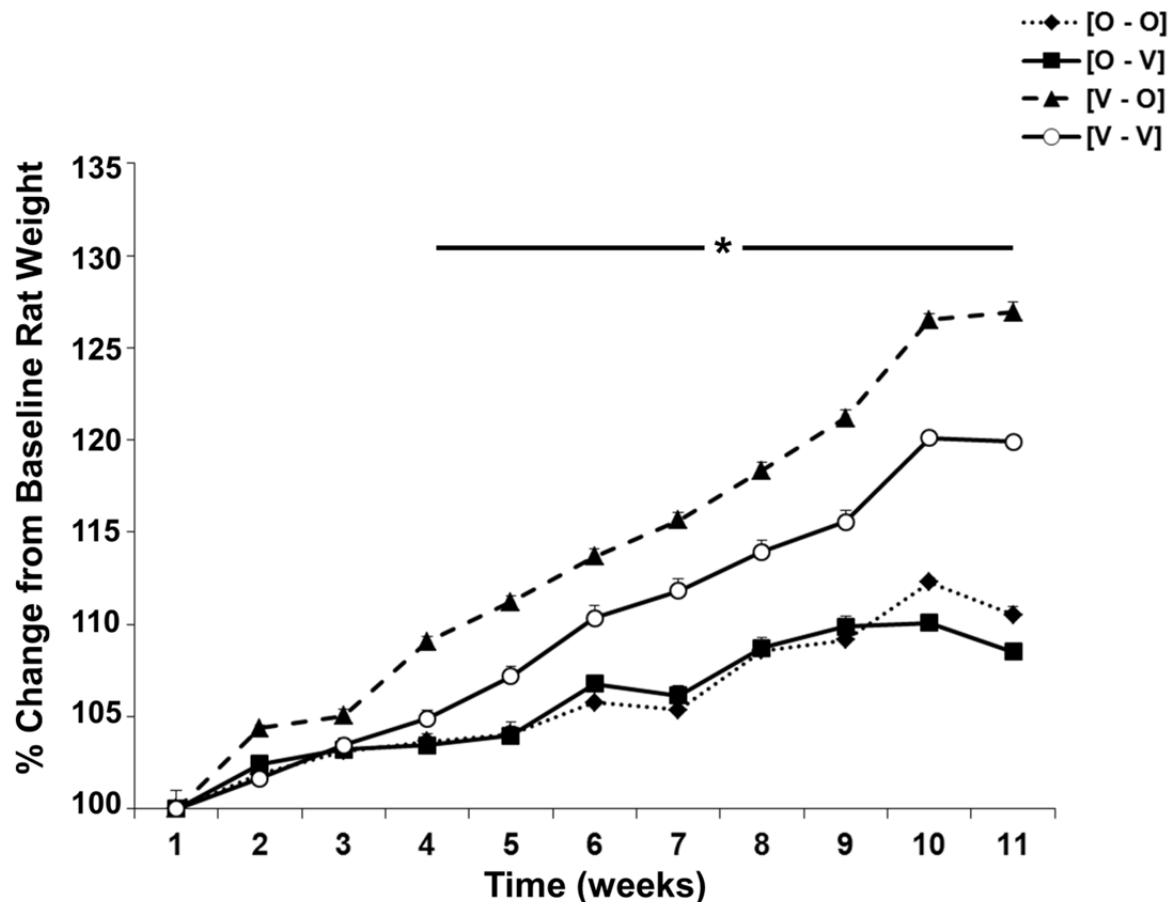
Fig**. 2.22**: *The effects of chronic olanzapine on rat weights from week 1 (baseline) through to week 11.* All rats were weighed on a daily basis and the percentage weight gain from baseline values was calculated for each week. Values represent group means ±SEM.
*$p < 0.05$ difference between rats treated during the week with olanzapine vs. vehicle (challenge treatment groups collapsed, i.e. [V-V] & [V-O] vs. [O-V] & [O-O]).

Extracted tissue weights:

As described above, body tissues were extracted at the end of the study and weighed.

Because there was significantly greater weight gain in the animals that were treated with

vehicle during the week, organ weights were analyzed both as absolute weights, and also

corrected for individual body weight (Table **2.6**).

Tissue	Treatment	Absolute Weight (mg)	Corrected Weight (mg)
Liver	[O – O]	1726.9 ± 90.4***‡‡	5.9 ± 0.2***
	[O – V]	1820.4 ± 54.7***‡	6.6 ± 0.2**
	[V – O]	2128.4 ± 83.7	6.3 ± 0.3**
	[V – V]	2419.4 ± 113.8	7.6 ± 0.3
Kidneys	[O – O]	1950.9 ± 64.7***‡	6.7 ± 0.2**
	[O – V]	1924.0 ± 55.6***‡	6.9 ± 0.1*
	[V – O]	2199.1 ± 74.6	6.5 ± 0.2***
	[V – V]	2434.4 ± 108.2	7.6 ± 0.3
Adrenal Glands	[O – O]	114.9 ± 3.8	0.4 ± 0.01*‡
	[O – V]	117.4 ± 7.3	0.4 ± 0.02*‡‡
	[V – O]	117.9 ± 5.4	0.3 ± 0.02
	[V – V]	116.9 ± 5.8	0.4 ± 0.02
Heart	[O – O]	1103.8 ± 57.0*	3.8 ± 0.1**
	[O – V]	943.9 ± 33.4‡	3.4 ± 0.1
	[V – O]	1196.6 ± 64.3*	3.5 ± 0.1*
	[V – V]	958.5 ± 117.2	3.0 ± 0.3
Gast. Muscle	[O – O]	1615.7 ± 65.6***‡‡‡	5.6 ± 0.2
	[O – V]	1657.2 ± 49.6**‡‡	6.0 ± 0.2
	[V – O]	1934.7 ± 50.1	5.7 ± 0.2
	[V – V]	1928.3 ± 61.0	6.0 ± 0.1
Peri. Fat Pad	[O – O]	2924.3 ± 481.7	10.0 ± 1.4
	[O – V]	2720.2 ± 364.8	9.8 ± 1.2
	[V – O]	4218.5 ± 1104.5	11.7 ± 2.3
	[V – V]	2329.1 ± 432.7	7.2 ± 1.2
Retro. Fat Pad	[O – O]	3655.0 ± 669.0	12.3 ±2.0
	[O – V]	3628.4 ± 491.4	13.0 ± 1.5
	[V – O]	5624.8 ± 867.5	15.9 ± 1.6
	[V – V]	4038.9 ± 616.2	12.5 ± 1.7
Ing. Fat Pad	[O – O]	2370.4 ± 511.4	8.1 ± 1.6
	[O – V]	1564.9 ± 313.0	5.6 ± 1.0
	[V – O]	2271.9 ± 336.2	6.5 ± 0.7
	[V – V]	1488.7 ± 238.6	4.6 ± 0.7

Table **2.6**: *Absolute and corrected tissue weights for olanzapine drug treated rats.*

[O – O] = chronic olanzapine, challenge olanzapine; [O – V] = chronic olanzapine, challenge vehicle; [V – O] = chronic vehicle, challenge olanzapine; [V – V] = chronic vehicle, challenge vehicle; Gast. Muscle = gastrocnemius muscle; Peri. Fat = periovarian fat pad; Retro. Fat Pad = retroperitoneal fat pad; Ing. Fat Pad = Inguinal fat pad. Rats were chronically treated with olanzapine (15 mg/kg-daily i.p) or vehicle for 5 consecutive days. Once a week, all rats were subjected to an IGTT where each rat was challenged with either

olanzapine or vehicle. Tissues of each rat were extracted, weighed and stored. Values represent group means ± SEM.

In terms of the absolute tissue weights, there was a significant main effect of weekly drug treatment on the liver [$F_{(1, 31)} = 35.87$; $p < 0.001$], kidney [$F_{(1, 31)} = 26.37$; $p < 0.001$] and gastrocnemius muscle [$F_{(1, 31)} = 29.39$; $p < 0.001$], in which tissue weights were larger in vehicle treated animals ([V-V] and [V-O]) than in olanzapine treated rats ([O-O] and [O-V]). There was a main effect of drug challenge treatment on heart weight [$F_{(1, 31)} = 8.16$; $p < 0.01$], whereby rats challenged once per week with olanzapine ([V-O] and [O-O]) had a larger heart. When tissue weights were corrected for individual body weights, the difference in kidney and gastrocnemius muscle weights lost significance, but liver weights remained significantly smaller in animals treated weekly with olanzapine ($p = 0.01$) by 10%. Similarly, corrected heart weights remained larger in olanzapine challenged rats ($p < 0.05$). Interestingly, corrected adrenal gland weights were significantly greater in rats treated weekly with olanzapine than vehicle ($p < 0.01$), by approximately 15%.

2.4.4 Discussion

In the present study, we examined the effects of chronic treatment with either high dose olanzapine or vehicle over a 10 week period on sensitivity to the metabolic effects of a challenge with the same antipsychotic drug. Animals were challenged once per week, after a 48 hour washout period, with either olanzapine (15 mg/kg) or vehicle. Consistent with

114

previous studies [64, 72, 73, 77], we noted that in drug naïve animals, acute exposure to olanzapine resulted in significant metabolic dysregulation. This was evident as increased fasting glucose levels, impaired glucose clearance in response to a glucose challenge, and an increase in insulin resistance measured by HOMA-IR. In rats treated throughout the week with olanzapine, the level of glucose intolerance and insulin resistance remained consistent over time in response to the olanzapine challenge: fasting glucose levels, hyperglycemia during the glucose tolerance test and insulin resistance never changed significantly over the 10 weeks from baseline values, indicating that the physiological pathways remained completely functional, and there was no toxic effect of the drug. In strong contrast were rats that were treated with vehicle during the week but challenged once per week acutely with olanzapine. These animals showed a dramatic increase in metabolic dysregulation beyond the rats treated during the week with olanzapine, which included all measures of glucose dysregulation and insulin resistance. This was evident by the second week of testing, but a small drop in the magnitude of the effect during weeks three and four meant that the effect lost significance, until week five through the remainder of the study, whereupon this group displayed consistently greater metabolic dysregulation than all other groups. This was specifically due to an increase in metabolic dysregulation by this group of animals compared to baseline values, reflecting an increase in the magnitude of the effect over time.

While the effects of olanzapine and other antipsychotic drugs on metabolic dysregulation have been reported previously after either acute *or* chronic treatment [70, 71, 93], this is the first study to examine the effects of drug treatment longitudinally on a weekly basis on both glucose intolerance and insulin resistance. Previous studies have measured metabolic effects in naïve or chronically treated animals, but typically at a single time point

after drug treatment [75, 78, 79, 81]. This is often due to the limitations of sophisticated procedures for measurement of insulin resistance, such as the euglycemic clamp, in which the difficulty of keeping multiple indwelling catheters viable for an extended period restricts studies to a single drug treatment. The glucose tolerance test, as used in the present study, is a well validated technique that is widely used in both clinical and preclinical studies of Type II DM [67, 107], and is an optimal procedure for repeated testing of metabolic dysregulation in the same animals. Our observation that rats treated chronically during the week with olanzapine over 10 weeks displayed the same degree of metabolic dysregulation at the end of the experiment as on the first exposure is therefore important for a number of reasons. It provides additional validation of multiple prior studies that have measured the effects of olanzapine after only acute treatment [64, 71-73]. The clinical adverse effects of olanzapine in patients are normally assessed after at least 12 weeks of drug treatment [172], and therefore the legitimacy of studying acute effects in rodents could be called into question. The present findings indicate that at least for olanzapine, glucose intolerance and insulin resistance as measured by current techniques do not change over time due to factors such as drug tolerance [178]. The costs associated with extended dosing of atypical antipsychotics in preclinical studies can be considerable, including both drug and animal housing, and thus the present findings provide additional support for the use of acute dosing models to understand the biological basis of antipsychotic drug-induced metabolic side-effects.

The major novel finding of the present study is that intermittent exposure to a high dose of olanzapine once per week caused a significantly greater increase in metabolic dysregulation than did chronic weekly treatment with the drug. This effect was evident by the second week of testing, and consistent by the fifth week of treatment; not only were

metabolic effects greater than in rats treated weekly with olanzapine, but also greater than their own baseline response. As such, this increased response represents a "sensitization" to the metabolic effects of the drug. To our knowledge, this has never been reported previously, and represents a potentially important novel finding. Due to the lack of prior longitudinal studies of antipsychotic drug-induced metabolic dysregulation, it is uncertain whether this effect is unique to olanzapine or may occur with other atypical antipsychotic drugs. The physiological basis of this phenomenon is beyond the scope of the present study. However, we believe that this is unlikely to reflect pharmacokinetic differences between groups, such as changes in plasma drug levels. As olanzapine in the present study was administered intraperitoneally, bypassing first-past metabolism, any differences in plasma levels would likely be due to drug metabolism. Olanzapine is metabolized principally by cytochrome CYP1A2, and to a lesser extent by CYP2D6 and CYP3A4, as well as via glucuronidation by uridine diphosphate glucuronyltransferase [169]; there is no evidence in the literature which indicates that intermittent treatment with olanzapine can modify the levels of these enzymes, but future research should address this possibility.

Interestingly, chronic treatment with olanzapine caused animals to gain weight at a slower rate than vehicle treated animals. This stands in contrast to the clinical scenario where olanzapine causes weight gain [179, 180]. Rodent models of antipsychotic drug-induced weight gain represent a controversial area of research, which we have addressed in detail recently [125]. Many preclinical studies have observed weight gain after olanzapine treatment [75, 86, 181-184]. However, the effects are not always consistent, and even in the same research group, using the same species and drug doses, weight gain may [81] or may not [185] be observed, depending on variables such as diet. Indeed, numerous variables may

impact on weight gain, including drug dose, duration of treatment, route of administration, diet and housing [125]. In the present study, female Sprague-Dawley rats treated with olanzapine for five consecutive days per week for 10 weeks failed to gain weight at the same rate as controls, despite no difference in food consumption. Weight gain between the two treatment groups started to differ from each other significantly during the fourth week of chronic drug treatment. In general, greater effects of olanzapine on weight gain are often observed in the earlier stages of drug treatment; for example, Pouzet and colleagues (2003) noted that in female rats, daily treatment with a dose of 20 mg/kg of olanzapine caused initial weight gain over the first two weeks that was no longer significant by the end of the third week. Similarly, in the study by Albaugh and colleagues (2006), olanzapine induced weight gain occurred rapidly over the first two weeks, but by 30 days of treatment, the difference between groups had substantially diminished. Many of the studies that have observed weight gain did not extend treatment past three weeks [75, 81, 83, 181, 186], at a time when our animal groups did not differ by weight. Studies that have used extended drug treatment of over four weeks or greater are less likely to observe drug-induced weight gain. Two separate studies reported an absence of olanzapine-induced weight gain in female rats after a duration of four weeks of chronic drug treatment [79, 185]. The former of these studies reported that female rats treated with olanzapine for four weeks did not increase total body weight but displayed enhanced visceral adiposity compared to controls, despite no change in food intake. Two studies that evaluated chronic olanzapine treatment in male rats reported similar results to the present study. They noted that rats treated chronically with olanzapine exhibited overall reductions in body weight gain, despite an increase in intra-abdominal adipose tissue mass [84, 187]. This was due mainly to a decrease in lean muscle mass, possibly as a result

of chronically reduced locomotor activity in the antipsychotic drug treated animals. We noted that olanzapine treatment decreased the size of the gastrocnemius muscle (the only muscle we measured) and our findings are thus consistent with a reduction in overall muscle mass compared to controls.

In conclusion, the current results describe for the first time the longitudinal effects of chronic treatment with the atypical antipsychotic drug olanzapine on metabolic dysregulation. Glucose intolerance and insulin resistance occur acutely and remain stable over a period of 10 weeks when adult female rats are treated with a high dose of the drug during the week, independent of changes in body weight. The major novel finding of this study is that when rats are treated only intermittently with olanzapine, once per week, there is a sensitization of the metabolic dysregulation that occurs within two weeks and which remains stable by the fifth week of treatment. This finding may have important clinical implications: "intermittent" rather than "continuous" treatment with olanzapine, and possibly other antipsychotic drugs in humans, might increase the metabolic side-effects of these compounds, putting patients at increased risk of cardiometabolic disorders. The issue of treatment adherence with antipsychotic drugs in the clinical population remains a major challenge [170, 188], and if the current preclinical findings extend to patient populations, patients may be increasing the adverse effects of antipsychotic drugs by not taking their medication on a daily basis. However, Remington and colleagues have recently demonstrated that treatment with specific antipsychotic drugs every second day is equally as efficacious as daily treatment [189, 190]. Further study of this phenomenon is therefore needed, particularly because most preclinical studies do not achieve steady state plasma levels of drugs [143], and so the direct comparison of intermittent and continuous drug treatment in rodents to human

equivalent dosing remains uncertain. Additional future research should include the interval

between drug treatments required to induce sensitization, the effects of a range of lower drug

doses, different antipsychotic drugs and the underlying physiological mechanisms

responsible.

Chapter 3: Investigation of ameliorative pharmacological treatments on antipsychotic drug-induced metabolic dysregulation

A version of this chapter has been published and is reprinted from:

3.1 Study 5: Differential effects of 3 classes of antidiabetics drugs on olanzapine-induced glucose dysregulation and insulin resistance in female rats

3.1.1 Overview

Despite the similarity of SGA-induced metabolic syndrome to other forms of prediabetes, the paucity of knowledge about the underlying physiology of the condition has hindered the development of optimal treatment strategies for controlling metabolic dysregulation. Nevertheless, health care providers have recognized the serious nature of SGA-induced metabolic side-effects and sought to ameliorate them through various interventions. Consistent with the Type II DM literature, some success has been obtained using lifestyle changes, including exercise and dietary modifications [191]. However, these procedures may be more challenging in the psychiatric population [192], and therefore the mainstay of treatment remains the use of antidiabetic drugs. A number of different antidiabetic drugs are currently used to treat metabolic syndrome [193, 194] and Type II DM, but unlike antipsychotic drugs that all work primarily through a similar mechanism in regards to clinical efficacy [blockade of dopamine D_2 receptors [195]], the antidiabetic drugs operate through diverse physiological pathways. For instance, the efficacy of metformin (a biguanide), is mediated in part by an AMPK-dependent kinase (AMPK) signaling pathway, which does not directly stimulate insulin secretion [196]. The main mode of action for rosiglitazone (a thiazolidinedione), involves the activation of the peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear transcriptional protein that belongs to the family of PPARs, which regulate genes involved in lipid and glucose metabolism [197]. Rosiglitazone-induced

acute effects are also independent of direct insulin release [198]. In contrast to both metformin and rosiglitazone, glyburide (a sulfonylurea) directly increases insulin secretion in the pancreas by inhibiting the ATP sensitive potassium channel in beta cells [199]. It is therefore important to determine if specific classes of antidiabetic drugs are more efficacious in treating SGA-induced metabolic syndrome, as this form of metabolic dysregulation may be more or less sensitive to individual antidiabetic drugs.

