THE DIABETOGENIC EFFECTS OF ANTIPSYCHOTIC MEDICATIONS: FROM RODENTS TO HUMANS

by

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Abstract

A growing body of literature has linked atypical antipsychotics (AAPs) to an increased propensity for weight gain and metabolic disturbances, including type 2 diabetes. While weight gain is a leading risk factor for diabetes, evidence suggests that AAPs may influence glucose homeostasis independently of changes in adiposity. These ‘direct’ drug effects have been consistently supported by animal models, where following even a single dose of certain AAPs immediate effects are observed with noted perturbations on insulin sensitivity, and insulin secretion. However, the mechanisms underlying these effects remain poorly understood. Also, the translational value of the acute dosing rodent model has not been established in humans. As such, we set out to first elucidate mechanisms of these ‘direct’ effects by deconstructing antipsychotic receptor binding
profiles using selective antagonists and gold standard clamping techniques to examine
effects on glucose metabolism. We also investigated antipsychotic administration directly
into the brain in rodents to tease out central vs. peripheral effects on glucose metabolism.
Finally, we examined whether the effects of a single dose of olanzapine on glucose
metabolism could be replicated in healthy humans, independently of adiposity or the
confounding effects of the illness of schizophrenia. Our findings suggest that cholinergic,
serotonergic, and dopaminergic pathways may be involved in antipsychotic-induced
glucose dysregulation. We also suggest that such effects may be mediated in part through
the central nervous system. Our results in humans suggest that acute drug effects may be
less pronounced than in rodents, failing to note an effect on insulin sensitivity or
secretion, but observing other early perturbations in lipid and glucose metabolism. Taken
together, the work here begins to elucidate mechanisms underlying the diabetogenic risk
associated with AAPs, findings which have important implications given the widespread
use of these drugs, as well as the increased mortality attributable to cardiovascular
disease that defines those with schizophrenia.
ACKNOWLEDGEMENTS

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CHAPTER 1
LITERATURE REVIEW

1.0 Schizophrenia:
Schizophrenia is considered to be a chronic psychiatric illness characterized by variability in symptoms and course although disruptive functioning, both social and vocational, is shared in common by many with the illness. The disorder usually begins before age 25, typically requiring lifelong medication (Freedman, 2003). Affecting up to 1 in 100 individuals in the population, the early age of onset and chronic nature make for a significant social and economic burden to the health care system and society. The direct costs of schizophrenia have been estimated in the range of 3.5% of national health expenditures (Knapp, Mangalore, & Simon, 2004). Equally concerning, indirect costs due to lost productivity (attributable to morbidity and premature mortality) have been estimated to exceed direct costs (Knapp et al., 2004). Indeed, patients with a diagnosis of schizophrenia die 12-15 years before the average population, resulting in the observation that schizophrenia is associated with more loss of lives than cancer or many other physical illnesses (Saha, Chant, & McGrath, 2007).

Emerging early in the 19th century, reports describing patients presenting with catatonic, paranoid, and hebephrenic symptoms paved the way for work by Emil Kraepelin who in the early 1900’s grouped together the noted symptoms under the label of “dementia praecox”. Kraeplin’s definition captured the notion of the early and progressive, degenerative nature of this illness, distinguishing it from individuals presenting with more prominent mood symptoms which were linked to improved prognostic outcomes. In 1911, Eugene Bleuler coined the term “schizophrenia”, reflecting his notion of a “schism”, or splitting, of the mind. Bleuler also went on to describe fundamental/primary symptoms including ambivalence, autism, loosening of associations, and changes in affect. As well, he identified accessory symptoms, including those that Kraepelin considered the major components of dementia praecox e.g. hallucinations and delusions. Bleulers’ shift in terminology was also a reflection of a less pessimistic view regarding the illness’ outcome.
Today, schizophrenia is characterized clinically by the presence of hallucinations, delusions, disorganized speech/behaviour, negative symptoms, and social/occupational dysfunction (American Psychiatric Association 2000) (Table 1). Psychosis, prototypically described by delusions and hallucinations (also categorized as positive symptoms), is by no means exclusive to schizophrenia. This is reflected by the various diagnostic categories of psychotic disorders referenced in the DSM-IV and ICD-10 (Table 2). Criteria distinguishing the different categories of psychotic disorder are based on duration, presence of mood symptoms, associated medical conditions or substance use, and level of dysfunction. Although schizophrenia is diagnostically considered a single disease, it probably comprises a group of heterogeneous disorders. Similarly, diagnostic classifications of psychosis exist within the caveat of noted overlaps in genetic liability (Craddock, O'Donovan, & Owen, 2009; Kendler & Diehl, 1993; Lichtenstein et al., 2009), suggesting common underlying etiology. Taken together, these observations supporting shared genetics, as well as some evidence of the presence of attenuated forms of psychosis in ~5% of healthy individuals (van Os, Linscott, Myin-Germeys, Delespaul, & Krabbendam, 2009), have argued for changes in the DSM-V and ICD-10, shifting from a cross-sectional, phenomenological definition, to one which can also incorporate dimensional, longitudinal indicators. Moreover, the definition will likely shift to include a broader clinical perspective to factor changes in functional outcome. In this regard, we may expect to see less focus on positive symptoms and greater attention to other domains such as volition and drive, (the negative symptom dimension), as well as alterations in neurocognition. While the relationship between negative and cognitive symptom domains continues to be a subject of debate, what has become clearer is that together these domains may represent the illness core, and collectively may impact most on functional outcome and recovery (Foussias & Remington, 2010).
Table 1: The DSM-IV diagnostic criteria for schizophrenia (American Psychiatric Association, 1994)

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<th>Diagnostic Criteria for Schizophrenia: DSM-IV Criteria</th>
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<td><strong>A. Characteristic symptoms:</strong> Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated):</td>
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<td>1. delusions</td>
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<td>2. hallucinations</td>
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<td>3. disorganized speech (e.g., frequent derailment or incoherence)</td>
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<td>4. grossly disorganized or catatonic behavior</td>
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<td>5. negative symptoms, i.e., affective flattening, alogia, or avolition</td>
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**Note:** Only one criterion A symptom is required if delusions are bizarre or hallucinations consist of a voice keeping up a running commentary on the person's behavior or thoughts or two or more voices are conversing with each other.

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

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

**D. Schizoaffective and mood disorder exclusion:** Schizoaffective disorder and mood disorder with psychotic features have been ruled out because either 1) no major depressive, manic, or mixed episodes have occurred concurrently with the active-phase symptoms; or 2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.

**E. Substance/general medical condition exclusion:** The disturbance is not due to the direct physiologic effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

**F. Relationship to a pervasive developmental disorder:** If there is a history of autistic disorder or another pervasive developmental disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).
Table 2: Main diagnostic categories of psychotic disorders according to DSM-IV (American Psychiatric Association, 1994)

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<td>• Schizoaffective disorder</td>
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<td>• Schizophreniform disorder</td>
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<td>• Delusional disorder</td>
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<td>• Brief psychotic disorder</td>
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<th>Affective psychoses:</th>
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<td>• Major Depressive disorder with psychotic features</td>
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<th>Substance-induced psychotic disorder</th>
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<tr>
<td>Psychotic disorder due to a general medical condition</td>
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<tr>
<td>Psychosis not otherwise specified</td>
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1.1 Pathophysiology

Currently, schizophrenia is predominantly seen as a biological disorder, and in the face of this there has been a growing focus on brain neurobiological mechanisms. Genetic epidemiologic data (e.g. greater concordance among monozygotic vs. dizygotic twins) point to a significant heritable component accounting for approximately 70% of the risk (Tsuang, 2000). In addition to genetics, an environmental component likely accounts for the remaining 30% of risk, including perinatal insults and psychosocial stressors (Cannon, Jones, & Murray, 2002; Hoek, Brown, & Susser, 1998; Khashan et al., 2008).

The advent of neuroimaging techniques has identified subtle brain changes, including loss of grey matter, ventricular enlargement, as well as alterations in white matter tracts (Ellison-Wright & Bullmore, 2009; Glahn et al., 2008; Vita, De Peri, Silenzi, & Dieci, 2006). Other lines of investigation have pointed to abnormalities in evoked potentials, demonstrating decreased brain responses to new sensory stimuli, with a subsequent inability to suppress brain activity to repeated stimuli, suggesting dysfunctional information processing at higher brain levels (Bramon et al., 2005; Patterson et al., 2008).
Similarly, underlying immunological abnormalities have been associated with schizophrenia; for example, a recent meta-analysis comparing schizophrenia patients with data from healthy controls have suggested aberrations in vivo in interleukin-1-Receptor A (IL-1RA), sIL-2R, and IL-6 (Potvin et al., 2008). As will be discussed later in this work, data supporting an inflammatory syndrome in schizophrenia must however be interpreted with caution given possible confounding effects of factors related to schizophrenia (obesity, smoking, stress, etc.). As it stands, no biomarkers for the illness have been identified, speaking to the lack of sensitivity and specificity even with the more reproducible biologic abnormalities (Allen, Griss, Folley, Hawkins, & Pearlson, 2009). As such, no objective test exists for the diagnosis of schizophrenia, leaving clinicians to rely on reference criteria in the DSM-IV and ICD-10.

1.2 Treatment

1.2.1 Conventional, first generation or ‘typical' antipsychotics
Lending the earliest, most robust support for schizophrenia as a biological disorder was the serendipitous discovery in the 1950’s that chlorpromazine, a phenothiazine derivative, had antipsychotic activity (Kurland, 1955). In the wake of this finding, it was established that blockade of dopamine accounted for the therapeutic mechanism of the observed antipsychotic effects (Carlsson, 1978), leading to the argument that schizophrenia might represent a disorder of hyperdopaminergic activity. This was later supported by experimental data suggesting that drugs increasing dopamine in the brain could exacerbate or induce psychosis (Curran, Byrappa, & McBride, 2004; Ellison & Eison, 1983; Lieberman, Kane, & Alvir, 1987). Furthermore, clinical antipsychotic potency was found to correlate with affinity for the D2 Receptor (Creese, Burt, & Snyder, 1976; Seeman, Lee, Chau-Wong, & Wong, 1976). Taken together, findings highlighted schizophrenia as a disorder of hyperdopaminergic activity and the importance of D2 blockade in antipsychotic activity, prompting the development of increasingly potent dopamine D2 antagonists. This shift from broader heterogeneous receptor binding drugs like chlorpromazine to more selective D2 antagonists like haloperidol gave rise to the concept of ‘low potency' and ‘high potency’ agents, respectively. Trials soon emerged to
support the superiority of this earliest group of antipsychotics, now referred to as ‘conventional’, ‘typical’, or ‘first generation’ antipsychotics (FGA), over placebo (Klein, 1969).

Unfortunately, concerns began to emerge regarding various side effects, the most debilitating paradoxically linked to D₂ blockade. More specifically, abnormal movements were associated with dopamine blockade at the level of the nigrostriatal dopaminergic pathways, while treatment effects were tied to their effects at the level of the mesolimbic-mesocortical dopaminergic pathways. This group of adverse motor side effects encompassed the more imminent risk of parkinsonian-like side effects (extrapyramidal symptoms or EPS), akathisia, and dystonia, as well as a longer term risk of tardive dyskinesia, a potentially irreversible side effect affecting up to 25% of treated individuals (J. M. Kane, Woerner, Borenstein, Wegner, & Lieberman, 1986; J. M. Kane et al., 1984) (Casey, 1991). Concerns around these D₂-related side effects, in combination with evidence of limited clinical efficacy, encouraged a search for more effective and better tolerated compounds (Kerwin, 2000), leading ultimately to clozapine and the newer, so called “second-generation” antipsychotics.