To date, the effects of most of the main classes of antidiabetic drugs on SGA-induced metabolic dysregulation remain undetermined in preclinical models. It is important to perform such studies, as findings may not only provide knowledge about the biological pathways that are affected, but also offer insights into optimal treatment approaches in the clinic.

We therefore conducted the present study to determine the effects of three of the most commonly used classes of oral hypoglycemic drugs (i.e., biguanides, thiazolidinediones and sulfonylureas) on the metabolic dysregulation caused by the SGA drug olanzapine. Olanzapine is a widely-used SGA drug with a low propensity for neurological side-effects that proved to be superior in controlling psychosis and preventing rehospitalization compared to other SGA drugs in a major head-to-head trial [200]. However, enthusiasm for the use of olanzapine is tempered by evidence that it causes significant metabolic side-effects, which may be second only to clozapine in severity [201, 202]. We therefore tested the effects of the three different antidiabetic drugs metformin, rosiglitazone and glyburide on glucose intolerance and insulin resistance caused by acute treatment with olanzapine in a rat model that we have used previously.

3.1.2 Materials & methods

Animals

Adult female Sprague-Dawley rats (Charles River, Montreal, Canada) initially weighing 250 to 275 g were pair-housed and maintained on a 12-hour light-dark cycle (lights on at 07:00h) in a temperature controlled colony at $22 \pm 1^{\circ}C$. Rats were allowed to habituate to the UBC colony for one week prior to experimental testing. Food and water were available *ad libitum*. Animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Approval by the University of British Columbia's Animal Care and Use Committee was established for all procedures.

Drugs

The doses of olanzapine (7.5; 15 mg/kg, IP, herein labeled as "lower" and "higher" dose) [purchased from Toronto Research Chemicals Inc, Toronto, ON, Canada] were carefully chosen to represent the middle-to-upper range of pharmacological relevant levels *in vivo*, and are based on previously reported behavioral studies [64, 103, 143, 203]. The vehicle solution for olanzapine consisted of 50% polyethylene glycol 400, 40% distilled water and 10% ethanol (PEG solution). Olanzapine was administered via the intraperitoneal route in a volume of 1 ml/kg as a single injection, at 60 min prior to the glucose challenge (see below, Section 2.4). The doses of metformin (100; 500 mg/kg, PO), rosiglitazone (6; 30 mg/kg, PO) [purchased from Toronto Research Chemicals Inc., Toronto, ON, Canada] and glyburide (2; 10 mg/kg, PO) [purchased from Sigma-Aldrich Inc., St. Louis, MO, USA] were based on

124

previous preclinical studies [204-206] and represented a five-fold range from low to high

doses in the acute setting of various antidiabetic animal models. The vehicle solutions for

metformin and rosiglitazone consisted of heated 0.9% saline (which was allowed to cool

before administration), whereas the vehicle for glyburide consisted of PEG solution. All

hypoglycemic drugs were administered via the PO route (gastric gavage) as a single daily

administration for two consecutive days. The duration of oral hypoglycemic drug treatment

was set to 2 consecutive days to ensure that baseline fasting metabolic parameters (measured

both before and after olanzapine administration) and postprandial measures could be

examined under antidiabetic drug exposure. All solutions were compounded fresh daily, and

the use of all other chemical compounds were commercially available and of reagent grade.

Baseline intraperitoneal glucose tolerance test (IGTT) (see Fig. **3.1**)

Prior to the administration of the first antidiabetic trial (metformin), all rats were subjected to

a baseline glucose tolerance test (day 1). Briefly, animals were wrapped in a towel to

minimize stress and a small drop of saphenous venous blood was procured through the use of

a 25 gauge needle for the baseline blood glucose measurement at t = 0 min. Subsequently, all

animals received a glucose challenge (1 g/kg/ml, IP) followed by repeated sampling of blood

glucose readings at t = 15, 45, 75 and 105 min. All blood glucose measurements were

determined by a handheld glucometer (One Touch Ultra). Rats were left untreated from days

2-7 before the first antidiabetic drug administration (day 8) and the subsequent

Intraperitoneal glucose tolerance test (IGTT; day 9). As the present longitudinal study

exposed the rats consecutively to three different antidiabetic drugs which could theoretically

have residual carryover effects, a similar "washout" procedure was performed one week after

each drug treatment (rats were left untreated during that week after each olanzapine/antidiabetic drug trial, days 10 -14). Any putative carryover effects would be detected as a change in IGTT results in the absence of drug challenge.
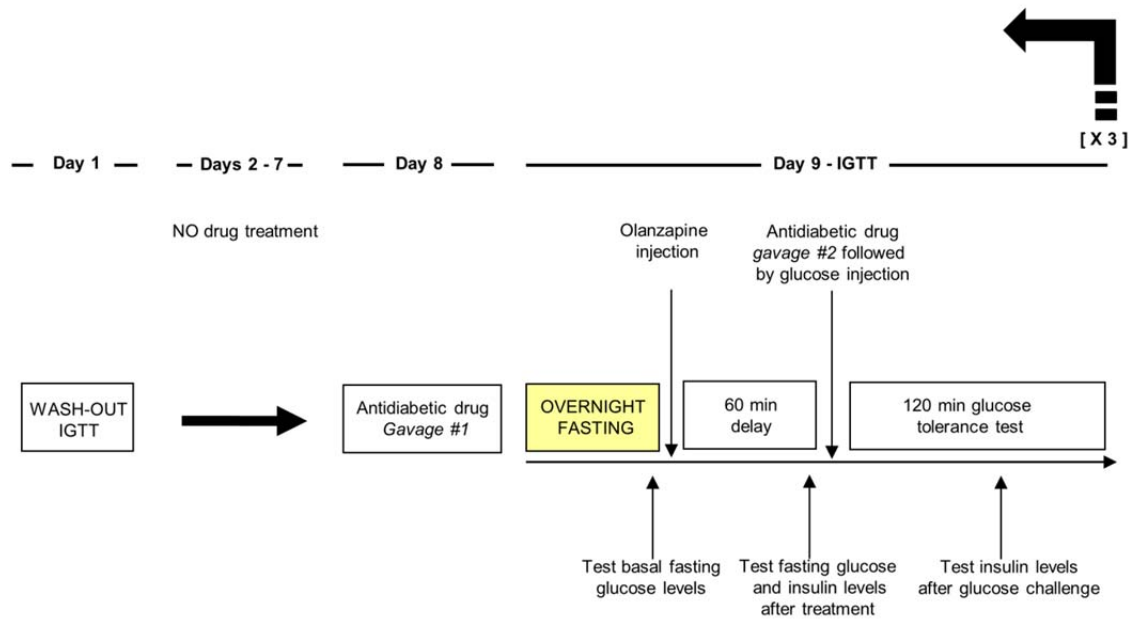
Fig. **3.1**: *Experimental protocol describing the treatments with an oral hypoglycemic drug before the intraperitoneal glucose tolerance test (IGTT) with acute olanzapine treatment.*

Acute antidiabetic treatment (see Fig. **3.1**).

Rats (n = 8-10 per group) were rank ordered based on the baseline IGTT and the initial total body weight, and they were then randomized into one of nine treatment groups: higher dose olanzapine (15 mg/kg) and higher dose metformin (500 mg/kg), higher dose olanzapine and lower dose metformin (100 mg/kg), higher dose olanzapine and no metformin (0.9% saline vehicle), lower dose olanzapine (7.5 mg/kg) and higher dose metformin, lower-dose olanzapine and lower dose metformin, lower dose olanzapine and 0.9% saline, no olanzapine

(PEG vehicle solution) and higher dose metformin, no olanzapine and lower dose metformin, and no olanzapine and no metformin (0.9% saline vehicle).

Each rat received a single gavage administration of either metformin or 0.9% saline on Day 8 (at 11:00h). On Day 9, rats that were fasted overnight (16 ± 2 h) had their baseline blood glucose levels measured and then received a single intraperitoneal injection of either olanzapine (7.5; 15 mg/kg) or PEG vehicle (t = 0 min). After a 60 min delay, animals were subjected to a 100 µL saphenous blood draw, whereby plasma was prepared by centrifugation of whole blood (10, 000 RPM, 10 min, 4°C) and stored at -80°C for the analysis of insulin levels. The animals then received the second dose of metformin or vehicle by gavage (60 min post-olanzapine administration), followed thereafter by an intraperitoneal challenge injection of glucose (1 g/ml/kg). Glucose levels were then measured every 15 min for the duration of 120 min. This protocol was repeated identically for the two additional antidiabetic drugs, rosiglitazone (6, 30 mg/kg, PO) and glyburide (2, 10 mg/kg, PO). For the entirety of the study, each animal handler was blinded to drug treatment.

Insulin measurement by ELISA

Individual plasma samples extracted during Day 2 from each of the three antidiabetic IGTTs were analyzed for insulin content using the ultra sensitive rat insulin Enzyme-Linked ImmunoSorbent Assay (ELISA) kits (Crystal Chem Inc., IL, USA), as previously performed [64] and stated in this thesis (Section 2.1.2).

Insulin resistance

Determination of insulin resistance in rats was accomplished using the homeostatic model assessment of insulin resistance (HOMA-IR) equation, as previously reported in Section 2.1.2.

Statistical analysis

Variables were analyzed with a two-factor Analysis of Variance (ANOVA), with antipsychotic drug (two doses of olanzapine and vehicle) and antidiabetic drug (two doses and vehicle) as the between subjects factor, with alpha value set at $p < 0.05$. Individual glucose measurements at the 8 time points during the IGTT were integrated to generate a single area under the curve value. The variables analyzed included: fasting levels of glucose prior to and at 60 min after the antipsychotic drug challenge, the area under the curve (AUC) for the glucose tolerance test, fasting post-drug insulin and HOMA-IR values. When appropriate, LSD post-hoc tests were conducted. Data were analyzed with SPSS software, Chicago, IL.

3.1.3 Results

Olanzapine and Metformin

Fasting levels of glucose in the rats prior to olanzapine administration did not differ between the groups (Table **3.1**). However, fasting levels of glucose measured at 60 min after treatment with olanzapine, but before the administration of second metformin dose and the glucose

load, showed a highly significant effect of antipsychotic drug treatment [$F_{(2,63)} = 19.18$, $p < 0.0001$]. Post-hoc analysis indicated that all olanzapine treated groups had higher fasting glucose levels than the vehicle treated groups ($p < 0.001$) (Table **3.1**). Interestingly, the higher dose olanzapine treated rats that were not given metformin had higher fasting glucose levels than all other groups ($p < 0.05$), including the two other higher dose olanzapine treated groups that received metformin the day before. This suggests the possibility that the first day of treatment had a residual effect on glucose levels after challenge with the antipsychotic drug.

Oral Hypoglycemic Drug	Measure	Treatment Group								
		V - V	V - L	V - H	$O_{(7.5)}$ - V	$O_{(7.5)}$ - L	$O_{(7.5)}$ - H	$O_{(15)}$ - V	$O_{(15)}$ - L	$O_{(15)}$ - H
METFORMIN (100 & 500 mg/kg, PO)	G_0	4.8 ± 0.2	4.5 ± 0.4	4.7 ± 0.2	7.5 ± 0.8*	6.6 ± 0.6*	7.5 ±1.0*	10.0 ± 0.8* [a]	7.9 ± 1.0*	7.5 ± 0.6*
	I_0	18.0 ± 3.2	19.7 ± 1.4	19.8 ± 1.4	55.6 ± 5.0*	46.5 ± 4.2*	63.8 ± 7.8*	69.0 ± 5.8*	57.6 ± 6.5*	69.8 ± 12.9*
	HOMA-IR	3.9 ± 0.8	4.0 ± 0.3	4.2 ± 0.4	18.9 ± 2.9*	13.9 ± 2.1*	21.7 ± 4.2*	31.7 ± 4.7*	20.3 ± 4.0*	24.2 ± 4.8*
ROSIGLITAZONE (6 & 30 mg/kg, PO)	G_0	4.5 ± 0.2	5.0 ± 0.2	4.6 ± 0.3	7.9 ± 0.6*	6.2 ± 0.4*	6.7 ± 0.4*	7.1 ± 0.6*	6.8 ± 0.4*	7.2 ± 0.6*
	I_0	19.1 ± 1.8	22.4 ± 2.1	19.8 ± 2.1	43.8 ± 4.5*	43.1 ± 9.4*	48.0 ± 7.6*	54.4 ± 6.8*	51.7 ± 7.1*	44.1 ± 7.1*
	HOMA-IR	3.8 ± 0.4	4.9 ± 0.5	4.1 ± 0.6	15.6 ± 2.0*	11.9 ± 2.6*	15.0 ± 3.0*	18.0 ± 3.2*	16.0 ± 2.9*	14.7 ± 3.2*
GLYBURIDE (2 & 10 mg/kg, PO)	G_0	4.2 ± 0.2	2.5 ± 0.2*	2.4 ± 0.1*	6.5 ± 0.9*	5.4 ± 0.7	5.3 ± 0.8	7.6 ± 0.8*	6.9 ± 0.9*	6.2 ± 0.5*
	I_0	19.5 ± 1.8	42.6 ± 2.4*	35.0 ± 4.4*	61.2 ± 9.8*	45.5 ± 5.0*	66.3 ± 4.3*	62.2 ± 10.7*	67.8 ± 3.4*	83.8 ± 8.7*
	HOMA-IR	3.5 ± 0.4	4.8 ± 0.3	3.7 ± 0.4	19.7 ± 4.9*	11.2 ± 2.0*	15.9 ± 2.6*	21.7 ± 4.4*	21.0 ± 3.0*	23.0 ± 2.8*

Table **3.1**: *Mean concentration of fasting glucose, insulin and HOMA-IR scores in rats treated with oral hypoglycemic drugs.*
I_0 = fasting insulin levels (µU/ml); G_0 = fasting glucose levels (mmol/L); HOMA-IR = homeostasis model assessment of insulin resistance (µU·mmol) / (ml·L); H = high-dose hypoglycemic; HOMA-IR = homeostatic model assessment of insulin resistance; L = low-dose hypoglycemic; O = olanzapine; $O_{(15)}$ = olanzapine, 15 mg/kg; $O_{(7.5)}$ = olanzapine, 7.5 mg/kg; V = vehicle.

Rats were treated with vehicle or olanzapine (7.5; 15 mg/kg) on Day 2. Values represented as group means ± SEM.
* = significantly different from V – V group, $p < 0.05$.
a = significantly different from $O_{(15)}$ – L and $O_{(15)}$ – H groups, $p < 0.05$

130

Analysis of insulin levels post-olanzapine but prior to the metformin and glucose load indicated a significant main effect of antipsychotic drug treatment [$F_{(2,63)} = 29.71$, $p < 0.0001$], whereby insulin levels were significantly increased in all groups treated with olanzapine (Table **3.1**). Insulin resistance was calculated using the HOMA-IR equation. The ANOVA indicated a significant main effect of olanzapine treatment on HOMA-IR values [$F_{(2,63)} = 24.36$, $p < 0.0001$], whereby they were significantly higher in all groups treated with olanzapine compared to those treated with vehicle; HOMA-IR values were also significantly higher in the 15 mg/kg dose olanzapine groups than the 7.5 mg/kg dose groups ($p < 0.05$). The effects of metformin on olanzapine-induced glucose dysregulation were directly assessed in the IGTT (Figure **3.2**). The ANOVA indicated significant main effects of both olanzapine [$F_{(2,63)} = 24.796$, $p < 0.0001$] and metformin [$F_{(2,63)} = 5.146$, $p < 0.01$] treatment in the IGTT. Post-hoc analysis revealed that olanzapine produced a dose-dependent increase in the glucose values during the IGTT, with the 15 mg/kg dose causing the greatest degree of glucose intolerance ($p = 0.01$). Both doses of metformin caused a significant reduction in olanzapine-induced glucose intolerance ($p < 0.005$), however, this effect did not differ between the two doses of metformin. Glucose levels were still higher in groups that received olanzapine as well as metformin relative to those that did not receive olanzapine ($p < 0.05$), reflecting a partial rather than full reversal of glucose intolerance.
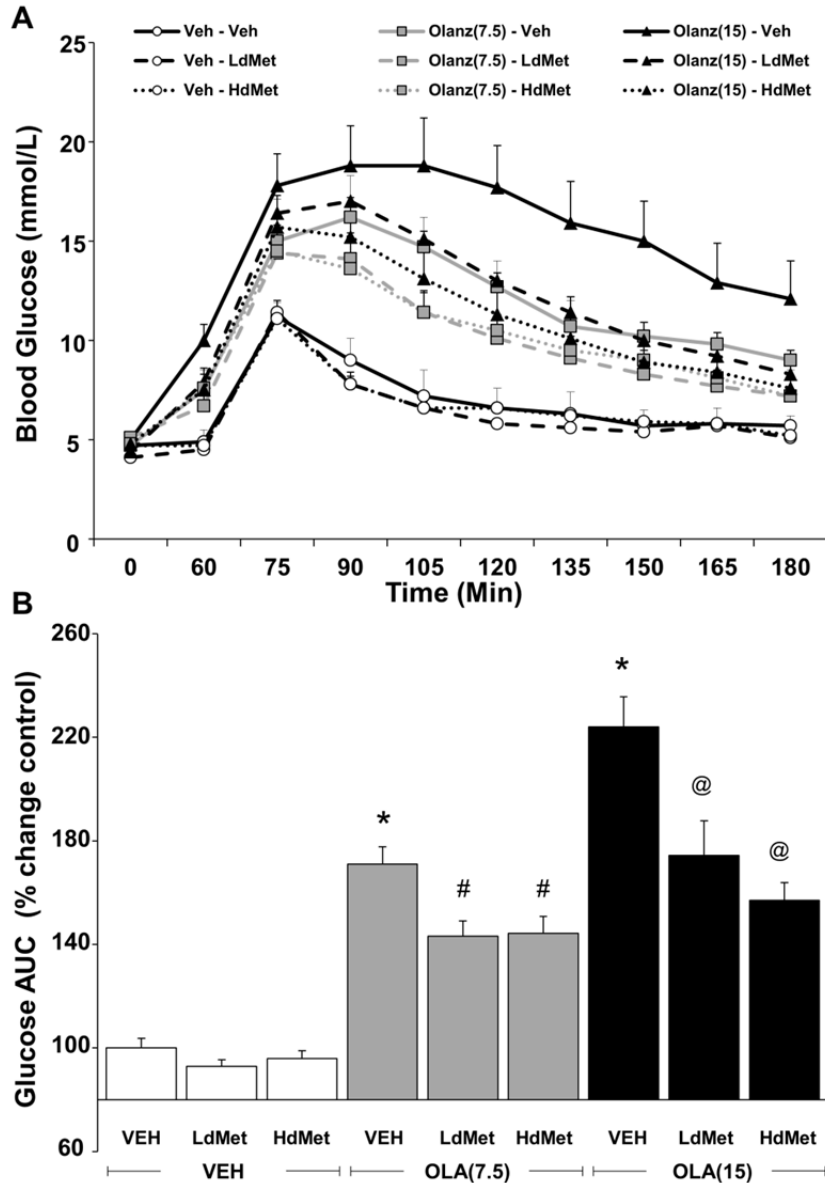
Fig. 3.2: *The acute effects of metformin treatment on olanzapine-induced glucose intolerance in adult female rats*. On the first day, animals (n = 8 - 10 per group) received a single gavage of metformin (100; 500 mg/kg, PO). The following day, glucose levels were recorded prior to olanzapine treatment (7.5; 15 mg/kg, IP) in overnight-fasted rats at Time 0, and again 60 min following olanzapine injection. Immediately following this glucose measurement, all rats received a second gavage of metformin. Subsequently, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2 hours. Significance values not included (**A**). Total cumulative glucose levels for each treatment group are summed as "area under the curve" and are presented as *percent change from vehicle control* (**B**). Values represent group means ± SEM. * = significantly greater than vehicle only treated animals, p < 0.001; # = significantly greater than vehicle-only treated animals (p < 0.01) but lower than animals treated with 7.5 mg/kg olanzapine but no metformin, p < 0.05; @ = significantly

greater than vehicle-only treated animals ($p < 0.01$) but lower than animals treated with 15 mg/kg olanzapine but no metformin, $p < 0.05$.