1.2.2 Second generation or ‘atypical’ antipsychotics

Clozapine, the first so-called ‘atypical’ antipsychotic (AAP) or second generation antipsychotic (SGA) was introduced into clinical practice in the late 1960s. Its ability to effect an antipsychotic response with minimal EPS became the sine qua non of atypicality. However, it was withdrawn from the market soon after its introduction following reports of deaths later linked to agranulocytosis (Idanpaan-Heikkila, Alhava, Olkinuora, & Palva, 1977). It was ultimately findings of superior efficacy over FGAs in treatment-refractory patients (J. Kane, Honigfeld, Singer, & Meltzer, 1988), that led to clozapine’s reintroduction in the 1990’s. Characterized by a relatively low affinity for the D₂ receptor, and a heterogeneous receptor binding profile to serotonin (5-HT₂ₐ, 5-HT₂ₐ, 5-HT₆, 5-HT₇), dopamine (D₄), muscarinic (M₁,M₂,M₃,M₄,M₅), adrenergic (α₁- and α₂-subtypes) and other biogenic amine receptors (Roth, Sheffler, & Kroeze, 2004), clozapine challenged the notion that only D₂ antagonism was essential to antipsychotic
efficacy (Brunello, Masotto, Steardo, Markstein, & Racagni, 1995). In turn, efforts to find a “safer” clozapine, devoid of the risk of agranulocytosis, have led to the discovery and approval of a number of newer “atypical” agents (asenapine, olanzapine, quetiapine, zotepine, aripiprazole, paliperidone, risperidone, sertindole, aripasidone, amisulpride). At present, several different pharmacological models exist to account for “atypicality”. These include a high ratio of serotonin receptor $5\text{HT}_{2A}$ to dopamine receptors $D_2$ antagonism (Wadenberg, Wikler, & Svensson, 2007), preferential mesolimbic binding (Pilowsky et al., 1997), and rapid dissociation from the $D_2$ receptor (Kapur & Seeman, 2001). Attenuation of excessive blockade of dopamine in the striatum, characterized by the FGAs as a class, is a feature shared in common by these different models.

Despite initial evidence supporting superiority of SGAs over FGAs, emerging studies, including both efficacy and effectiveness trials, have called into question this claim (Jones et al., 2006; R. S. Kahn et al., 2008; Lieberman et al., 2005; Rosenheck et al., 2003). It has been argued that the initial evidence suggesting treatment advantages of SGAs may be attributable, at least in part, to methodological artifacts including use of high comparator doses of haloperidol, predisposing to secondary negative symptoms and/or treatment discontinuation (Tandon & Nasrallah, 2006). Whether differences between first and second generation agents exist remains an issue of debate and investigation. For example, it is generally established that first episode patients have a better treatment response compared to those in later stages of the illness (D. G. Robinson et al., 1999), and respond well irrespectively of the agent used (Armenteros & Davies, 2006; R. S. Kahn et al., 2008; Salimi, Jarshkog, & Lieberman, 2009; Sikich et al., 2008). On the other hand, they may also be more prone to treatment emergent side-effects (D. G. Robinson, Woerner, Delman, & Kane, 2005). With respect to specific symptom domains, it appears that all antipsychotic medications have robust effects on positive symptoms (Leucht, Arbter, Engel, Kissling, & Davis, 2009), reflected in the fact that all antipsychotics share in common $D_2$ antagonism (Kapur & Remington, 2001; Seeman et al., 1976). However, reduction of negative and cognitive symptoms remains a challenge, with no demonstrable efficacy against primary, or so-called “deficit” symptoms regardless of agent (Kirkpatrick, Fenton, Carpenter, & Marder, 2006). While some data
suggest improvement in attention in association with antipsychotic treatment, effects on other relevant cognitive domains remain inconsistent (Green & Braff, 2001).

Direct comparisons between various SGAs provide divergent results with respect to efficacy (Leucht, Komossa et al., 2009; Tandon et al., 2008); however, there is general agreement that clozapine demonstrates clinical superiority over both first and other second generation agents in treatment refractory schizophrenia (Chakos, Lieberman, Hoffman, Bradford, & Sheitman, 2001; Leucht, Arbter et al., 2009; Lewis et al., 2006; McEvoy et al., 2006). Among existing SGAs, there is also evidence of superior efficacy, albeit less robust, for olanzapine over other agents in chronic schizophrenia (Johnsen & Jorgensen, 2008; Komossa et al., 2010; Leucht, Komossa et al., 2009; Lieberman et al., 2005).

1.2.3 Second generation antipsychotics and metabolic side effects
Given a reduced burden of motor-related side effects as compared to the FGAs (Correll, Leucht, & Kane, 2004; Jeste et al., 1999; Leucht, Pitschel-Walz, Abraham, & Kissling, 1999), it was hoped that SGAs would offer better tolerability than their earlier counterparts. Unfortunately, initial enthusiasm in this regard has been tempered by a growing concern regarding a high incidence of metabolic side effects, including weight gain, increased triglycerides/cholesterol, and diabetes (Allison & Casey, 2001; Mackin, 2005; H. Nasrallah, 2003; H. A. Nasrallah & Newcomer, 2004; Wirshing, Spellberg, Erhart, Marder, & Wirshing, 1998). It has subsequently been noted that liability for weight gain and metabolic side effects differs between the SGAs; clozapine and olanzapine have the greatest weight gain liability, followed by risperidone and quetiapine, with newer medications, ziprasidone and aripiprazole for example, noted to have the least propensity for weight gain and associated metabolic disturbances (Allison et al., 1999; Lieberman et al., 2005). Despite differences between individual agents, serious concerns emerged in association with reports of diabetes, diabetic ketoacidosis (DKA) and death in conjunction with use of SGAs (Jin, Meyer, & Jeste, 2002), leading regulatory bodies such as the FDA (Food and Drug Administration) to demand a monograph warning identifying these risks as a class effect. That said, the field has now
recognizes that a dichotomy associating FGAs with EPSE and SGAs with metabolic side effects may be overly simplistic. For example, chlorpromazine was historically linked to diabetes (Charatan & Bartlett, 1955; Dynes, 1969), and more recently has been shown to have comparable metabolic adverse effects to clozapine, during both initial and long-term exposure (Girgis et al., 2011; Lieberman et al., 2003). Similarly, high potency FGAs are likely to have a similar metabolic risk profile to newer agents considered to be lower risk for metabolic concerns (e.g. aripiprazole and ziprasidone).

Further to this point, neither high potency FGAs nor “lower liability” SGAs are devoid of metabolic risk. This is exemplified in first-episode populations and those early on in treatment. In a one year follow-up report of a Comparison of Atypicals for First Episode (CAFÉ) study, significant weight gain (>7%) occurred in 80% of cases with olanzapine, 57.6% with risperidone, and 50% with quetiapine, with mean weight gains of 10kg, 6kg and 5kg respectively (Patel et al., 2009). In a two year follow-up study of first-episode patients with little or no prior exposure to antipsychotic medications, comparison of haloperidol to olanzapine demonstrated that while olanzapine was associated with more rapid and greater weight gain (15kg), treatment with haloperidol was by no means benign, with an associated 7kg weight gain (Zipursky et al., 2005). A 12 week, open label, randomized trial in drug naïve first episode subjects found olanzapine was associated with 7.5kg in weight gain, as compared to risperidone (5.6kg), and haloperidol (3.8kg) (Perez-Iglesias et al., 2007). Interestingly, in a two year follow-up of this study, the difference in weight gain across medications disappeared i.e. olanzapine (10.9kg), risperidone (8.9kg), haloperidol (9.7kg) (Perez-Iglesias et al., 2008), suggesting that treatments differed more by pattern of weight gain rather than the final amount of weight gain. A ten week cohort involving youth with 1 week or less of medication exposure found weight increased by 8.5kg with olanzapine, 6.1kg with quetiapine, 5.3 kg with risperidone, 4.4 kg with aripiprazole, and a 0.2 kg increase in an untreated comparison group (Correll et al., 2009). Collectively, the data involving both short-term and long-term evidence comparing olanzapine or risperidone in chronic patients as well as those experiencing a first-episode, demonstrate a 3 to 4-fold larger magnitude of weight gain in those early on in illness (Alvarez-Jimenez et al., 2008). While the seminal meta-analysis
of the literature examining weight gain liability, reviewing 81 articles, reported average weight gains of 4.45kg for clozapine, 4.15kg for olanzapine, 2.1kg for risperidone, and 0.04kg for ziprasidone over a 10 week period, the present data would suggest that effects of underlying illness and previous treatments in chronic patients may result in an underestimation of impact of antipsychotics on weight gain, as well as an overestimation of differences between agents.

To summarize, neither first or second generation antipsychotics represent a homogenous group of compounds. Nonetheless, introduction of the AAPs or SGAs has highlighted concerns surrounding drug-related weight gain and serious metabolic side effects.

1.3 Metabolic pathology and psychotic illness:

Increased rates of obesity, diabetes, and dyslipidemias in patients with schizophrenia are well-established findings. Compared with the general population, patients with schizophrenia have a lifespan shortened by as much as 15 years, a finding largely attributable to higher risk of cardiovascular disease (CVD) (Hennekens, Hennekens, Hollar, & Casey, 2005). While antipsychotic medications contribute to the metabolic profile of this population, there are many other factors which can amplify the severity of metabolic dysfunction seen in these individuals. First, there may be effects related to the underlying disease process of schizophrenia. As reviewed elsewhere, even prior to the introduction of antipsychotics, a link between metabolic dysregulation and psychotic illness was noted, in particular with respect to risk of diabetes (Kohen, 2004). Some more recent studies (T. A. Cohn et al., 2006; Fernandez-Egea et al., 2008; Ryan, Collins, & Thakore, 2003; Sengupta et al., 2008; Spelman, Walsh, Sharifi, Collins, & Thakore, 2007; Venkatasubramanian et al., 2007), although not all, (Arranz et al., 2004; Sengupta et al., 2008; Zhang, Yao, Liu, Fang, & Reynolds, 2004) examining first episode drug-naïve individuals have suggested an underlying risk of diabetes. Similarly, studies examining drug naïve patients, or those early in treatment, with respect to adiposity in comparison to matched healthy controls have yielded mixed results, with some studies suggesting that patients may have increased abdominal fat (Ryan, Flanagan, Kinsella, Keeling, & Thakore, 2004; Sengupta et al., 2008; Thakore, Mann, Vlahos, Martin, &
Reznek, 2002), while others have found no differences (Zhang et al., 2004). Genetic factors may confer additional risk, as reflected by the observation that diabetes occurs in 30% of relatives of those with schizophrenia (Mukherjee, Schnur, & Reddy, 1989). It is also likely that other factors can increase the risk of metabolic problems; for example, stress has been linked to the metabolic syndrome (Bjorntorp, 2001). Likewise, a myriad of other illness-associated factors, including poor dietary habits, lower socioeconomic status, inactivity, and exceedingly high smoking rates, may contribute to the metabolic vulnerability of this population (Brown, Birtwistle, Roe, & Thompson, 1999).