Olanzapine and Rosiglitazone

Comparison of glucose levels in the baseline IGTT and the washout IGTT after metformin treatment (i.e., one week before and one week after metformin treatment) indicated no carryover effect of drug treatment, and so animals were re-randomized to two days of treatment with rosiglitazone the following week.

Fasting levels of glucose in the rats on the second day of rosiglitazone treatment prior to olanzapine administration did not differ between the groups. Olanzapine increased fasting levels of glucose measured at 60 min after antipsychotic drug treatment [$F_{(2,63)} = 23.29$, $p < 0.0001$] (Table **3.1**). This reflected in increased glucose levels for the olanzapine treated groups compared to groups not treated with olanzapine ($p < 0.001$). Fasting insulin levels were similarly increased in all olanzapine treated groups compared to vehicle treated rats [$F_{(2,63)} = 17.31$, $p < 0.0001$] (Table **3.1**). Analysis of HOMA-IR values revealed a significant effect of olanzapine [$F_{(2,63)} = 17.29$, $p < 0.0001$], whereby HOMA-IR values were significantly higher in all groups treated with olanzapine. Whereas HOMA-IR values were lower in all groups that had received rosiglitazone on the previous day, this effect did not approach significance, unlike with metformin.

Analysis of the data from the IGTT indicated that there was both an effect of treatment with olanzapine [$F_{(2,63)} = 27.95$, $p < 0.0001$] as well as an effect of treatment with rosiglitazone [$F_{(2,63)} = 5.43$, $p < 0.01$]. Similar to the effects of metformin, both doses of rosiglitazone caused a significant reduction in glucose intolerance in olanzapine treated rats

(p < 0.01) (Figure **3.3**), but did not completely reverse glucose intolerance as glucose levels

still remained significantly higher than those in rats not treated with olanzapine (p < 0.05).

Fig. **3.3**: *The acute effects of rosiglitazone treatment on olanzapine-induced glucose intolerance in adult female rats*. On the first day, animals (n = 8 - 10 per group) received a single gavage of rosiglitazone (6; 30 mg/kg, PO). The following day, glucose levels were recorded prior to olanzapine treatment (7.5; 15 mg/kg, IP) in overnight-fasted rats at Time 0, and again 60 min following olanzapine injection. Immediately following this glucose measurement, all rats received a second gavage of rosiglitazone. Subsequently, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1

g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2 hours. Significance values not included (**A**). Total cumulative glucose levels for each treatment group are summed as "area under the curve" and are presented as *percent change from vehicle control* (**B**). Values represent group means ± SEM. * = significantly greater than vehicle-only treated animals, $p < 0.001$; # = significantly greater than vehicle-only treated animals ($p < 0.01$) but lower than animals treated with 7.5 mg/kg olanzapine but no rosiglitazone, $p < 0.05$; @ = significantly greater than vehicle-only treated animals ($p < 0.01$) but lower than animals treated with 15 mg/kg olanzapine but no rosiglitazone, $p < 0.05$.

Olanzapine and Glyburide

Comparison of glucose levels in the washout IGTTs before and after treatment with

rosiglitazone indicated no difference in glucose tolerance, and therefore the rats were re-

randomized to treatment with glyburide the following week.

Analysis of fasting glucose levels on the second day of glyburide treatment revealed a

highly significant main effect of antidiabetic drug treatment [$F_{(2,63)} = 32.79$, $p < 0.0001$],

unlike with metformin and rosiglitazone. This was due to a large reduction of approximately

50% in fasting glucose levels in animals treated with the glyburide, demonstrating that

glyburide has hypoglycemic actions even at 24 hours after administration (Table **3.1**). At 60

min following treatment with olanzapine there was a main effect of treatment with both

olanzapine [$F_{(2,63)} = 29.11$, $p < 0.0001$] and glyburide [$F_{(2,63)} = 4.66$, $p < 0.05$] on fasting

glucose levels. Both doses of olanzapine caused increases compared to vehicle treated rats (p

< 0.001). The effect of glyburide, representing residual effects from the first day of treatment,

was evident as decreased fasting glucose levels compared to rats not treated with the

antidiabetic drug: while all glyburide treated groups showed decreases, this was only

significant in the rats not treated with olanzapine. Fasting insulin levels revealed main effects

of both olanzapine treatment [$F_{(2,63)} = 23.59$, $p < 0.0001$] and glyburide treatment [$F_{(2,63)} =$

3.15, $p < 0.05$] 24 h previously (Table **3.1**). Olanzapine, relative to vehicle, caused an

135

increase in insulin levels ($p < 0.001$), Glyburide treatment 24 h previously increased insulin

levels, but only in the higher (10 mg/kg) groups ($p < 0.05$). Insulin resistance, measured by

HOMA-IR, exhibited a main effect of olanzapine treatment [$F_{(2,63)} = 26.65$, $p < 0.0001$] but

no effect of glyburide, as olanzapine increased HOMA-IR values. Glucose intolerance during

the IGTT, following the second dose of glyburide, also revealed a main effect of olanzapine

treatment [$F_{(2,63)} = 39.35$, $p < 0.0001$], but no effect of glyburide treatment (Figure **3.4**). As

previously, olanzapine caused an increase in glucose intolerance, regardless of glyburide

treatment group, with both doses of olanzapine increasing glucose intolerance significantly (p

< 0.0001). While glyburide decreased glucose levels in the non-olanzapine treated animals,

this effect did not quite achieve significance, and it had no effect in olanzapine treated

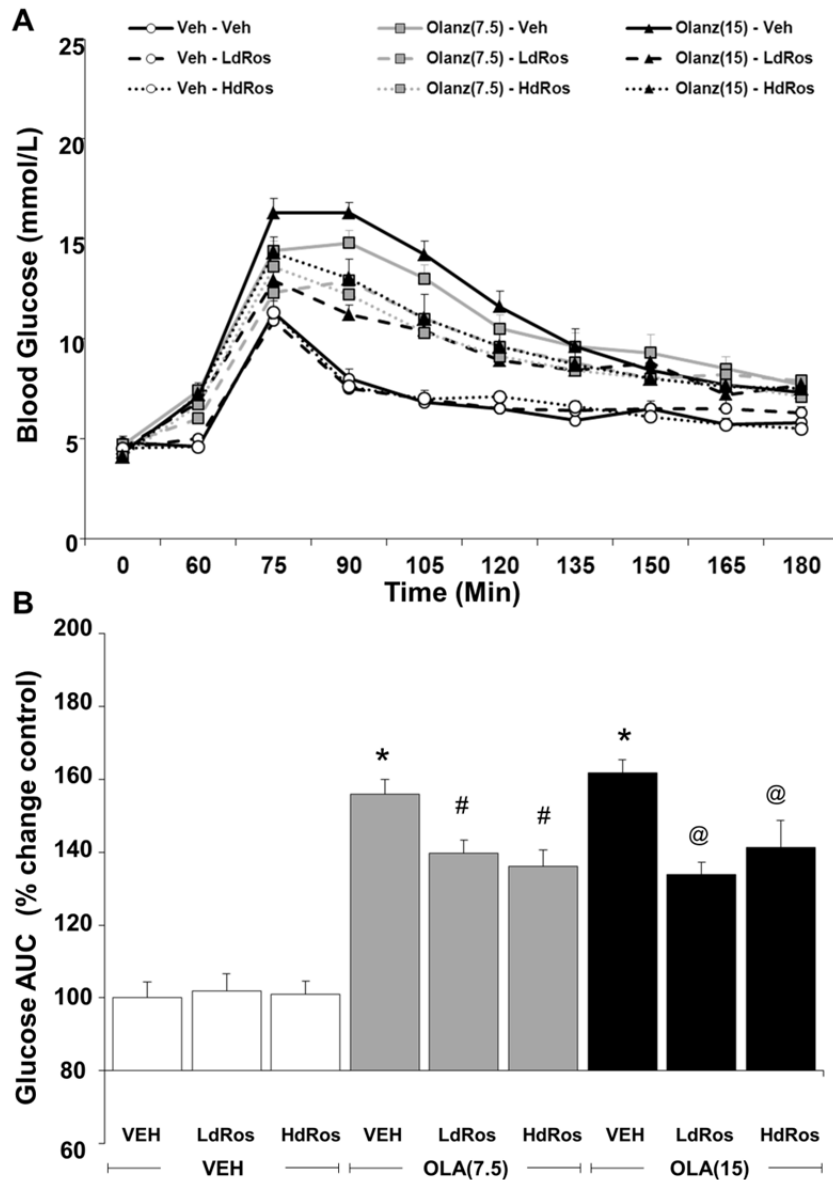animals, unlike with metformin and rosiglitazone.
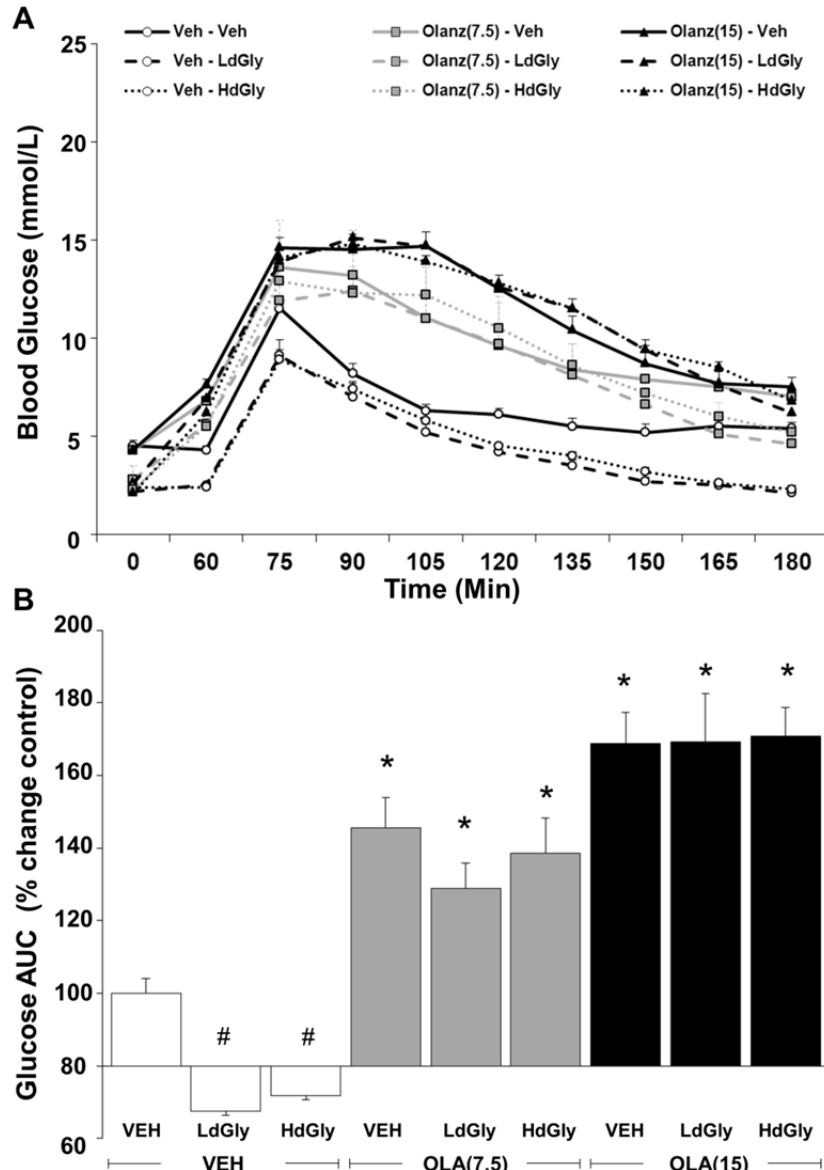
Fig. **3.4**: *The acute effects of glyburide treatment on olanzapine-induced glucose intolerance in adult female rats*. On the first day, animals (n = 8 - 10 per group) received a single gavage of glyburide (2; 10 mg/kg, PO). The following day, glucose levels were recorded prior to olanzapine treatment (7.5; 15 mg/kg, IP) in overnight-fasted rats at Time 0, and again 60 min following olanzapine injection. Immediately following this glucose measurement, all rats received a second gavage of glyburide. Subsequently, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2 hours. Significance values not included (**A**). Total cumulative glucose levels for each treatment group are summed as "area under the curve" and are presented as *percent change from vehicle control* (**B**). Values represent group means ± SEM. # = significantly lower than vehicle-only treated rats, $p < 0.05$; * = significantly greater than vehicle-only treated rats, $p < 0.01$.

Interestingly, the magnitude of the response to olanzapine during the IGTT showed a slight reduction with time across the entire study, as AUC glucose levels modestly (but non-significantly) declined with both doses of olanzapine between the first exposure to olanzapine (with metformin, Fig. **3.2**) and the second exposure (with rosiglitazone, Fig. **3.3**), although there was no further drop between the second and third olanzapine exposure (with glyburide, Fig. **3.4**).

3.1.4 Discussion

In the present study, we tested the effects of three distinct classes of oral hypoglycemic drugs on glucose dysregulation and insulin resistance in adult female rats that were treated with lower and higher doses (7.5 mg/kg and 15 mg/kg) of the SGA drug olanzapine. The hypoglycemic drugs were administered once daily for two consecutive days, and included a biguanide (metformin), thiazolidinedione (rosiglitazone) and sulfonylurea (glyburide).

A major conclusion from the present set of results is that olanzapine-induced glucose dysregulation can be alleviated, in part, by antidiabetic drug mechanisms that are independent of direct insulin release. Under the current experimental conditions, improvement of glucose tolerance and hyperglycemia was demonstrated by both metformin and rosiglitazone, but not glyburide treatment. It is unlikely that the two doses of glyburide used in these experiments were too low to have an effect, as these are doses commonly used efficaciously in other rat models of metabolic dysregulation and Type 2 diabetes [204]. Furthermore, our wide, five-fold dose range of glyburide reduced fasting glucose levels by almost 50% and nearly doubled plasma insulin levels in the rats not given olanzapine relative

to the corresponding levels of control animals, consistent with glyburide's known insulin secreting action. It appears that increasing insulin levels alone is insufficient to decrease the glucose dysregulation induced by olanzapine. Working through mechanisms independent of direct insulin release, metformin and rosiglitazone were able to cause a respective 39-54% and 29-50% decrease in glucose intolerance in the IGTT. It is not known whether higher doses of these two antidiabetic drugs could reverse olanzapine-induced glucose dysregulation still further and completely ameliorate glucose intolerance. The effects of metformin and rosiglitazone were not dose-dependent, as the higher dose of each drug did not have a greater effect, and so doses might have to be substantially higher to produce additional effects on glucose dysregulation. It is also unlikely that more extended dosing could produce a greater effect, as our pilot studies found no further benefit to extending hypoglycemic drug treatment beyond one week (data not shown). It is possible that the inability of these drugs to completely reverse olanzapine-induced glucose dysregulation reflects the complex physiological effects of the antipsychotic and pathogenic effects through multiple pathways.

Consistent with previous studies in rats, olanzapine treatment caused significant metabolic dysregulation [71, 73, 80, 81, 83, 85, 97, 126, 129, 207], evident as elevated fasting glucose levels, insulin resistance reflected in greater HOMA-IR values, and glucose intolerance in the IGTT. We assessed the effects of metformin, rosiglitazone and glyburide on these metabolic side-effects in rats for the first time in the literature. Metformin showed an effect on glucose dysregulation after the first day of treatment: fasting glucose levels were decreased after treatment with the higher dose of the antipsychotic, and there was also a strong trend towards decreased HOMA-IR values. Importantly, after the second dose, metformin significantly reduced glucose intolerance in the IGTT, although values still

remained above those of controls. Rosiglitazone did not exhibit effects after the first day of treatment, but the second dose of the drug resulted in a reduction of glucose intolerance in the IGTT similar to metformin, causing a significant reduction of glucose intolerance but, again, not a complete return to control values. In contrast, glyburide had a strong hypoglycemic effect on fasting glucose levels in rats not treated with olanzapine, indicating the potent effect of the drug 24 hours after its first administration. However, the drug did not decrease fasting glucose levels after olanzapine treatment, and unlike the other two antidiabetic drugs, glyburide had no effect on glucose intolerance in the IGTT. Previously, we have reported that intermittent treatment with olanzapine can cause a sensitization of glucose intolerance. This was not observed in the present study, likely due to factors including the duration of treatment, re-randomization of animals after each antidiabetic, injection regimen and potential influence of exposure to antidiabetic drugs.