To summarize, the association between metabolic abnormalities, CVD and schizophrenia itself is by no means straightforward. It becomes important to tease apart the roles of medications and illness, with the goal of assessing to what extent, and by which mechanisms, medications may be contributing to these troublesome side effects. Prior to a discussion of plausible pathways by which these side effects occur, relevant physiology and methods which can potentially be employed to measure medication-induced perturbations will be reviewed with particular focus on glucose metabolism.

2.0 Glucose metabolism:

2.1 Physiology of glucose metabolism

Complex arrays of endocrine and neuronal signals underlie the coordination of energy homeostasis and normal glucose metabolism. Central to regulation of whole-body glucose homeostasis is the tightly coordinated control of insulin action and secretion.

Glucose, a key regulator of insulin secretion, enters from the blood into the pancreatic β-cells through the GLUT2 receptor/transporter and undergoes glycolysis. The catabolism of glucose increases the ATP/ADP ratio, triggering the closure of the ATP sensitive potassium (K$_{ATP}$) channel and resulting in depolarization of the cell membrane. This, in turn, activates voltage gated Ca$^{2+}$ channels, causing an influx of Ca$^{2+}$. The increased intracellular Ca$^{2+}$ leads to protein kinase activation, terminating in fusion of insulin
containing vesicles to the plasma membrane and insulin exocytosis (Kalant H, 2006). Insulin secretion may also be triggered by other pathways and agents besides glucose stimulus, for example by free fatty acids (FFAs) (Prentki, Tornheim, & Corkey, 1997), glucagon-like polypeptide (GLP-1) (Thorens, 1995), and cholinergic agents mediated through the muscarinic M₃ receptor (Persaud, Jones, Sugden, & Howell, 1989).

In healthy individuals, glucose stimulation results in a characteristic biphasic insulin secretory response, with the first (acute) phase starting 1-2 min after glucose challenge, lasting approximately 10 minutes (Perley & Kipnis, 1967). A second phase occurs approximately 10 min after the initial bolus and tends to persist so long as the hyperglycemia is present. The second phase response is composed of pre-existing insulin granules, as well as newly synthesized granules.

Insulin is a small protein, composed of two amino acid chains connected to each other through disulfide linkages. Synthesis of insulin occurs in the β-cell, through the action of specific endopeptidases (PC2 and PC3), which convert a precursor molecule, called proinsulin, to split products (des-64,65-proinsulin, and des-64,65-proinsulin). The split products consist of 3 domains (A, B, C), and are processed via binding of the A and B chain (forming insulin) and cleavage of the C chain (C-peptide) (Neerman-Arbez, Cirulli, & Halban, 1994). Both C-peptide and insulin are packaged into granules, and co-secreted. While C-peptide is biologically inactive, its clearance is not affected by changes in hepatic insulin sensitivity, whereas that of insulin will be. As such, it is a useful marker of “pre-hepatic” insulin secretion (Eaton, Allen, Schade, Erickson, & Standefer, 1980).

Although insulin affects a wide range of physiological processes, its best established role is that of glucose regulation. In response to a rise in plasma glucose levels, insulin is secreted and will bind to its transmembrane receptor, resulting in a cascade of events that maintain normoglycemia. The insulin receptor (IR) is a transmembrane single polypeptide chain and belongs to the receptor tyrosine kinase family. Insulin binds to the insulin receptor and allosterically activates tyrosine kinase in the intracellular domain. The insulin receptor undergoes autophosphorylation on multiple tyrosine residues and
phosphorylates insulin receptor substrates (IRS), resulting in their activation and recruitment of multiple downstream signaling proteins (see figure 1) (Kalant H, 2006). This generates unique insulin responses which vary based on cell type. For example, in skeletal muscle and adipose tissue stimulation of the phosphoinositide 3-kinase (PI3K) pathway will enhance glucose utilization by regulating the expression or subcellular localization of glucose transporters, GLUT 4 and GLUT 1, likewise stimulating the storage of glucose as glycogen or fat (Kalant H, 2006). In pancreatic β-cells, this same cascade has been suggested to promote β-cell survival (Tuttle et al., 2001).

The molecular signaling pathways activated by insulin ultimately mediate energy metabolism in many tissues. In liver and muscle, insulin activates glycogen synthase and inhibits glycogen phosphorylase, consequently increasing the synthesis of glycogen from glucose while simultaneously inhibiting enzymes which promote breakdown of glycogen. Glycogen is a polysaccharide composed of multiple branches of glucose residues, which on short notice can be converted to energy. In the liver, insulin stimulates glucokinase which phosphorylates glucose to glucose-6-phosphate, hence trapping glucose entering from the bloodstream into the hepatocytes. The decreased plasma glucose concentration due to the phosphorylation creates a concentration gradient between the plasma and the liver cells, further facilitating the movement of glucose into hepatocytes. The overall action of insulin is to inhibit gluconeogenesis (glucose production from non-carbohydrate precursors) and glycogenolysis (breakdown of glycogen to glucose), reducing plasma glucose levels. In the adipocytes, insulin promotes triglyceride synthesis and inhibits lipolysis (primarily through inhibition of lipases, including hormone sensitive lipase (HSL), and adipose triglyceride lipase (ATGL), preventing the breakdown of triglycerides to free fatty acid (FFA) (Kalant H, 2006).