The selective effects of metformin and rosiglitazone versus glyburide on glucose homeostasis are largely consistent with the known effects of olanzapine on glucose dysregulation. Accumulating evidence suggests that the pathogenesis of SGA drug-induced glucose dysregulation stems mainly from inadequate hepatic glucose control [71, 72, 128], reflecting hepatic insulin insensitivity. In Type 2 DM, hepatic insulin insensitivity is associated with both hyperglycemia and hyperinsulinemia [208], similar to the present findings with olanzapine. Defects in liver metabolism, including decreased insulin-mediated hepatic glucose uptake (HGU) and/or increased hepatic glucose output (HGO) are central to the onset of hyperglycemia. Hyperinsulinemic-euglycemic clamp studies have demonstrated that olanzapine significantly decreased hepatic insulin sensitivity and increased HGO in rodent models [71-73]. For both metformin and rosiglitazone, *in vitro* evidence indicates that

140

suppression of liver HGO, to a considerable degree, is mediated independently of the effects of insulin [209, 210]. In comparison, sulfonylureas such as glyburide produce their therapeutic effects by directly stimulating insulin secretion from the pancreas, giving rise to sustained levels of circulating insulin [199]. In theory, the increased levels of insulin caused by treatment with glyburide should stimulate insulin-dependent processes that lower glucose levels in response to a hyperglycemic state, such as HGU, peripheral glucose disposal, and inhibition of glucogenic responses including HGO. However, the clear failure of glyburide to affect olanzapine-induced hyperglycemic fasting glucose levels and glucose intolerance in the IGTT strongly suggests that the therapeutic effects of metformin and rosiglitazone occur via their insulin-independent mechanisms. As metformin's pharmacological action involves suppressing HGO by curtailing gluconeogenesis, in addition to enhancing peripheral glucose utilization [211, 212], there are shared physiological pathways between both antipsychotic and antidiabetic drug that may explain the effects observed in the glucose tolerance test. The 'cellular energy sensing' AMPK signaling pathway has been proposed as one valid mechanism of action for metformin's antidiabetic effects. As an enzyme involved in regulating glucose and lipid metabolism [213], differential regulation of AMPK by metformin has been observed between the periphery and the brain. Both liver and muscle AMPK activity is increased by metformin exposure, facilitating inhibition of lipogenesis, gluconeogenesis and increased glucose uptake [214, 215]. Metformin can also block hypothalamic AMPK activity, resulting in anorexigenic effects [216, 217]. In light of this, several recent studies have documented elevated levels of phosphorylated hypothalamic AMPK after chronic olanzapine treatment [218], and interestingly, have associated these to olanzapine-induced weight gain and increased food intake [219]. There is also emerging

evidence that suggests metformin modulates multiple components of the incretin axis via an AMPK-independent mechanism. Enhanced plasma levels of the insulinotropic hormone glucagon-like peptide 1 (GLP-1) have been reported after metformin treatment in humans and preclinical models [220-222]. Among other beneficial antidiabetic effects, GLP-1 is known to suppress the hyperglycemic action of glucagon, which in turn, contributes to decreased HGO and lower circulating glucose levels. Likewise, several recent studies conducted by Smith and colleagues have demonstrated that olanzapine-, clozapine- and quetiapine-induced glucose dysregulation is associated with impaired GLP-1 production and enhanced glucagon secretion, leading to stimulated HGO [70]. These studies together with our present findings, suggest mutual targets for both metformin and antipsychotic drug action, and implicate the importance of altered insulin counter-regulatory hormones in SGA drug-induced glucose disturbances. The opposing effects of SGA drugs and metformin on glucagon, GLP-1 and AMPK may explain why hypoglycemic drug treatment has only been partially successful in relieving SGA drug-induced metabolic side-effects in the clinic [223]. Rosiglitazone, via activation of PPARγ receptors, causes reduced expression of genes required for hepatic gluconeogenesis, such as pyruvate carboxylase and glucose-6-phosphatase [224]. Consequently, rosiglitazone enhances suppression of HGO and increases peripheral glucose disposal [208, 225].

To our knowledge, four other studies have determined the effects of antidiabetic agents on SGA drug-induced glucose intolerance. In the Lykkegaard et al. study [93], treatment of female rats with liraglutide, a human GLP-1 analogue, alleviated metabolic indices, including olanzapine-induced glucose intolerance. There was no effect on fasting plasma insulin levels, but importantly, only a single dose of both olanzapine and liraglutide

were tested. Two other studies confirmed that clozapine-induced glucose intolerance and hyperglycemia can be modulated by antidiabetic treatment. In the first of these, treatment with the GLP-1 receptor agonist exendin-4 significantly decreased glucose levels in the glucose tolerance test after treatment with an acute 10 mg/kg dose of clozapine [70]. The second study assessed the effects of three different PPARγ modulators (glimepiride, rosiglitazone and fenofibrate) on ziprasidone-, clozapine-, and chlorpromazine-induced hyperglycemia and hyperinsulinemia in mice [226]. Rosiglitazone and glimepiride reduced hyperglycemia in chlorpromazine-treated animals, while all three PPARγ modulators reduced clozapine-induced increases in glucose levels, with the greatest effect attributed to rosiglitazone. One additional study by Adeneye et al. examined the chronic effects of both metformin (20 mg/kg) and glyburide (0.1 mg/kg) pretreatment on risperidone-induced weight gain, hyperglycemia, insulin resistance and dyslipidemia in male rats. After 60 days of pretreatment, metformin significantly reduced weight gain, fasting hyperglycemia, hyperinsulinemia and dyslipidemia; whereas glyburide had no significant effect on the above metabolic parameters [227]. Our results are therefore consistent with the two latter studies, and also largely consistent with the human clinical literature. Human studies have confirmed that metformin may help alleviate some of the metabolic sequelae of olanzapine. A recent meta-analysis concluded that metformin has modest effects on olanzapine-induced weight gain [228], while another meta-analysis that included multiple SGAs determined that metformin reduces but does not fully reverse drug-induced insulin resistance [223]. There is less evidence regarding the clinical efficacy of rosiglitazone, likely due in part to ongoing concern about cardiovascular side-effects of the drug [229]. However, a clinical trial noted that rosiglitazone significantly improved glycemic control in patients treated with olanzapine

[230]. To our knowledge, there has been no reported evaluation of glyburide on the metabolic sequelae of olanzapine or other SGAs, but given our current findings, we would not expect this agent to be efficacious.

Limitations:

The principal limitation of the current study is the evaluation of only a single SGA drug, despite testing multiple doses of both antipsychotic and antidiabetic treatments. Whereas most SGA drugs cause metabolic dysregulation, the extent to which all such drugs produce effects through shared pathways remains unknown. It will therefore be necessary in future studies to determine whether the current findings with olanzapine generalize across this entire class of SGAs, including newer drugs.

Conclusion:

The present study shows that both metformin and rosiglitazone, but not glyburide, can mitigate glucose intolerance caused by olanzapine in female rats. These findings are consistent with those reported in preclinical and clinical studies. Our findings indicate that drugs that influence hepatic glucose metabolism are most effective. Further studies using representative drugs from other classes of antidiabetic drugs and different models of SGA-induced metabolic abnormality are needed to elucidate the biological basis of SGA-induced metabolic sequelae, and how antidiabetic drugs reverse these side effects. Future research should also examine multi-drug antidiabetic combinations, as routinely occurs in the clinical setting [231], to identify optimal treatment strategies that may guide future clinical studies.

3.2 Study 6: A pilot study evaluating the effects of exenatide treatment on olanzapine-induced glucose dysregulation

3.2.1 Overview

GLP-1 is an incretin hormone that is secreted from the intestinal L-cells in response to a meal, to enhance glucose disposal, stimulate glucose-dependent insulin secretion and promote β-cell survival and function [232]. Exenatide; a synthetic GLP-1 receptor agonist, has been reported to increase insulin secretion, decrease elevated glucagon secretion and improve satiety in patients with Type II DM [233, 234]. Quite often though, exenatide is administered as an adjunctive diabetic treatment with other antidiabetic drug classes such as metformin, to further improve glycemic profiles of Type II DM patients [235]. However, to our knowledge, the effect of exenatide treatment on olanzapine drug-induced metabolic side-effects has not been evaluated in humans.

In this pilot experiment we examined the acute effects of exenatide on olanzapine-induced glucose intolerance using our animal paradigm. We chose five different doses of exenatide (5.0, 2.0, 0.5, 0.25 and 0.05 µg total) to establish a dose-response curve. The main purpose of this study was to estimate the 'optimal' dose of exenatide in our rodent model, so that future studies could focus on the effects of antidiabetic drug combination treatment (i.e., exenatide and metformin or exenatide and rosiglitazone) on olanzapine-induced glucose intolerance. We hypothesized that female rats exposed to acute olanzapine would display the characteristic hyperglycemia and glucose intolerance, while animals that were administered exenatide treatment in combination with olanzapine would show a dose-response reduction in both fasting and post-prandial glucose levels during the IGTT.

3.2.2 Materials & methods

Animals

Adult female Sprague-Dawley rats (Charles River, Montreal, Canada) were pair-housed and maintained on a 12-hour light-dark cycle in a temperature controlled colony. Food and water were available *ad libitum*. Animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Approval by the University of British Columbia's Animal Care and Use Committee was established for all procedures. The animal cohort was not treatment naïve and was re-used from the previous existing study.

Drugs

The doses of olanzapine (10 mg/kg, i.p) [purchased from Toronto Research Chemicals Inc, Toronto, ON, Canada] were carefully chosen to represent the middle-to-upper range of pharmacological relevant levels *in vivo*, and are based on previous studies conducted by our group. The vehicle solution for olanzapine consisted of 50% polyethylene glycol 400, 40% distilled water and 10% ethanol (PEG solution). Olanzapine was administered via the intraperitoneal route in a volume of 1 ml/kg as a single injection, at 60 min prior to the glucose challenge. The doses of exenatide (0.05, 0.25, 0.5, 2 and 5 μg total, i.p) [purchased from Sigma-Aldrich Inc., St. Louis, MO, USA] were based on the previous preclinical study [Smith et al. (2009)] and represented a 100-fold range from low to high doses in the acute setting of various antidiabetic animal models. The vehicle solution for exenatide consisted of 0.9% saline. Exenatide was administered via the intraperitoneal route as a single daily

administration for two consecutive days. The duration of exenatide drug treatment was set to two consecutive days to ensure that baseline fasting metabolic parameters (measured both before and after olanzapine administration) and postprandial measures could be examined under antidiabetic drug exposure (as per Chapter 3.1). All solutions were made fresh daily, and all other chemical compounds used were commercially available and of reagent grade.

Baseline intraperitoneal glucose tolerance test (IGTT)

Prior to the administration of exenatide, all rats were subjected to a baseline glucose tolerance test (day 1), as described previously in Section 3.1.2 of this thesis. Immediately after the baseline IGTT, rats were left untreated from days 2-7 before the exenatide drug treatment regimen (day 8) and the subsequent IGTT (day 9).

Acute exenatide treatment

Rats (n = 4 per group) were rank ordered based on the baseline IGTT and the initial total body weight, and they were then randomized into one of six treatment groups: olanzapine (10 mg/kg) and vehicle (0.9% saline), olanzapine and exenatide (total dose, 5 µg), olanzapine and exenatide (total dose, 2 µg), olanzapine and exenatide (total dose, 0.5 µg), olanzapine and exenatide (total dose, 0.25 µg), and olanzapine and exenatide (total dose, 0.05 µg).

Each rat received a single i.p administration of exenatide or 0.9% saline on Day 8 (at 11:00h). On Day 9, rats that were fasted overnight (16 ± 2 h) had their baseline blood glucose levels measured and then received a single intraperitoneal injection of olanzapine (t = 0 min). The animals then received the second dose of exenatide or vehicle by i.p injection (60 min post-olanzapine administration), followed thereafter by an intraperitoneal challenge injection

of glucose (1 g/ml/kg). Glucose levels were then measured every 15 min for the first hour, followed by every 30 min for the last two time points. For the entirety of the study, each animal handler was blinded to drug treatment.

Statistical analysis

Variables were analyzed with a two-factor Analysis of Variance (ANOVA), with antipsychotic drug (olanzapine) and exenatide (five doses and vehicle) as the between subjects factor, with alpha value set at $p < 0.05$. Individual glucose measurements at the 6 time points during the IGTT were integrated to generate a single area under the curve value. The variables analyzed included: fasting levels of glucose prior to and at 60 min after the olanzapine drug challenge and the area under the curve (AUC) for the glucose tolerance test. Data were analyzed with SPSS software, Chicago, IL.

3.2.3 Results

IGTT

Groups did not differ on the initial baseline IGTT values. Plasma glucose levels were measured on Day 9, at 60, 75, 90, 105, 135 and 165 min after olanzapine treatment (Fig. **3.5**). Repeated-measures ANOVA indicated a main effect of time on glucose levels [$F(5, 25) = 9.49$, p<0.001] as glucose levels decreased during 60 to 120 min after injection. Although a dose-response trend was demonstrated at all time points, there was no significant effect of exenatide treatment on plasma glucose levels [$F(5, 25) = 0.61$, NS].
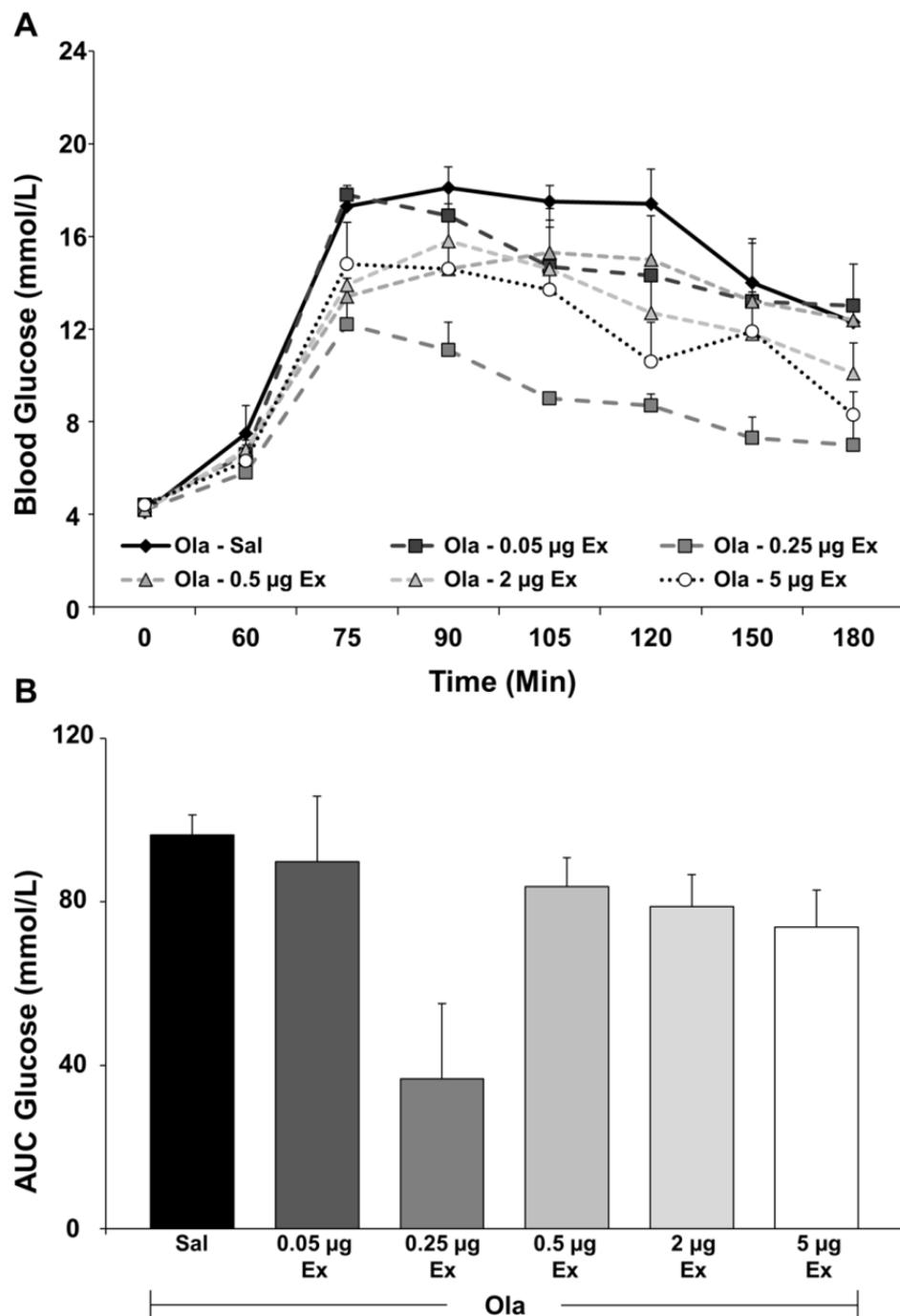
Fig. **3.5:** *The acute effects of exenatide treatment on olanzapine-induced glucose intolerance in adult female rats*. On the first day, animals (n = 4 per group) received a single injection of exenatide (0.05, 0.25, 0.5, 2.0 or 5.0 µg total, IP) or vehicle. The following day, glucose levels were recorded prior to olanzapine treatment (10 mg/kg, IP) in overnight-fasted rats at Time 0, and again 60 min following olanzapine injection. Immediately following this glucose measurement, all rats received a second injection of exenatide. Subsequently, all rats were subjected to a glucose tolerance test by receiving an intraperitoneal challenge injection of 1 g/ml/kg of glucose, and blood glucose levels were measured every 15 min for the next 2

hours. (**A**). Total cumulative glucose levels for each treatment group are summed as "area under the curve" (**B**). Values represent group means ± SEM.

3.2.4 Discussion

In the present pilot study, we tested the effects of five different doses of the GLP-1 receptor agonist exenatide on glucose dysregulation in adult female rats treated with olanzapine. Exenatide was administered once daily for 2 consecutive days. Although limited by the number of animals per group, this pilot study allowed for the estimation of the 'optimal' dose for use in future studies that focus on antidiabetic drug combination treatment.

Consistent with our previous studies, olanzapine at the dose of 10 mg/kg caused significant hyperglycemia and glucose intolerance during the IGTT [64, 236]. To our knowledge, this is the first reporting of exenatide treatment on olanzapine-induced glucose dysregulation in animals. The data is not conclusive. Moreover, it is not clear from the data if the effect of 0.25 μg total is real or an outlier, as one animal in the 0.25 μg treatment group had to be omitted from analysis due to injection error. Speculatively, a dose-response trend may exist for exenatide treatment (the larger the dose, the greater the reduction in fasting and postprandial glucose levels), and implies that a higher dose of 5 μg total may be best for future work. However, a follow-up experiment should be performed with a larger treatment group number to achieve appropriate statistical power and to confirm these exploratory results. Complementary measures of metabolic indices, such as fasting insulin, glucagon and endogenous GLP-1 levels should also be obtained to quantify the actions of acute exenatide treatment.

When compared to the human incretin GLP-1 hormone, exenatide is substantially more resistant to the di-peptidyl peptidase (DPP)-4 (the endogenous degradation enzyme for GLP-1), resulting in a longer elimination half-life and an overall more potent glucoregulatory effect [237]. Due to these differences, exenatide exerts a multitude of pharmacodynamic properties in several organs including the pancreas (to increase glucose-dependent insulin secretion and bio-synthesis, and to decrease glucagon secretion), the hypothalamus and stomach (to slow gastric emptying to promote satiety) and the liver (to decrease hepatic glucose output) [237]. Although complementary measures, such as insulin measurements were not obtained during this experiment, these data suggest that exenatide is decreasing hyperglycemia and improving glucose intolerance through several mechanisms at peripheral sites (pancreas and liver) in addition to central mediated effects. Likewise, these effects were only partial, as the restoration of glucose metabolism was not achieved. It is likely that the present doses used were not high enough; however a dose of 2 µg and greater represents the mid-to-high end of the spectrum in regards to preclinical studies of both Type II DM and SGA-induced glucose impairments [70, 238, 239].

Interestingly, the four different antidiabetic drug treatments currently tested in this thesis all exert hypoglycemic actions via different mechanisms. As with metformin and rosiglitazone, exenatide exerts marked hypoglycemic effects on the liver, albeit through different downstream effector pathways. Exenatide treatment in diabetic mice caused increased hepatic glucokinase protein expression and activity (which was more pronounced in the presence of hepatic insulin resistance), which would facilitate glucose transport and breakdown of glucose within the liver [240]. Furthermore, and in parallel to what we observed with metformin and rosiglitazone, the study by Dhanesha and colleagues illustrated

that exenatide actions were parallel to, but independent of, the direct actions of insulin. Therefore, we hypothesize that SGA drugs are associated with hepatic insulin resistance (increased HGO in the presence of high glucose and insulin levels), and antidiabetic drugs that act on the liver to facilitate glucose-uptake or slow down glucose output independently of insulin appear to improve the blood glucose profile of animals treated with olanzapine.

To our knowledge, only two other studies have determined the effects of GLP-1 agonist treatment on SGA drug-induced glucose dysregulation. In the first of these, a single injection of exenatide (5 µg total) significantly reduced clozapine-induced elevations in post-prandial plasma glucose and glucagon levels in male rats [70]. The authors of this study demonstrated that GLP-1 treatment can relieve glucose dysregulation primarily by decreasing circulating glucagon levels, thereby decreasing HGO. The latter study reported that liraglutide (0.2 mg/kg), a human GLP-1 analog, improved olanzapine-induced weight gain, fat accumulation and glucose tolerance in female rats [93]. This second study demonstrates the ameliorative actions of GLP-1 treatment, however it was difficult to tease apart if the action of liraglutide was via directly decreasing glucose intolerance or a secondary effect from reduced body weight gain.

It is therefore of interest to examine the combined effects of several antidiabetic drug treatments (i.e., exenatide and metformin) on acute olanzapine-induced glucose dysregulation to determine if antidiabetic polypharmacy is an effective ameliorative intervention against SGA drug-induced glucose derangements. Future work of this kind should determine the optimal dose of exenatide when used with metformin or rosiglitazone. Doses of both antidiabetic drugs need to be tapered, and should consist of a range of several doses to attain proper dose-response effects. Complementary measures of fasting hormones and expression

levels of key glucoregulatory proteins (e.g. GLUT4, GLUT1, glucokinase, pyruvate

carboxylase and glucose-6-phosphatase) should be evaluated in parallel. Knowledge of the

benefits of multidrug antidiabetic combinations in a valid animal model of glucose

dysregulation may aid in future clinical studies.

Chapter 4: Investigation of ameliorative non-pharmacological interventions on antipsychotic drug-induced metabolic dysregulation

A version of this chapter has been submitted to a peer-review journal for publication:

4.1 Study 7: Routine exercise ameliorates the metabolic side-effects of treatment with the atypical antipsychotic drug olanzapine in rats

4.1.1 Overview

Antipsychotic drugs represent the principal pharmacological treatment for psychotic disorders. There has been a widespread recent increase in the use of antipsychotic drugs for additional psychiatric indications [10, 241], with 1-year prevalence of drug use now exceeding 3.5% of the population in some countries [242]. The newer, second generation antipsychotic drugs (SGAs) are associated with fewer of the neurological side-effects observed with the first generation drugs, but concerns have been raised about their metabolic side-effects. Numerous clinical studies have shown that SGAs cause weight gain, hyperlipidemia and insulin resistance, resulting in high rates of prediabetes [243]. For example, one recent study of adult psychiatric inpatients observed that nearly 50% of patients treated with antipsychotics met criteria for prediabetes or Type 2 diabetes mellitus (DM) [28]. Importantly, the key symptoms of glucose intolerance and insulin resistance may be caused by SGAs independently of any weight gain. Studies have noted many new-onset cases of diabetes in the absence of major weight-gain in psychiatric patients [56], while acute treatment with SGAs in non-psychiatric subjects causes rapid-onset glucose intolerance [45, 46, 51]. These metabolic side-effects not only increase morbidity and mortality in patients treated with SGAs, but also contribute to lower rates of medication adherence [244].

Options to control metabolic side-effects in patients who are treated with SGAs include the use of anti-diabetic medications. The most efficacious of these for weight gain is

metformin, while both sibutramine and topiramate can facilitate weight loss [245, 246]. Metformin and rosiglitazone can also reduce glucose intolerance and insulin resistance in patients treated with SGAs [230, 247]. Nevertheless, both weight gain and glucose intolerance are only partially reversed by anti-diabetic drugs, and many of these medications carry additional health risks [229]. Management of metabolic dysregulation therefore typically includes lifestyle modification. In particular, it is well established that interventions which include routine aerobic exercise-training can enhance insulin sensitivity in both healthy and insulin-resistant subjects [248, 249]. In non-psychiatric diabetic patients, exercise has been shown to exert its beneficial effects on hyperglycemia through multiple mechanisms, with one of the most consistent findings being an increase in levels of the glucose transporter 4 (GLUT4) in skeletal muscle [250, 251]. Exercise has been reported to benefit patients who experience SGA-induced weight gain [252, 253]. However, the physiological pathways of the beneficial effects of exercise in patients with prediabetes or Type 2 DM whose etiology is based on treatment with SGAs remain almost entirely unknown.

Fortunately, the metabolic side-effects of SGAs have been accurately modeled in preclinical rodent paradigms [125]. Antipsychotics with greater metabolic liability in humans exert potent metabolic dysregulation in the animal paradigms [64, 73, 77, 118, 121], and these models are therefore useful in helping to understand the biological basis of metabolic side-effects. We and others have recently shown that specific classes of anti-diabetic drugs can partially reverse glucose intolerance in SGA-treated rats [227, 254], similar to effects observed in humans. To our knowledge, there has never been a preclinical study of the effects of exercise on metabolic dysregulation caused by a SGA.

The purpose of the present study was therefore to determine whether chronic aerobic exercise could ameliorate the effects of a SGA on glucose intolerance. We and other research groups have shown previously that the drug olanzapine, which is one of the most commonly prescribed SGAs, causes robust glucose intolerance and insulin resistance in female rats. We therefore examined the effects of chronic aerobic exercise, using activity wheels, on metabolic dysregulation caused by daily treatment with olanzapine for 9 weeks. Glucose sensitivity was assessed weekly using the glucose tolerance test. At the end of the study, changes in abdominal fat and levels of GLUT4 in skeletal muscle were also measured.

4.1.2 Materials & methods

Experimental animals

Adult female Sprague-Dawley rats (250-275g; Charles-River, Montreal, QC, CA) were pair-housed under ambient temperature (22 ± 1ºC) with 12-hour light-dark cycle (lights on 07:00h). Food and water were available *ad libitum,* except prior to glucose tests. Animals were treated in accord with the 'Principles of laboratory animal care' (NIH publication no. 85-23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm) and UBC's Animal Care Committee.

Drug treatment

The dose of olanzapine (10 mg/kg; 1 ml/kg) [TRC Inc, Toronto, ON, CA] was based on our prior studies [64, 103]. Vehicle formulation was prepared daily (50% polyethylene-glycol

400, 40% distilled water and 10% ethanol). Rat weights were recorded daily. Chow consumption was recorded weekly and measured on a cage-by-cage basis (n = 2 per cage).

Weekly intraperitoneal glucose tolerance test (IGTT)

Prior to treatment, rats were subjected to a baseline IGTT [254]. Fasted animals (16 ± 2 hours) were wrapped in a towel to minimize stress, and saphenous venous blood was procured to measure baseline glucose levels at t = 0 min and then at t = 15, 45, 75 and 105 min after an intraperitoneal (i.p.) injection of glucose (1 g/kg/ml) using a handheld glucometer (One Touch Ultra). This baseline was used to rank-order animals for subsequent randomization to treatment groups.

In the main experiment, on Day 1 of Week 1, fasted animals received a baseline glucose measurement, followed by i.p injection of either olanzapine or vehicle (n = 30 per group). Sixty minutes after olanzapine, rats were subjected to the IGTT, with glucose levels measured every 15 min for 120 min. Additionally, saphenous blood draws (200μL) were obtained for plasma measurement of olanzapine levels (t = 60, 75, 120 min) on Week 4; blood samples were centrifuged (10,000 RPM, 10 min, 4ºC) and stored at -80ºC. On Day 1 of each subsequent Week, the IGTT was repeated identically following daily exercise (see below). A separate group of rats (n=6) were treated with 10 mg/kg olanzapine and blood procured at 1, 4, 8 and 24 hours after treatment to determine pharmacokinetic levels.

Exercise regimen and chronic drug treatment (see Figure **4.1** for sequence of events)

On Day 2 of Week 1, animals were randomized into six treatment groups (n = 8-10 per group): olanzapine and no exercise (sedentary), olanzapine and light exercise (1 hour), olanzapine and moderate exercise (3 hours), vehicle and no exercise, vehicle and light exercise, or vehicle and moderate exercise. Rats assigned to sedentary conditions remained within their home cage, while exercise animals were placed in individual activity wheels [Med-Associates, St. Albans, VT, USA]. Running wheel activity (total wheel revolutions) was recorded via an external electronic LCD counter. After exercise, all groups rested for 30 min, followed by administration of either olanzapine or vehicle. This procedure was repeated daily every Mon-Fri, followed by a two day "wash-out' period over the weekend to allow olanzapine levels to clear completely, for 9 consecutive weeks. As above, an IGTT was performed every Mon morning.
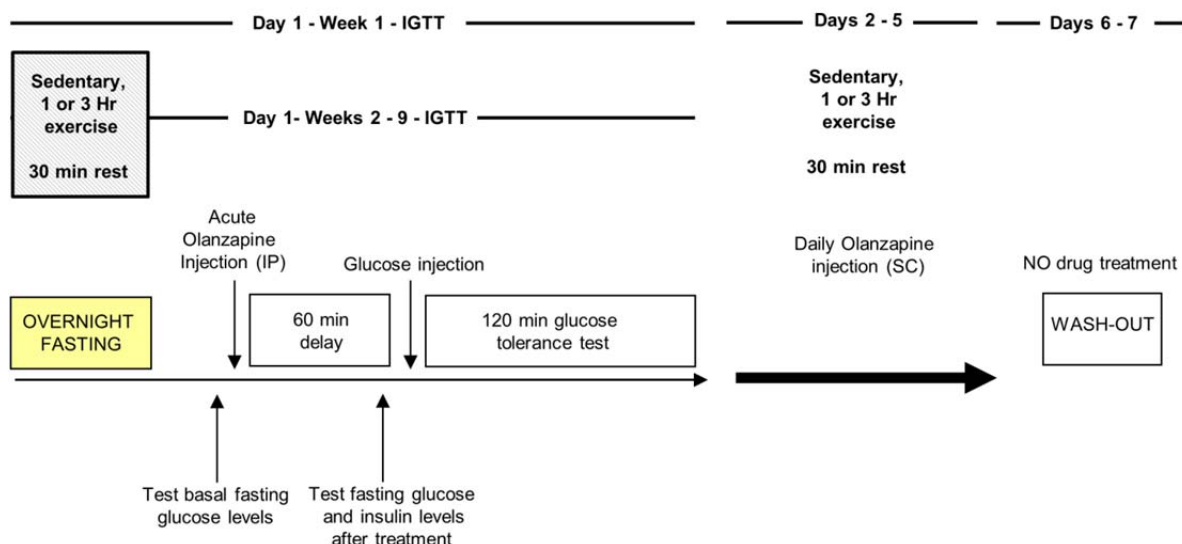
Figure **4.1**: *Experimental protocol describing the weekly exercise and treatment regimen with chronic olanzapine (10 mg/kg, s.c.) and the weekly Intraperitoneal Glucose Tolerance Test (IGTT).*

Determination of plasma olanzapine concentration

Standard solutions of olanzapine (2000-40000ng/ml) were prepared by dilution in 0.1M

orthophosphoric acid (OPA) to generate a standard curve. The internal standard (IS)

consisted of 2000ng/ml clozapine in OPA. A modified version of the single-step liquid-liquid

acid solution back-extraction with wash technique was used [255]. Samples were prepared

with 2.5μL of 2000ng/mL IS, 50μL ultrapure water and 5μL of 0.5M dibasic sodium

phosphate added to 47.5μL plasma. 800μL of 70:30 (v/v) diisopropyl ether:pentane was

added followed by vertical mixing, centrifugation and transfer of organic layer to 100μL

OPA. Samples were centrifuged and the organic layer discarded. 40μL of 70:30 (v/v)

diisopropyl ether:pentane was added, followed by mixing, centrifugation, and removal of the

residual organic layer. The remaining acidic aqueous layer was injected into the HPLC system.

HPLC-UVD

Olanzapine levels were analyzed by HPLC coupled to ultraviolet detection (UVD). A Shimadzu series HPLC system, including a SPD-20A UV/Vis detector, separated analytes on a C18-EPS column. Mobile phase contained 75% ultrapure water, 25% acetonitrile, 0.05M potassium dihydrogenphosphate, and 0.2% triethylamine, pH–adjusted to 3.4. A 20μl injection was loaded at 1.0ml/min. The UV-detector program was a 0–10 min sequence at 255 nm and 10–20 min sequence at 245 nm for acquisition of olanzapine and IS, respectively.

Tissue removal

Animals were euthanized 24 hours after the final IGTT via sodium pentobarbital overdose. Kidneys, adrenal glands, whole heart and intra-abdominal fat (perirenal, retroperitoneal, inguinal) were dissected out and weighed. Gastrocnemius muscle was extracted and stored at -80ºC for analysis.

Skeletal muscle tissue preparation and Western blot analysis

Frozen gastrocnemius muscle was homogenized in ice-cold lysis buffer (50mM Tris-HCl at pH 6.8, 1mM EDTA, 0.2% sodium dodecyl sulfate, and 1μg/ml protease inhibitor cocktail [Sigma-Aldrich, St.Louis, MO]). Homogenates were centrifuged (3,000 rpm, 20 min, 4°C) and total protein concentration determined by DC Protein Assay (Bio-Rad Laboratories,

Hercules, CA). Triplicate samples of 20µg of protein were separated by 10%-SDS-polyacrylamide gel-electrophoresis and transferred to activated polyvinylidene difluoride membranes. Equal amounts of lysates from 3T3-L1 cells expressing GLUT4 (sc-2243, Santa Cruz Biotechnology, Santa Cruz, CA) served as positive controls. Membranes were blocked in 5% milk in Tris-buffered saline containing 1% Tween-20 for 1hour at room temperature, followed by overnight incubation at 4ºC with anti-GLUT4 mouse monoclonal antibody (ab65267, Abcam, Cambridge, MA) diluted 1:1000. Blots were washed and incubated with peroxidase-conjugated goat anti-mouse IgG secondary antibody (1:5000 dilution; Jackson Laboratories, West Grove, PA). Protein bands were viewed by chemiluminescence (PerkinElmer, Walthman, MA) and captured using a LAS-3000 imager (Fujifilm Medical Systems, Stamford, CT). Band intensities were quantified by densitometric analysis.

Statistical analysis

Data were analyzed by ANOVA or t-test with significance set at $p < 0.05$. Tukey's post-hoc tests were used for follow-up analysis of significant main effects, using SPSS software (v18), Chicago, IL. Correlations were conducted using Pearson's Correlation.

4.1.3 Results

Weekly IGTT

Groups did not differ on the initial baseline IGTT values. Analysis of the weekly IGTT values throughout Weeks 1-9 by repeated-measures ANOVA indicated highly significant main effects of time [$F(8,400) = 3.48$, $p=0.001$], olanzapine treatment (drug or vehicle)

[F(1,50) = 275.78, p<0.001] and exercise status (sedentary, 1 or 3hours) [F(2,50) = 10.02, p<0.001]. Post-hoc analysis indicated that IGTT values remained stable in vehicle-treated rats, and did not change over time or differ between exercise treatments. Olanzapine treatment caused pronounced, immediate glucose intolerance, evident as a large increase in the "area-under-the-curve" of glucose levels in the IGTT (see Figure **4.2**). Glucose levels in olanzapine-treated rats did not differ between groups based on exercise treatment during the first three weeks, but after the fourth weekly IGTT, both the 1 and 3hour exercise groups displayed significantly lower IGTT values than the sedentary olanzapine-treated rats, and these group differences remained lower until the end of the study (see Figure **4.3**). IGTT values in the two exercise groups always remained significantly higher than vehicle-treated rats, indicating only a partial amelioration of olanzapine's effects on glucose tolerance. IGTT values showed slightly less improvement in the 3hour than the 1hour group, but this effect was not significant (p = 0.56).
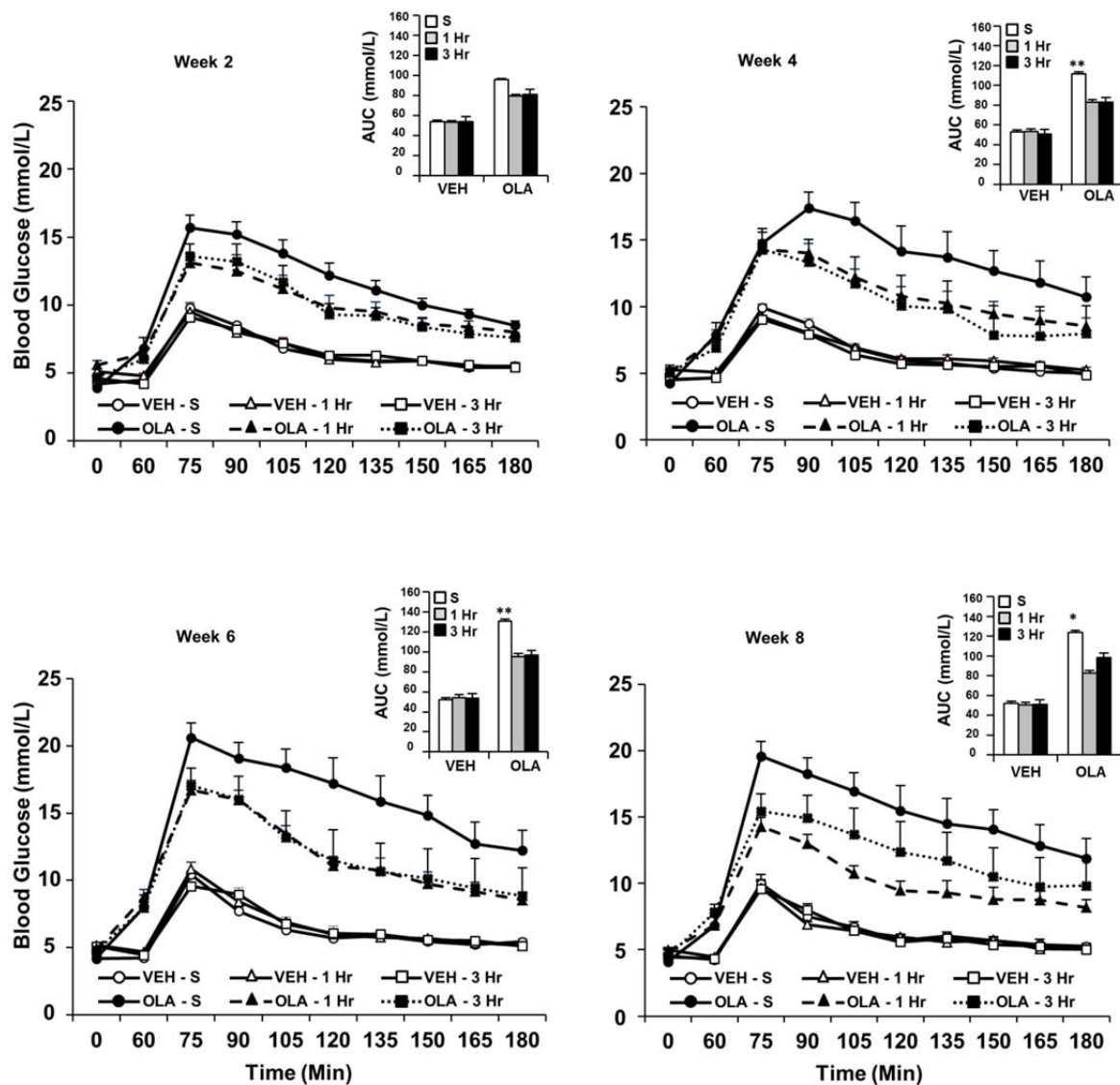
Figure **4.2**: *The effects of exercise on olanzapine-induced glucose intolerance for 9 consecutive weeks.* Adult female rats (n = 8-10 per group) received moderate exercise (3 Hour), light exercise (1 Hour) or sedentary conditions (S), followed by chronic administration of either olanzapine (10 mg/kg, s.c.) or vehicle for 5 consecutive days. This was followed by a 48 hour "wash-out" period. Subsequently, fasted animals then received a challenge intraperitoneal injection of either olanzapine (10 mg/kg) or vehicle. Glucose levels were recorded prior to treatment (Time 0) and then at 60 min. All rats were subjected to a glucose tolerance test (1 g/ml/kg of glucose, IP), and blood glucose levels were measured every 15 min for a 2 hour duration. This procedure was repeated for an additional 9 weeks. Total cumulative glucose levels for each treatment group are summed as "area under the curve" by graph insets (*top right of each graph*). Representative data shown for weeks 2, 4, 6 and 8. Values represent group means ± SEM. ** = significantly larger vs both exercise groups, $p < 0.001$, * = $p < 0.05$.