In a fasting state, glucose production is predominately produced by the liver via gluconeogenesis and glycogenolysis, ensuring maintenance of euglycemia and sufficient glucose supply to the central nervous system (CNS). This is mediated primarily by the actions of glucagon, a hormone with opposing actions to that of insulin, which is released by the pancreatic α-cell. Glucagon binds to glucagon receptor, a G-protein coupled
receptor (GPCR), which through increased cAMP levels activates protein kinase A (PKA), resulting in glycogen breakdown and release of glucose into the bloodstream. In the case of decreasing blood glucose, increasing sympathetic drive can result in release of epinephrine from the adrenal gland. Epinephrine can then bind to the to β2 adrenergic receptor in the liver and muscle, inducing cAMP-mediated PKA activation in an analogous manner to glucagon. Other hormones which can drive carbohydrate metabolism in a manner opposing insulin in order to raise blood glucose include estrogen and cortisol, and are often referred to as insulin-counter-regulatory hormones (Guyton, 2006).

The liver also has the ability to either synthesize lipids de novo (lipogenesis), or in the case of decreasing blood glucose to use them for energy by mitochondrial β-oxidation. Mitochondrial β-oxidation causes the breakdown of fatty acids to ketone bodies (i.e. acetoacetate and β-hydroxybutyrate) as well as acetyl CoA; the latter can be utilized for energy. In states of prolonged insulin deficiency, this process can result in excessive production of ketones and decreasing blood pH, which through disruptions in osmotic balance can be fatal if not treated promptly (Guyton, 2006).
2.2 Abnormal glucose metabolism and diabetes

Diabetes mellitus is a chronic disorder characterized by defects of insulin action and/or secretion. Current classification of diabetes is based on underlying etiology, and includes 4 clinical classes ("Diagnosis and classification of diabetes mellitus," 2005; Rodbard et al., 2007):

- Type I diabetes mellitus (DM1), which is characterized by autoimmune destruction and absolute insulin deficiency
- Type 2 diabetes mellitus (DM2), defined by insulin resistance and relative insulin deficiency
- Gestational diabetes (GDM), characterized by a first onset of glucose intolerance in pregnancy
- Other specific types, which include genetic defects in insulin action, β-cell function, endocrinopathies, drug- or chemical-induced perturbations, or genetic syndromes.
Type 2 diabetes mellitus remains the most prevalent form of diabetes, accounting for 90-95% of individuals with diabetes. It represents a complex metabolic disorder with evidence indicating significant roles for both β-cell function and insulin resistance. Insulin resistance is likely a defect which is present many years before diabetes is diagnosed, and is characterized by a decreased response to insulin in its key target tissues, including liver, muscle and adipose tissue (Warram, Martin, Krolewski, Soeldner, & Kahn, 1990). In the liver, insulin resistance results in an inability to properly suppress hepatic glucose production (HGP), contributing to fasting glycemia. In skeletal muscle, insulin resistance manifests itself through reduced glucose uptake as well as increased breakdown of glycogen stores. In adipose tissue, similarly to skeletal muscle, insulin resistance reduces glucose transport but, more importantly, results in loss of insulin’s suppressive effects on stored triacylglycerols (TAGs), resulting in release of FFAs into the circulation (Guyton, 2006). In turn, FFAs have a number of adverse metabolic consequences, including further detrimental effect on glucose uptake via inhibition of key glucose transporters (Boden & Shulman, 2002; Y. Kim, Tamura, Iwashita, Tokuyama, & Suzuki, 1994; Roden et al., 1996), and lipotoxic effects on various tissues, including the endocrine pancreas and cardiac tissues (Y. Lee et al., 1994). Similarly, this lipotoxicity explains why obesity/adiposity remains one of the best-established risk factors for DM2. Adiposity is associated with ectopic lipid accumulation in non-adipose tissues, including liver and muscle, where they are transformed to acyl-coenzyme A (acyl-CoA), then oxidized in mitochondria to generate ATP. When present in excess, acyl-CoA is metabolized to diacylglycerols and ceramides, compounds which can prevent IRS activation, leading to reduced glucose uptake and glycogen synthesis in muscles as well as increased HGP (Savage, Petersen, & Shulman, 2007). Furthermore, adipose tissue is increasingly being understood as an active endocrine organ and both obesity and DM2 are recognized to be associated with a low grade systemic inflammatory response, characterized by altered cytokine production and activation of inflammatory signaling pathways (Hotamisligil, 2006). The adipocytokines (proteins acting in an autocrine, paracrine, or endocrine fashion to influence several metabolic functions) secreted by adipose tissue have, in turn, been implicated in the development of insulin resistance. For
example, leptin and adiponectin are understood to be insulin sensitizers, while resistin, tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) as insulin antagonists (Pittas, Joseph, & Greenberg, 2004).

Predisposition and development of insulin resistance is dictated by a combination of genetic and environmental factors (gender, age, ethnicity fitness, diet, smoking, obesity) ("Canadian Diabetes Association 2008 clinical practice guidelines for the prevention and management of diabetes in Canada," 2008; "Diagnosis and classification of diabetes mellitus," 2005; "Diagnosis and classification of diabetes mellitus," 2012). Regardless of cause, as insulin resistance develops and progresses the β-cell initially compensates by increasing insulin production and secretion, thus maintaining normoglycemia. However, in predisposed individuals the pancreatic β-cell begins to decline in mass and function, with a corresponding decline in insulin. The initial manifestation of the latter is typically loss of the first phase insulin response, resulting in postprandial hyperglycemia, whereas fasting hyperglycemia tends to be a later phenomenon. The growing discrepancy between a decline in β-cell compensation and growing insulin resistance results in progression through the spectrum of corresponding clinical abnormalities (pre-diabetes, comprising both impaired fasting glucose and impaired glucose tolerance, then DM2). Table (3) describes the diagnostic criteria for diabetes and prediabetes according to the ADA ("Diagnosis and classification of diabetes mellitus," 2012).