164
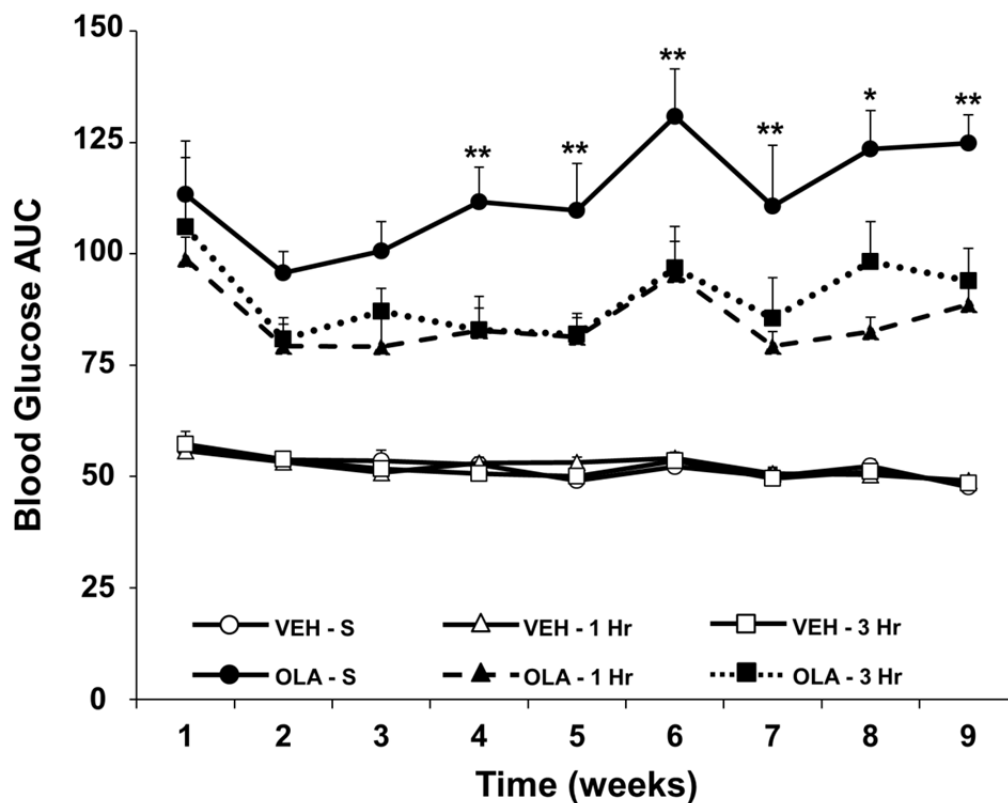
Figure **4.3**: *The effects of exercise on olanzapine-induced glucose intolerance for 9 consecutive weeks*. Total cumulative glucose levels for each treatment group are summed as "area under the curve" from week 1 (baseline) through to week 9. Values represent group means ± SEM. ** = significantly larger vs both exercise groups, $p < 0.001$, * = $p < 0.05$.

Weight gain & food consumption

Repeated-measures ANOVA indicated a significant main effect of time on body weights [$F(8,400) = 216.81$, $p<0.001$], as all groups showed weight gain over time (Figure **4.4 A**). However, there was no effect of olanzapine treatment [$F(1,50) = 0.89$, NS] or exercise status [$F(1,50) = 0.03$, NS], indicating no systematic effect of either of these factors on weight gain. By contrast, there was a significant main effect of time [$F(8,400) = 234.37$, $p<0.001$], olanzapine treatment [$F(1,50) = 5.89$, $p<0.05$] and exercise [$F(1,50) = 4.07$, $p<0.05$] on weekly food consumption (Figure **4.4 B**). When collapsed across weeks, sedentary vehicle-

treated rats ate the least, vehicle-treated exercising and olanzapine-treated sedentary rats ate

more, and olanzapine-treated exercising rats ate the most. This was not due to differences in

body weight, as an ANCOVA using midpoint bodyweights as covariate indicated all main

effects remained significant.
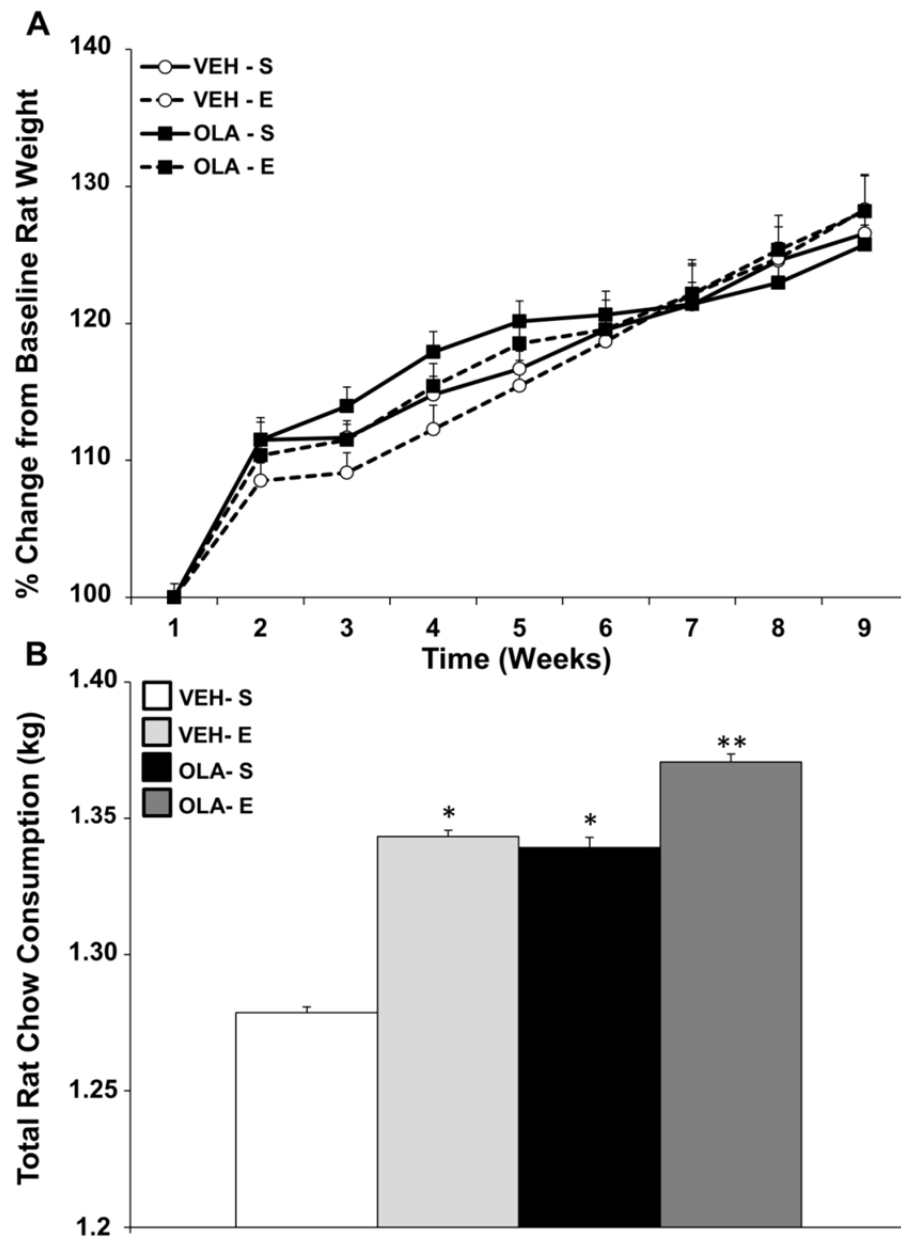
Figure **4.4**: *The effects of exercise and chronic olanzapine treatment on rat weights from week 1 (baseline) through to week 9.* All rats were weighed on a daily basis and the percentage weight gain from baseline values were calculated from sedentary (S) and

exercised (E) animals of each week (**A**). Total rat chow consumption was recorded on a weekly cage-by-cage basis (n = 2 rats per cage) and the total amount consumed for each treatment group are calculated from all weeks of study (**B**).Values represent group means ± SEM.. ** = significantly larger vs all other groups, $p < 0.001$, * = significantly larger vs vehicle-resting (i.e., no exercise) rats $p < 0.001$.

Activity

There were significant main effects of time [$F_{(8,264)} = 22.34$, $p<0.001$], exercise [$F_{(1,33)} = 9.48$, $p<0.005$] and olanzapine treatment [$F_{(1,33)} = 10.02$, $p<0.005$] on activity levels. As expected, rats in the 3hour groups ran more than the 1hour groups, and all groups showed increased activity over time (Table **4.1**). Interestingly, olanzapine-treated rats were less active than vehicle-treated animals throughout the study. This effect was not affected by the 2-day break over the weekend (i.e., levels of activity on the Mon did not differ from the rest of the week).

	Time (Weeks)	Treatment	
		VEH	**OLA**
	1	1458 ± 183	1333 ± 282
	2	1967 ± 204	1315 ± 210
	3	2593 ± 293	1466 ± 217
	4	2974 ± 629	1423 ± 409
Activity Counts	5	2546 ± 814	2546 ± 844
(1 Hour)	6	2216 ± 873	1454 ± 493
	7	3029 ± 677	1864 ± 513
	8	2745 ± 910	1693 ± 616
	9	2615 ± 537	1759 ± 500
	1	2789 ± 524	967 ± 209**
	2	3606 ± 746	1484 ± 275*
	3	5241 ± 797	2000 ± 483
	4	4937 ± 1976	2458 ± 558
Activity Counts	5	6177 ± 2622	2865 ± 649**
(3 Hours)	6	5961 ± 2072	2387 ± 1271*
	7	7622 ± 2461	3268 ± 484**
	8	6028 ± 1902	3548 ± 659
	9	6249 ± 1611	2831 ± 616**

Table **4.1**: *Mean activity levels for olanzapine-treated rats exposed to 1 or 3 hours of daily exercise.*

VEH = Vehicle; OLA = Olanzapine (10 mg/kg). Rats were exposed to either sedentary, 1 Hour or 3 Hours of daily exercise, followed by a single daily injection of olanzapine or vehicle. Activity counts were recorded after each daily session, five times per week for nine consecutive weeks. Values represented as weekly means ± SEM at t = 1 or 3 hours. * = p < 0.05 significantly different versus vehicle-treated group, ** = p < 0.005 significantly different versus vehicle-treated group.

Olanzapine levels

Plasma olanzapine levels were measured on Week 4, at 60, 75 and 120min after drug

treatment (Table **4.2**). There was a main effect of time on olanzapine levels [$F_{(2,50)} = 12.55$,

p<0.001] as drug levels decreased during 60 to 120min after injection. There was no main effect of exercise on plasma olanzapine levels [$F_{(2,25)} = 0.65$, NS].

Treatment		Time (Min)		
		60	75	120
Plasma [OLA], ng/ml	OLA - S	2220 ± 295	2086 ± 269	1884 ± 235
	OLA – 1 Hour	1918 ± 300	1798 ± 245	1688 ± 211
	OLA – 3 Hours	1934 ± 316	1863 ± 322	1524 ± 267

Table **4.2**: *Mean concentration of plasma olanzapine after 4 weeks of daily exercise in female rats.*

[   ] = concentration; OLA = Olanzapine 10 mg/kg; S = Sedentary. Rats were exposed to either sedentary conditions, 1 Hour or 3 Hours of daily exercise, followed by a single daily injection of olanzapine or vehicle. Values represented as means ± SEM at t = 60, 75 and 120 min during the IGTT on Week 4. No significant differences between groups.

Tissue weights

There was no effect of exercise or olanzapine treatment on kidney, adrenal or heart weights (Table **4.3**). However, there was a significant main effect of olanzapine treatment on the amount of perirenal [$F_{(1,50)} = 23.63$, p<0.001], retroperitoneal [$F_{(1,50)} = 11.01$, p<0.005] and inguinal [$F_{(1,50)} = 5.06$, p<0.05] fat. Post-hoc analysis indicated that the olanzapine/sedentary and olanzapine/3hr exercise groups had significantly more perirenal fat than all of the vehicle-treated groups, while retroperitoneal fat was greater in both the olanzapine/sedentary and olanzapine/1hour exercise groups than all vehicle-treated animals. Inguinal fat was greater in the olanzapine/3hour group compared to all vehicle-treated groups.

Tissue	Treatment	Absolute Weight (g)
Kidneys	VEH - S	2.417 ± 0.128
	VEH - 1 Hr	2.481 ± 0.081
	VEH - 3Hr	2.449 ± 0.047
	OLA - S	2.358 ± 0.065
	OLA - 1 Hr	2.382 ± 0.068
	OLA - 3 Hr	2.274 ± 0.057
Adrenal Glands	VEH - S	0.067 ± 0.002
	VEH - 1 Hr	0.072 ± 0.003
	VEH - 3Hr	0.072 ± 0.004
	OLA - S	0.075 ± 0.003
	OLA - 1 Hr	0.077 ± 0.005
	OLA - 3 Hr	0.067 ± 0.003
Heart	VEH - S	1.198 ± 0.046
	VEH - 1 Hr	1.217 ± 0.066
	VEH - 3Hr	1.295 ± 0.069
	OLA - S	1.245 ±0.034
	OLA - 1 Hr	1.295 ± 0.042
	OLA - 3 Hr	1.261 ± 0.061
Ing. Fat Pad	VEH - S	1.632 ± 0.143
	VEH - 1 Hr	1.548 ± 0.255
	VEH - 3Hr	1.585 ± 0.242
	OLA - S	1.836 ± 0.238
	OLA - 1 Hr	1.760 ± 0.196
	OLA - 3 Hr	2.391 ± 0.257*
Retro. Fat Pad	VEH - S	1.615 ± 0.154
	VEH - 1 Hr	1.520 ± 0.150
	VEH - 3Hr	1.765 ± 0.213
	OLA - S	2.760 ± 0.272*
	OLA - 1 Hr	2.417 ± 0.445*
	OLA - 3 Hr	1.852 ± 0.208
Peri. Fat Pad	VEH - S	1.988 ± 0.224
	VEH - 1Hr	1.962 ± 0.277
	VEH – 3Hr	2.121 ± 0.167
	OLA - S	3.964 ± 0.219*
	OLA - 1 Hr	2.763 ± 0.410
	OLA - 3 Hr	3.811 ± 0.759*

Table **4.3**: *Total organ weights for olanzapine drug treated rats with or without exposure to routine exercise.*

VEH - S = vehicle, sedentary; VEH - 1 Hour (Hr) = vehicle, 1 Hr exercise; VEH - 3 Hr = vehicle, 3 Hr exercise; OLA - S = olanzapine, sedentary; OLA - 1 Hr = olanzapine, 1 Hr exercise; OLA - 3 Hr = olanzapine, 3 Hr exercise; Peri. Fat = periovarian fat pad; Retro. Fat

Pad = retroperitoneal fat pad; Ing. Fat Pad = Inguinal fat pad. Rats were exposed to aerobic activity for either 0, 1 or 3 hours and chronically treated with olanzapine (10 mg/kg-daily s.c.) or vehicle for 5 consecutive days. Once a week, all rats were subjected to an IGTT where each rat was challenged with either olanzapine or vehicle. Tissues of each rat were extracted, weighed and stored. Values represent group means ± SEM. * = significantly different versus all vehicle-treated groups, $p < 0.05$.

GLUT4 levels

A discrete band at the appropriate molecular weight for GLUT4 was observed in the Western blots (Figure **4.5 A**). The ANOVA indicated a main effect of exercise [$F_{(2,50)} = 25.00$, $p<0.001$] on GLUT4 levels in the gastrocnemius muscle, as well as an exercise × olanzapine-treatment interaction [$F_{(2,50)} = 4.51$, $p<0.05$]. Post-hoc analysis indicated that in sedentary vehicle-treated rats, levels of GLUT4 were non-significantly ($p=0.09$) increased after 1hour of exercise, and elevated further after 3hours of exercise ($p<0.001$). In comparison, both the 1 and 3hour exercise groups who received olanzapine showed higher GLUT4 levels than olanzapine-treated sedentary rats ($p<0.001$) (Figure **4.5 B**). In olanzapine-treated rats, there was a strong negative correlation ($r = -0.57$, $p<0.005$) between GLUT4 levels and glucose levels on the final IGTT, indicating that higher GLUT4 levels were associated with decreased glucose intolerance (Fig **4.6**).
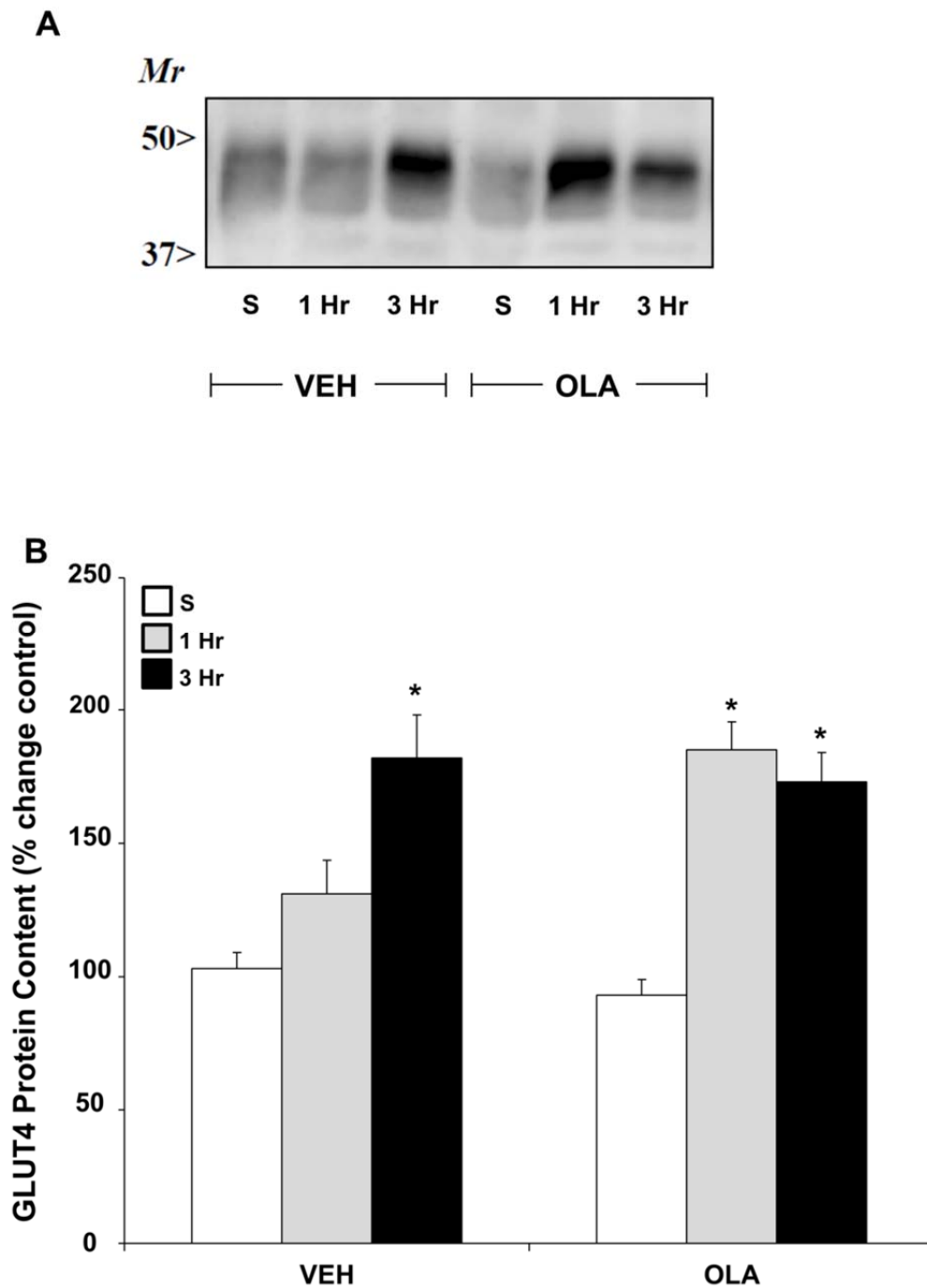
Figure **4.5:** *The effects of exercise and chronic olanzapine treatment on total immunodensity of GLUT4 in rat gastrocnemius muscle.* After 9 weeks of olanzapine treatment, gastrocnemius muscle samples from animals that received sedentary conditions (S), light (1 Hour), or moderate (3 Hours) exercise were assessed for total GLUT4 protein content. (**A**). Relative molecular masses (*Mr*) were estimated (in kDa) from in-gel prestained standards. (**B**) Representative immunoblot depicting the effects of exercise treatment on the expression

172

of GLUT4 protein in rat gastrocnemius muscle of olanzapine-treated rats. Values represent group means ± SEM. * = significantly larger vs resting (no-exercise) rats, $p < 0.01$.
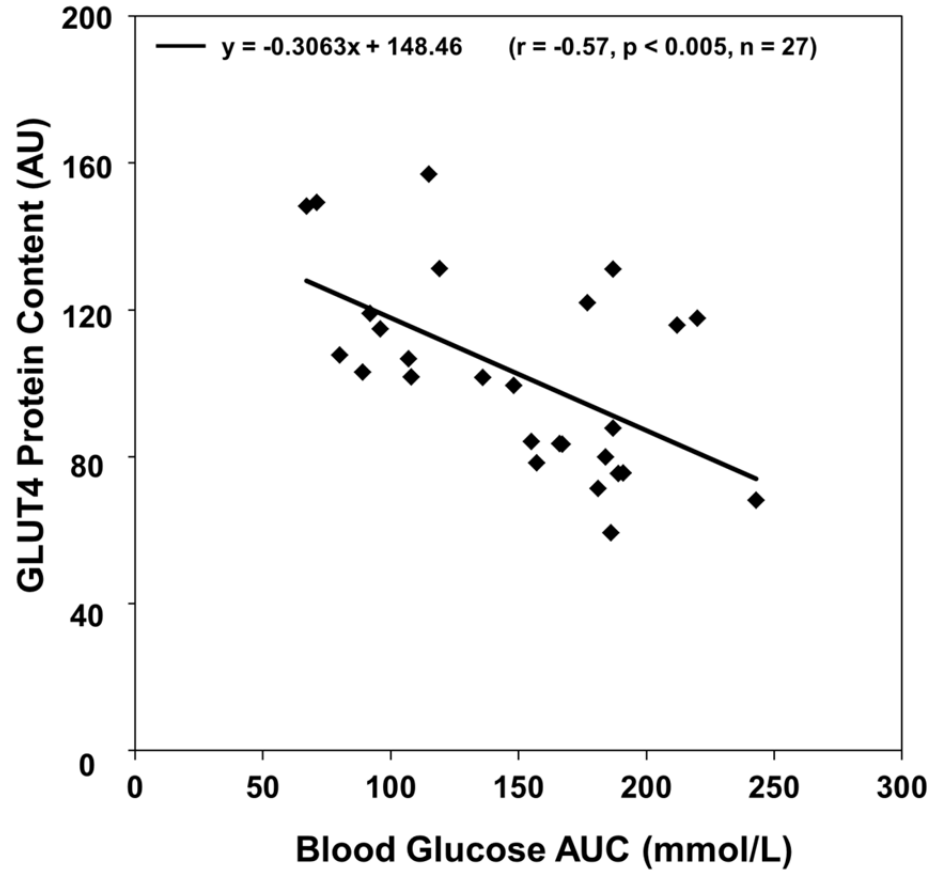
Figure **4.6:** *Negative correlation between GLUT4 protein levels in rat gastrocnemius muscle and blood glucose AUC in IGTT.* After 9 weeks of olanzapine treatment, gastrocnemius muscle samples (n = 27) from animals that received sedentary conditions (S), light (1 Hour), or moderate (3 Hours) exercise were assessed for total GLUT4 protein content. Pearson's correlation analysis was conducted on data obtained from GLUT4 protein levels vs blood glucose area under the curve (AUC). Values represent group means of the arbitrary units (AU) obtained from skeletal muscle immunoblots vs individual blood glucose AUC values obtained from Week 9 of the intraperitoneal glucose tolerance test (IGTT).

4.1.4 Discussion

In the present study, we assessed the effects of routine exercise on the metabolic side-effects of chronic treatment with the SGA drug olanzapine. Rats that were treated daily with 10 mg/kg of olanzapine Mon-Fri exhibited pronounced glucose intolerance in the IGTT, which remained stable in magnitude when tested weekly over a 9 week period. Animals treated with olanzapine that were able to exercise daily for either 1 or 3 hours from Mon-Fri displayed a significant reduction in glucose intolerance by the fourth week of exercise, and this effect lasted until the end of the study. There was no effect of exercise on glucose tolerance in vehicle-treated rats. There was no effect of olanzapine treatment on weight gain, despite greater food consumption in olanzapine-treated animals, although animals treated with the SGA exhibited significantly greater amounts of abdominal fat at the end of the study. Activity levels were lower in olanzapine-treated rats, but still remained high in absolute terms. At the conclusion of the study, animals that had been allowed to exercise had significantly higher levels of GLUT4 in skeletal muscle, and higher levels of this protein in olanzapine-treated rats were strongly associated with decreased glucose intolerance.

The findings with the IGTT reconfirm the powerful effects of the SGA olanzapine in animal models of glucose dysregulation [64, 65, 71, 72, 75, 83, 127-129, 254], which parallel the effects seen in humans [125]. The glucose tolerance test evaluates glucose "intolerance" by measuring the capacity of the fasted subject to return glucose levels to baseline after a glucose challenge; the procedure is commonly used in both clinical and preclinical studies of prediabetes and Type 2 DM [107]. The glucose tolerance test is viewed positively for its physiological relevance and practicality in measuring loss of glycemic control, and for its

174

accuracy in predicting how glucose will be regulated after a meal [67]. Importantly, the IGTT permitted us to conduct repeated, weekly measures of glycemic control, which other procedures, such as the hyperinsulinemic-euglycemic clamp (HIEC), would not have made possible over an extended period. We have shown previously in rats that there is a strong correlation between olanzapine-induced glucose intolerance measured with the IGTT and olanzapine-induced insulin resistance measured by the HIEC [236]. The magnitude of glucose intolerance we observed is consistent with known effects in humans. For example, in a recent double-blind, placebo-controlled crossover trial of acute olanzapine in non-psychiatric patients, 3-day treatment with a clinical dose of olanzapine caused significant impairments in the glucose tolerance test [51], whereby the glucose area-under-the-curve increased by 42%, which is comparable with our present findings in rats.

In the current study, we did not observe a significant effect of olanzapine treatment on weight gain. Data regarding the effects of SGAs on weight gain in rodents have been much less consistent than in humans. We have previously reviewed in detail many of the studies that examined weight gain in rats after chronic SGA treatment [125]. Some SGAs, such as clozapine, do not induce weight gain, despite causing substantial weight gain in humans. For olanzapine, a majority of preclinical studies observe some degree of weight gain, but these are typically in shorter duration studies, during the first 2-3 weeks of treatment [118, 127, 184]. Longer studies are less likely to report significant increases in weight [79, 185]. A casual examination of the longitudinal pattern of body weights in our study (Figure 4.6) hints that olanzapine-treated rats exhibited greater body weight from weeks 2-5, but not thereafter. Nevertheless, we did observe significant increases in adiposity at the conclusion of the study in olanzapine-treated animals. A number of recent preclinical

studies have reported increased adiposity in white adipose tissue (WAT) depots after repeated olanzapine treatment [116, 118, 121, 256]. In our study, we noted that olanzapine tended to increase visceral fat (perirenal and retroperitoneal) to a greater degree than subcutaneous (inguinal) fat. However, regional increases in WAT were dependent on exercise treatment in a complex manner that we cannot currently explain, and will require further study.

An important novel finding is that routine exercise increased levels of GLUT4 in the gastrocnemius muscle of SGA-treated animals. This effect was significant for both exercise groups in olanzapine-treated rats, as levels of GLUT4 almost doubled compared to sedentary rats, which parallels the observation that both 1 and 3 hours of exercise were equally effective in mitigating glucose intolerance. There is a large body of evidence indicating that the beneficial effects of exercise on glucose intolerance and insulin resistance in patients with Type 2 DM may be mediated partly through the activity and increased expression of GLUT4 [257]. The role of GLUT4 is manifold, and its beneficial effects on insulin resistance and hyperglycemia may involve its translocation to the cell membrane in addition to increased gene and protein expression in skeletal muscle and other tissues. We observed a strong and highly significant negative relationship between gastrocnemius GLUT4 levels and glucose intolerance in the IGTT, whereby higher levels of GLUT4 were associated with decreased glucose intolerance. As these findings are only correlational, the causal role of GLUT4 on olanzapine-induced glucose intolerance will require additional future study with techniques that directly modify expression of GLUT4, such as the muscle-specific GLUT4 knockout mouse [258]. Unlike the extensive Type 2 DM literature, the role of skeletal GLUT4 expression in patients treated with SGAs who exhibit metabolic dysregulation has not been

examined. Nevertheless, it is of interest that a recent genetic study observed an association between a polymorphism of the TBC1 domain family member 1 protein (a Rab-GTPase activating molecule that regulates GLUT4 trafficking) and antipsychotic-induced weight gain in patients treated with olanzapine or clozapine [259]. The present study therefore provides additional impetus to study the GLUT4 in this population, despite an etiology of metabolic dysregulation differing from Type 2 DM in non-SGA treated patients.

Alternative mechanisms may be considered with regards to the beneficial effects of exercise on olanzapine-induced glucose intolerance. It is unlikely that exercise increased the metabolism of olanzapine, such as through up-regulation of cytochrome P450 1A2 [260], thereby lowering plasma levels of the drug. We observed no significant difference in olanzapine levels between exercising and sedentary rats on Week 4, which is when exercise first demonstrated a significant improvement in glucose intolerance. Overall levels of visceral fat were lower in exercising rats, and this type of fat is much more strongly associated with metabolic dysregulation than subcutaneous fat [261]. It is possible that the beneficial effects of exercise occurred partly because they decreased or prevented the deposition of visceral fat, and this in turn improved glucose intolerance. However, glucose intolerance was present after first exposure to olanzapine, and did not worsen over time. Thus, the relationship between visceral fat and glucose intolerance remains uncertain, given that increases in visceral fat would be expected to occur over a matter of weeks, thus representing a less likely substrate for exercise than the GLUT4.

To our knowledge, the present study is the first to demonstrate beneficial effects of exercise on glucose intolerance caused by treatment with an SGA in otherwise normal animals. The study is consistent with our previous finding that the olanzapine-induced

glucose intolerance can be ameliorated, but not fully reversed, by treatment with the biguanide drug metformin and the thiazolidinedione drug rosiglitazone [130]. One group previously demonstrated that treatment with a high dose of the original first generation antipsychotic drug (FGA) chlorpromazine increased hepatic-specific insulin resistance, and exercise partially recovered insulin sensitivity in male rats [78]. However, to induce this effect, all rats first had to be 90% 'pancreatectomized' to induce a severe state of chronic hyperglycemia and loss of glucose-induced insulin release. Following a delay of 5-6 days for surgical recovery in preparation for the HIEC, the final assessment of metabolic indices was conducted only at a single time point at the end of the experiment. While useful as a model of the effects of an antipsychotic drug in patients with diabetes, pancreatectomized animals may not represent the most valid animal paradigm to investigate the impact of exercise intervention on drug-induced side-effects in the vast majority of psychiatric patients, who do not have severe loss of pancreatic function prior to starting drug treatment. Furthermore, olanzapine remains one of the most commonly used antipsychotic drugs worldwide, but use of chlorpromazine remains relatively infrequent.

In summary, the present study demonstrates the utility of routine aerobic exercise for treating the glucose intolerance caused by the widely-used SGA drug olanzapine. The current model suggests a potential role for GLUT4 in these effects, which indicates an important lead for clinical studies. The longitudinal design of the paradigm will allow for future studies to measure biochemical changes over time both before and after the beneficial effects of exercise become evident, to characterize key pathways for future therapeutic development. There is increasing concern that in previously healthy individuals, SGAs can cause weight-independent and drug-specific effects on glucose intolerance and insulin resistance. The

impact of these effects is widespread; for instance, a major recent clinical trial of antipsychotic drugs noted that in BMI-matched patients treated with SGAs, men were 85% and women 137% more likely to have metabolic syndrome than the normal subjects not treated with antipsychotics [152]. Given, in absolute terms, the very large – and increasing – number of patients treated with SGAs, understanding better the interventions that can ameliorate the metabolic side-effects of these drugs should remain a priority.

Chapter 5: General discussion

A version of this chapter has been published as:

Boyda, H.N., Procyshyn, R.M., Pang, C.C., Barr, A.M. (2012). Peripheral adrenoceptors: the impetus behind glucose dysregulation and insulin resistance. J Neuroendocrinol. 25(3): 217-28.

5.1 Summary

The central theme encompassing all 7 studies of this thesis examines the metabolic side-effects of SGA drugs, specifically glucose dysregulation and insulin resistance, in a female rat paradigm. The first study of this thesis indicates that acute APD treatments exert potent effects on glucose intolerance and insulin resistance, and that these effects are both dose- and time-dependent, and parallel the clinical situation. Drugs of high metabolic liability exert significant metabolic dysregulation compared to vehicle. The second study of this thesis examines the effects of novel SGA drugs. Using two complementary techniques, this is the first-ever preclinical study to determine that iloperidone is associated with substantial glucose intolerance and insulin resistance, while asenapine is devoid of glucose intolerance and insulin resistance, at least in the acute setting. The third study of this thesis examines the effects of APD combination treatment on glucose intolerance and insulin resistance, and indicates that administration of two APDs are associated with significantly more effects on glucose tolerance and insulin sensitivity, compared to either drug treatment alone. The fourth study determines the longitudinal effects of olanzapine on glucose intolerance and provides evidence that sensitization to the metabolic effects occur after intermittent treatment. The fifth and sixth studies examine pharmacological interventional treatment on acute glucose dysregulation. We provide evidence that different peripheral mechanisms of action of antidiabetic drug treatments affect olanzapine-induced glucose dysregulation differently, indirectly from insulin actions, which may be attributed to localized effects in the liver. The seventh study determines the non-pharmacological interventions of daily aerobic exercise on olanzapine-induced glucose intolerance. Evidence from this study suggests that insulin-

sensitive tissues, such as skeletal muscle, may play a role in olanzapine-induced glucose

dysregulation over a longitudinal time frame and are modulated after routine exercise.

This discussion will address these findings, their implications and limitations, and

provide future directions that will advance knowledge translation in this field of animal

models. In particular, I will focus on the actions of SGA drugs on glucose metabolism in the

periphery, as it has formed the bulk of my doctoral work. The precise mechanism(s) of action

of APD-induced metabolic side-effects in the periphery remain unknown. Therefore, I

dedicate a substantial portion of this discussion to hypothesizing which target organs are

being affected by SGA drugs to illicit effects on glucose metabolism.