Given the interdependence of insulin sensitivity and β-cell function in the development of diabetes, the argument has been made that they should always be considered in tandem. As described in studies conducted in healthy controls, there exists a hyperbolic relationship between insulin sensitivity and secretion (Bergman, Phillips, & Cobelli, 1981). Changes in sensitivity are compensated for by inverse changes in β-cell response; that is, if a product of sensitivity and secretion is calculated the result is a constant, termed the “disposition index”. The hyperbolic relationship has also been validated in individuals with normal glucose tolerance, but varying degrees of adiposity and hence insulin resistance (S. E. Kahn et al., 1993). Lending support to the central role of β-cell dysfunction in the pathogenesis of DM2, data from United Kingdom Prospective

**Table 3**: American Diabetes Association Diagnostic criteria for diabetes and pre-diabetes ("Diagnosis and classification of diabetes mellitus," 2012)

<table>
<thead>
<tr>
<th><strong>Diabetes:</strong></th>
</tr>
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<tbody>
<tr>
<td>1. Symptoms of hyperglycemia (polyuria, polydipsia, unexplained weight loss) AND</td>
</tr>
<tr>
<td>Casual Plasma glucose ≥200mg/dL (11.1mmol/L) OR</td>
</tr>
<tr>
<td>2. Fasting plasma glucose ≥ 126mg/dL (7.0mmol/L) OR</td>
</tr>
<tr>
<td>3. 2-hour plasma glucose ≥ 200mg/dL (11.1mmol/L) during a 75 gram oral glucose tolerance test (OGTT) OR</td>
</tr>
<tr>
<td>4. HbA1C ≥6.5%. The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program certified and standardized to the Diabetes Control and Complications Trial reference assay.</td>
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<table>
<thead>
<tr>
<th><strong>Pre-diabetes:</strong></th>
</tr>
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<tbody>
<tr>
<td>1. Impaired fasting glucose: Fasting plasma glucose between 100mg/dL (5.6mmol/L) and 125 mg/dL (6.9 mmol/L) OR</td>
</tr>
<tr>
<td>2. Impaired glucose tolerance: 2H plasma glucose in the 75gm OGTT between 140mg/dL (7.8 mmol/L) to 199mg/dl (11.0mmol/L) OR</td>
</tr>
<tr>
<td>3. HbA1C between 5.7-6.4%</td>
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**2.3 Overview of methods used to assess glucose metabolism**
There currently exist a variety of methods to assess glucose metabolism, including insulin sensitivity and β-cell function. With respect to insulin sensitivity, the hyperinsulinemic euglycemic clamp (HIEC) continues to be considered the gold standard (DeFronzo, Tobin, & Andres, 1979). The underlying principle of the HIEC is an intravenous insulin infusion (dosed per body surface area per minute), resulting in a new steady state, higher than fasting (i.e. hyperinsulinemic) insulin level. This should result in increased glucose disposal in muscle and suppression of hepatic glucose output. Blood glucose is frequently monitored under these conditions, while a variable glucose infusion maintains euglycemia. Based on the principle that HGP is suppressed, and that there is no net change in blood glucose, the glucose infusion rate should be equal to glucose disposal, allowing determination of whole body glucose disposal and an estimation of insulin sensitivity. Radioactive tracer infused during the clamp can also allow the separate determination of glucose production and utilization. A variant of the clamp, called the hyperglycemic clamp, allows for the determination of β-cell function as well as an estimation of peripheral tissue sensitivity. The hallmark of this technique is stimulation of the β-cell with the same concentration of hyperglycemia, allowing comparison of β-cell response through measurements of insulin and C-peptide across different subjects or treatment conditions (Elahi, 1996). Clamping techniques, however, are expensive, labor intensive, and lack practicality in the context of larger scale studies. Other existing methods include the insulin suppression test (IST) (Greenfield, Doberne, Kraemer, Tobey, & Reaven, 1981), the minimal model (or frequently sampled intravenous glucose tolerance test (FSIGTT)) (Bergman, Ider, Bowden, & Cobelli, 1979; Bergman et al., 1981), the homeostasis model assessment (HOMA) (Matthews et al., 1985)(24), and insulin tolerance test (ITT) (Grulet, Durlach, Hecart, Gross, & Leutenegger, 1993). The minimal model, which will be described in more detail in chapter 4, has been repeatedly shown to correlate strongly with euglycemic clamping techniques, and is generally considered less invasive and more cost-effective (Bergman et al., 1979; Bergman, Prager, Volund, & Olefsky, 1987; Saad et al., 1994). Briefly, it involves frequently sampling plasma glucose and insulin following a glucose challenge, data which is then entered into a computer model of glucose kinetics, allowing for a calculation of insulin sensitivity. Unlike the clamp, it does not require a continuous glucose infusion. It also allows for
determination of both insulin-dependent and insulin independent (i.e. glucose effectiveness) glucose utilization, and allows for estimation of β-cell function, which in the case of clamping techniques would require a separate hyperglycemic clamp. As a drawback, the minimod cannot distinguish between peripheral and hepatic glucose utilization; also, while it allows for assessment of β-cell function, it is not able to measure second-phase insulin secretion. In an IST, the subject is exposed to fixed-rate infusions of insulin and glucose, but plasma glucose is not “clamped” at a constant value. Instead, the underlying principle of the test is that in insulin sensitive individuals plasma glucose will decrease to a greater extent over time than in insulin resistant subjects (Greenfield et al., 1981).