5.2 Proposed biological targets for antipsychotic drug-induced metabolic side-effects

5.2.1 Insulin-sensitive tissues

To fully appreciate the roles of each target tissue and their contribution to glucose regulation,

a brief synopsis of mammalian physiology is provided. Among organs that make up

mammalian anatomy, the brain, heart, pancreas, liver, kidney, skeletal muscle and adipose

tissue are all considered highly insulin-sensitive. Of these, pancreatic, hepatic, skeletal

muscle and adipose tissue are by far the most important for regulating peripheral glucose

turnover and are capable of responding both directly and indirectly to insulin's action. To

initiate insulinotropic effects, insulin binds to its tyrosine kinase receptor and activates

downstream insulin receptor substrate (IRS) adaptor proteins, followed thereafter by

activation of various kinases including phosphoinositide 3-kinase (PI3K) and protein kinase

B (Akt) [262]. The principal hypoglycemic actions of insulin involve suppression of HGO and facilitation of peripheral glucose uptake by membrane-associated GLUT4 in skeletal muscle and adipose tissue [263]. Additional regulating factors of plasma blood glucose include the endogenous incretin hormones GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), both of which are released from the L-cells of the small intestine and primarily facilitate glucose bioavailability and clearance after an oral challenge (for detailed review, see [264]). By contrast, the ability of hyperglycemia alone to influence its own clearance is a major determinant of hormone-independent mechanisms of whole-body glucose disposal in hepatic and pancreatic tissues [265]. Since several different SGAs affect glucose metabolism and insulin resistance to varying degrees, insulin sensitive tissues represent target peripheral sites for the underlying physiological effects of SGA drug-induced glucose derangements.

5.2.2 Pancreas

Insulin Secretion

From the results obtained in this thesis in addition to others, impairments in insulin secretion represent a major feature in SGA drug-induced metabolic side-effects, prediabetes and Type II DM, emphasized by compensatory and sustained hyperinsulinemia that progressively declines over time [266]. Pancreatic β-cells are sensitive to blood glucose levels and are equipped with several facilitative glucose transporters, primarily GLUT1, -2 -3 and -9, which mediate glucose-stimulated insulin secretion [267]. McCulloch and colleagues measured GLUT mRNA expression in both human and mouse pancreatic tissue and determined that

GLUT1 and 3 are abundant in human tissue versus the predominant expression of GLUT2 in murine islets [268]. While glucose stimulates its own removal from the circulation during acute settings, chronic hyperglycemia generates deleterious effects on pancreatic β-cells and is considered to be accountable for the gradual deterioration of β-cell function. One underlying mechanism for hyperglycemia-induced glucotoxicity implicates activation of the carbohydrate response element-binding protein (ChREBP), a transcription factor that controls glycolytic and lipogenic genes, thus stimulating lipid accumulation, apoptosis and decreased insulin production [269].

A recent clinical open-label prospective study by Chiu and colleagues studied the longitudinal metabolic effects of 8 weeks of olanzapine treatment on pancreatic beta cell function in non-obese SGA-naïve patients with schizophrenia [178]. As assessed with the intravenous GTT, olanzapine treatment triggered a biphasic reaction of pancreatic beta cell function, where insulin was initially suppressed but subsequently rebounded via hyperinsulinemia. Another clinical report confirmed similar effects of biphasic insulin secretion after chronic olanzapine treatment [270]; these studies highlight the importance of periodic individual patient-risk assessments for hyperinsulinemia, which can be completed through use of the standard GTT and blood sampling technique.

Glucagon Secretion

Induction of SGA drug-induced glucose dysregulation and insulin resistance may also involve pancreatic α-cell dysregulation, signified by elevated fasting glucagon levels and deficient suppression of postprandial glucagon secretion [271]. Type II DM is characterised by α-cell insulin resistance: hyperglucagonemia is detected in combination with normal or

elevated insulin levels and chronic exposure to hyperglycemic states directly impairs insulin-mediated signaling in the pancreas [272]. Furthermore, high levels of glucagon counteract insulin's actions primarily at the site of the liver, where HGO is significantly enhanced by means of increased glycogenolysis and gluconeogenesis, while hepatic glucose uptake (HGU) and storage remains impaired [273].

However, within the last decade, a large body of clinical data has suggested that incretin-based therapies including GLP-1 receptor agonists are improving many of the pathophysiological abnormalities associated with pancreatic hyperglucagonemia and glucotoxicity [274]. In one randomized placebo-controlled phase 3 clinical trial, the GLP-1 agonist exenatide was associated with progressive corrections in glycemic control and pancreatic β-cell function, and interestingly, restored hepatic enzymatic function in diabetic patients after 3 years of treatment [275]. The rationale behind GLP-1 analog treatments for SGA drug-induced metabolic side-effects is relatively new [276], with a lack of clinical reports in humans. However, Smith and colleagues have been particularly interested in these analog compounds and have used rat paradigms to study the beneficial effects primarily on clozapine-induced glucose derangements (previously discussed in several chapters of this thesis) [70, 129]. From these preclinical studies, clozapine and olanzapine have been shown to induce glucagon secretion with parallel decreases in GLP-1 levels; and therefore patients on these medications may likely benefit from GLP-1 agonist therapy to relieve the additive hyperglycemic effects due to enhanced circulating glucagon levels. Nevertheless, clinical trials of this nature are warranted to determine the specific effects in humans.

5.2.3 Liver

Hepatic Glucose Uptake

During normal postprandial conditions, when portal insulin levels are high, the liver extracts approximately one third of ingested glucose to ensure glycogen synthesis and storage [277]. Thus, impaired insulin-mediated HGU is coupled to the pathophysiological development of whole-body hyperglycemia and peripheral insulin resistance. The close association of four key enzymes that either retain glucose inside the hepatocyte (glucokinase and glycogen synthase) or promote the release into the circulation (glucose-6-phosphatase and glycogen phosphorylase) regulate glucose homeostasis [278]. Direct impairments in enzymatic activities, such as suppressed glucokinase activation, generates irregular hepatic glucose flux and contributes to impaired glucose uptake in prediabetic individuals [279]. Generally speaking, the term hepatic insulin resistance has been utilized to describe the overproduction of hepatic-derived glucose (see below, *Hepatic Glucose Output*) but could theoretically be used to describe improper insulin-mediated hepatic glucose clearance from blood to hepatocyte. However, both clinical and preclinical literature on SGA drug treatment has yet to examine this topic in detail. Thus, further research on altered HGU in humans that are currently on SGA drug treatment is necessary.

Hepatic Glucose Output

One of the primary defects contributing to hyperglycemia is the gross overcompensation of HGO, as evident by increased gluconeogenesis and glycogenolysis [280]. The cause for inappropriately elevated rates of HGO remains unknown, but to a large extent, is brought

about by hepatic insulin resistance. This defect has yet to be confirmed in persons undergoing SGA drug therapy. Conversely, one clinical report has suggested that APD naïve patients with schizophrenia already display hepatic insulin resistance, as evident from increased HGO, compared to matched controls [281]. Therefore, some patients may already be predisposed to metabolic side-effects and SGA drug therapy may be causing further exacerbations in glucose metabolism.

It is now known that forkhead box transcription proteins including FoxO1, and peroxisome proliferative activated receptor-γ co-activator 1 (PGC-1α) are required for HGO, and represent viable targets in several animal models of insulin-signaling deficiencies. Through use of wild-type and mutant alleles of FoxO1 and PGC-1α in isolated hepatocytes, Puigserver and colleagues demonstrated the important interactions between these two transcriptional factors [282]. This evidence suggests that insulin-mediated Akt phosphorylation disrupts the interaction between these proteins promoting nuclear exclusion of FoxO1, and ultimately reduces the expression of enzymes required for HGO (glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK)) [282, 283]. Intriguingly, preclinical data on FoxO1 knockout mice report significant reductions in gluconeogenesis and decreased expression of G6Pase and PEPCK mRNA [284]. Similarly, novel transcriptional factors including FoxO6 have recently been implicated with insulin-dependent regulation of hepatic gluconeogenesis, where the selective knockdown of FoxO6 in insulin-resistant livers of diabetic mice reduced the uncontrolled HGO [285]. Of important clinical interest, two single nucleotide polymorphisms (SNPs) of the FOXO1 gene (rs2721068 and rs2297627) were associated with β-cell dysfunction and glucose intolerance in a sample of both Type II diabetic and nondiabetic human subjects [286]. Together, these

genetic studies implicate the role of genetic variation in the development of glucose dysregulation and verify a critical interaction between insulin and the downstream protein machinery. To our knowledge, clinical studies have yet to examine the genetic associations between increased HGO and SGA drug use, but this topic represents a key area for future genetic research. Nevertheless, as discussed throughout this thesis, numerous preclinical studies have confirmed that olanzapine and clozapine treatment are associated with increased HGO and hepatic insulin resistance [69, 71, 73], and indicate that the liver is the primary target for peripheral SGA drug-induced glucoregulatory effects.

5.2.4 Skeletal muscle

Glucose Uptake

After the infusion of a glucose load *in vivo*, skeletal muscle mass is accountable for 75% of total body glucose disposal [287], and therefore is significantly implicated in the development of peripheral insulin resistance. In fact, skeletal muscle insulin resistance is often evident before overt pancreatic defects and hyperglycemia [288]. Under normal glycemic conditions, insulin-stimulated glucose uptake remains the rate-limiting-step for glucose utilization in skeletal muscle. The signaling cascade involves insulin receptor-induced activation-of IRS proteins, coordinated regulation of vesicle trafficking machinery and activation of PI3K and Akt to mainly increase the translocation of GLUT4 to muscle cell surface [289]. It has been repeatedly shown that skeletal muscles composed primarily of slow oxidative fibers (e.g. soleus) have increased GLUT4 isoform content, and therefore display increased insulin sensitivity compared to fast glycolytic fibers (e.g. extensor digitorum

longus) [290]. To illustrate the importance of GLUT4 in insulin-mediated glucose uptake, heterozygous GLUT4 knockout mice are hyperglycemic, hyperinsulinemic, and display significant muscle insulin resistance [291]. The results from Study 7 of this thesis confirm GLUT4's powerful effect of glucose disposal, and indicate that olanzapine treatment may be directly impairing whole-body glucose disposal in skeletal muscle, while routine exercise partially protects the glucose clearance pathway. A preclinical study conducted by Panariello and colleagues demonstrated that L6 muscle cells exposed to clozapine treatment caused inhibition of insulin-stimulated glucose uptake, whereby clozapine impaired the insulin receptor tyrosine kinase activity and downstream Akt phosphorylation cascade [292]. Mitigation of skeletal muscle insulin resistance is thus imperative for improving systemic glucose homeostasis. Preclinical evidence strongly suggests that interventions which directly promote insulin sensitivity at insulin-sensitive tissues, such as routine aerobic exercise, should be incorporated into psychiatric patients' therapeutic regimen.

5.2.5 Adipose tissue

Glucose Uptake

The mammalian adipose tissue pool is characterised into two different types of fat: white adipose tissue (WAT) and brown adipose tissue (BAT). Even though BAT is richly innervated by the sympathetic nervous system and is therefore essential for physiological purposes including cold-induced thermogenesis [293], peripheral insulin resistance, to a considerable degree, is attributed to defective signaling in WAT. This is primarily because WAT is the body's main repository for excess energy (in the form of triglycerides) and many

hormones and cytokines that regulate insulin-sensitivity are directly secreted from adipocytes [294]. Excessive amounts of dysfunctional WAT can advance the initial stages of obesity and metabolic dysregulation [295]. During increased postprandial levels of glucose, insulin activates GLUT4-mediated glucose transport to promote the re-esterification of triglycerides [296]. Interestingly, when rats are challenged with a continuous insulin infusion to mimic hyperinsulinemia, mRNA levels of GLUT4 are elevated in WAT with a parallel decrease in skeletal muscle [297]. Aside from direct glucose uptake, insulin also exerts potent effects on lipolysis, such as inhibiting the mobilization of fatty acids through activation of its downstream kinase [298]. Consequently, this can secondarily affect blood glucose levels through decreasing the supply of fatty acids and glycerol to the liver, which reduces *de novo* glucose synthesis. Thus, WAT represents a tissue that is modified by both insulin and counter regulatory hormones (such as catecholamines) to buffer the transition between glucose uptake and lipogenesis.

Many clinical and preclinical studies have provided evidence that SGA drugs enhance visceral adiposity (increased WAT) [76, 299, 300], as well as increase circulating lipids in the bloodstream [301, 302]. Thus, hypertriglyceridemia leads to enhanced circulating FFAs, which indirectly induces HGO. A recent preclinical study demonstrated that after a single dose of either clozapine (25 mg/kg) or olanzapine (5 mg/kg) to female rats, an up-regulation of gluconeogenic and lipid-related gene expression occurred in both liver and WAT depots, with concurrent hyperglycemia, increased FFAs and hyperglucagonemia [119]. Furthermore, olanzapine was shown to enhance both glucose and FFA uptake into WAT to further increase lipogenesis in rats, and suggests that increased accumulation of fuel into storage depots promotes adiposity [303]. Increased adipose tissue has been highly correlated with all four

metabolic risk factors: glucose dysregulation, insulin resistance, hypertriglyceridemia, and hypertension. Likewise, many psychiatric patients have poor nutritional and lifestyle habits, placing them at higher risk for metabolic syndrome, Type II DM and cardiovascular disease.

5.2.6 Concluding remarks

Despite accumulating evidence regarding SGA drug use and the associated contributing defects in glucose metabolism, complete understanding of the cellular-specific effects of individual compounds remain to be elucidated. The most consistent preclinical findings, and those that are most homologous with clinical literature, suggest that dysfunctional biological pathways in the pancreas (hyperinsulinemia and hyperglucagonemia), intestine (reduced GLP-1 levels), WAT (indirect HGO and enhanced visceral adiposity), skeletal muscle (peripheral insulin resistance) and the liver (increased HGO and hepatic insulin resistance) are direct triggers of peripheral glucose dysregulation and insulin resistance. There is good evidence that APDs of higher metabolic risk affect these pathways to different degrees, leading to the variable spectrum of metabolic abnormalities associated with the individual SGA drug compound. However, follow-up studies should be conducted to determine the specific downstream targets in various insulin-sensitive tissues, and to delineate specific mechanisms of SGA drug-induced side-effects.

5.3 Limitations of the studies

As indicated throughout sections of this thesis, it has proven challenging to develop an animal model that reliably displays APD-induced metabolic side-effects in its entirety, and therefore, the model is left with several limitations. Perhaps the most substantial limitation to the current animal paradigm is the lack of consistent weight gain demonstrated from chronic administration of APDs with high metabolic liability. Interestingly, a recent report indicated that when the SGA drug olanzapine (7.5 mg/kg) is administered via osmotic mini pump to female rats for two weeks, significantly more weight gain and visceral fat accumulation occurs compared to the subcutaneous or intraperitoneal routes [256]. In addition, the duration of drug treatment and pharmacokinetics have also become an apparent factor in drug-emergent weight gain in rodents. Generally, the majority of rodent studies that report olanzapine-induced weight gain do so in the first three weeks of drug treatment [75, 81, 83, 181, 186], while studies that extend drug treatment to greater than four weeks do not observe significant body weight gain [79, 185]. Nevertheless, several metabolic sequelae including hyperglycemia, glucose intolerance and insulin resistance are reliably observed both in the acute and chronic setting. The availability of an animal model that characterizes all pathological and phenotypic features of APD-induced metabolic syndrome is therefore a crucial need that remains to be addressed.

As the majority of studies contained within this thesis are acute in nature, the short-term study of APD-induced glucose dysregulation may not fully represent the clinical situation, where patients are on antipsychotic medication for life. However, chronic studies in animals are complicated by various variables, including behaviour (such as cyclic feeding

192

times and normal weight gain with age) and dosing patterns (single daily treatment vs. continuous dosing). The rationale behind choosing the appropriate dose in preclinical models is complicated by the fact that there is no 'clinically relevant dose' in animals, which is a major short-coming. Nearly all research groups choose the dose based on previous studies of behavioural paradigms (e.g. PPI) or from qualitative studies on D_2 receptor occupancy ratios in brain regions such as the striatum. The doses used in the current thesis are those obtained from such preclinical models, in addition to our pilot dose-response experiments.

Another weakness of the above studies conducted in this thesis involves the use of rodent phenotypes that do not mirror the clinical situation. Persons affected by schizophrenia are thought to have pathophysiological dysfunctions within the brain, which are polygenic in nature and result from the interplay between specific genes and environmental stressors. For example, evidence suggests that dysfunctional Akt signaling in the brain may be associated with improper neuronal development and degeneration, and is a postulated etiological mechanism for many mental health disorders [304]. Furthermore, several candidate susceptibility genes including disrupted-in-schizphrenia-1 (DISC-1), neuregulin-1 (NRG-1), Akt-1 and catechol-O-methyltransferase have been related to schizophrenia through genetic linkage and association studies, which have been further studied in genetically modified murine models [304, 305]. All the animals used in the present thesis were healthy naïve rodents that did not contain any predisposed genetic modification or behavioural deficit(s). We chose the present model because we wished to identify the direct SGA drug-induced effects in order to control for the unnecessary pathophysiological effects that would arise from using genetically modified paradigms. However, the validity of animal models of

schizophrenia to assess the metabolic side-effects of SGA drugs remains a key future

endeavor for investigation.

5.4 Future directions

The research in this thesis has shed light on APD-induced glucose and insulin abnormalities,

and importantly, has demonstrated that these effects are *independent* of weight gain. The

results of these studies suggest that the presence of the APD compound in the body is the

causal factor for both glucose and insulin dysregulation, both of which occur via weight-

independent mechanisms. However, it is unknown whether secondary downstream events

further exacerbate the metabolic side-effects. For instance, it is known that acute clozapine

administration in both humans and animals induces hyperlipidemia. Whether increased levels

of lipids have a direct effect on glucose levels during treatment with different APD

compounds remains to be determined. In this regard, future work should evaluate whether

particular metabolic traits, such as chronic hyperlipidemia, hypertension or pre-developed

weight gain have any effects on SGA drug-induced glucose intolerance or insulin resistance.

Achievement of such effects could be conducted through direct administration (i.e., infusion

of lipids through osmotic mini-pump) or through engineered diabetic animal models such as

the obese Zucker or the obese spontaneously hypertensive Koletsky rats [306]. This would

reveal whether or not existing co-morbidities have additive or synergistic effects, as many

patients with schizophrenia already have a predisposition to such conditions.

Evidence indicates the peripheral administration of SGA drugs causes peripheral

effects on end organ targets such as the liver and WAT; however the exact connection

between these effects and those caused through central mediated mechanisms remain an area of active interest [128]. In particular, it would be interesting to examine whether central administration of SGA drugs differed in response to the IGTT or HIEC, and whether or not ameliorative pharmacological and non-pharmacological interventions reduced the metabolic dysregulation to similar degrees. Consequently, use of animal models of SGA drug-induced glucose dysregulation and insulin resistance remains strong and has spawned clinical trials to investigate interventions to ameliorate these effects [307, 308]. Animal models can provide novel insight into underlying physiological mechanisms that give rise to metabolic side-effects. Ultimately, such knowledge may guide future clinical testing of compounds that specifically target symptoms of metabolic syndrome, thereby improving medical therapy and patient compliance by reducing inadvertent drug-induced side-effects.

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