Indirect or surrogate measures of insulin sensitivity include the HOMA method, as well as the oral glucose tolerance test (OGTT). In large scale epidemiological studies, or in cases where repeated assessment of insulin sensitivity is needed, the HOMA method is often considered the most practical method (Matthews et al., 1985)(24). It employs a mathematical model predictive of glucose and insulin concentrations in cases of varying degrees of insulin resistance or β-cell dysfunction. Although less precise than the minimod method or clamping techniques, data suggest that HOMA estimates correlate with HIEC results (Bonora et al., 2000; Katsuki et al., 2001; Matthews et al., 1985). It also allows for an estimation of β-cell function, termed the HOMA-B. In the OGTT, subjects are challenged with a 75 gm dose of glucose or dextrose, and glucose and insulin levels are measured several times after the initial glucose administration. Various mathematical simulations have then been employed to calculate indices of insulin sensitivity based on kinetics of glucose and insulin (Gutt et al., 2000; Mari, Pacini, Murphy, Ludvik, & Nolan, 2001; Matsuda & DeFronzo, 1999), as well as approximations of an acute insulin secretory response (Hansen et al., 2007). While the OGTT may be considered simpler, more physiological and less invasive than any of the direct techniques of measuring insulin sensitivity, several challenges exist with this approach. First, the range of insulin values can vary widely between laboratories and individuals, and challenges exist in the interpretation of results based on a lack of rigorously defined normal ranges of insulin values (Robbins et al., 1996). Similarly, the
OGTT is not solely a reflection of insulin sensitivity but rather the culmination of many other processes that can affect plasma patterns of insulin and glucose after glucose ingestion. These include rates of glucose absorption, β-cell response to glucose and incretins, and insulin independent effects on glucose uptake and production (Hucking, Watanabe, Stefanovski, & Bergman, 2008). As such, interpretation of OGTT-derived values of insulin sensitivity warrant caution based on confounds by factors other than insulin-sensitivity changes per se (Hucking et al., 2008).

3.0 Atypical agents and glucose dysregulation:
DM2 is associated with significant long-term sequelae, including CVD. As reviewed by the Canadian Diabetes Association (CDA), a recent epidemiologic analysis projected that prevention of diabetes in males could reduce cardiovascular mortality in the entire US population by 9.0% (Narayan et al., 1999). Data from the US similarly indicate that 28% of cardiovascular expenditures are attributable to DM2 ("Economic costs of diabetes in the U.S. In 2007," 2008). Even hyperglycemia occurring below threshold for diagnosing diabetes is associated with greater risk of cardiovascular complications (Laakso, 1999).

Further, insulin resistance and DM2 are often considered to be central to the pathophysiology of a broader underlying disorder; the metabolic syndrome (MS), a constellation of distinctive risk factors predictive of CVD (Ford, 2004). Although a number of definitions of the syndrome have been proposed (differing based on inclusion of different factors and thresholds), it remains a strong a predictor of both DM2 and CVD (Balkau, Valensi, Eschwege, & Slama, 2007). While obesity may exaggerate the MS phenotype, insulin resistance represents a key element that, independent of weight, can lead to disturbances in lipids, blood pressure, and dysglycemia, which characterize the MS (Gallagher, Leroith, & Karnieli, 2010). It is estimated that patients with schizophrenia carry a 2-5 fold risk in the prevalence of DM2 as compared to the general population (De Hert, Peuskens, & van Winkel, 2009; Dixon et al., 2000), in keeping with a 2-fold increase in the prevalence of MS (McEvoy et al., 2005). Recent work by our group has underscored the scope of this problem, demonstrating in a sample of patients
with schizophrenia that the prevalence of diabetes was three times, and risk of MS almost two times, the rate observed in a comparative control sample (T. Cohn, Prud'homme, Streiner, Kameh, & Remington, 2004).

Although AAP-associated weight gain is a leading risk factor in the development of glucose dysregulation, evidence has also pointed to direct medication-related effects in this already at-risk population. This has been supported by a significant number of case reports of DKA, occurring early in treatment and prior to weight gain, with improvements in hyperglycemia following discontinuation, and in some cases, reoccurrence with medication re-challenge (Jin et al., 2002). While the physiology of DKA is complex, ketoacidosis is characterized by a profound deficiency in insulin secretion, suggesting that antipsychotics may influence pathways of pancreatic β-cell function. In keeping with antipsychotic-related effects on β-cell function, two prospective studies in antipsychotic-naive patients treated with AAPs reported increases in fasting glucose and/or decreases in insulin sensitivity as measured by HOMA (Fernandez-Egea, Miller, Garcia-Rizo, Bernardo, & Kirkpatrick, 2011; Oriot et al., 2008), alongside a disproportionally lower increase in compensatory insulin secretion than would be expected for the change in glycemia. Similarly, a prospective study in patients with schizophrenia being switched from treatment with conventional antipsychotics to treatment with olanzapine or risperidone reported significantly decreased insulin response during an intravenous glucose tolerance test, independent of changes weight (Chiu, Chen, Liu, & Lu, 2006).

While pharmacoepidemiological data examining associations between antipsychotic medications and diabetes have produced conflicting results, the majority have reported a higher incidence of diabetes in patients treated with antipsychotics as well as differential associations between agents, with a higher incidence among patients taking atypical antipsychotics (Buse et al., 2003; De Hert et al., 2008; Gianfrancesco, White, Wang, & Nasrallah, 2003; Kornegay, Vasilakis-Scaramozza, & Jick, 2002; Koro et al., 2002). For example, a recent meta-analysis, controlling for independent risk factors of diabetes (i.e. BMI, family history of diabetes, ethnicity, age, physical activity, social economic status),
reported an increased risk of diabetes with SGAs (clozapine, risperidone, quetiapine, clozapine) as compared to FGAs in schizophrenia (M. Smith et al., 2008). Differential effects between antipsychotics supports the notion of direct antipsychotic effects on glucose homeostasis; however, and as reviewed by Scheen and De Hert, data from pharmacoepidemiological databases must be interpreted with caution given limitations in the quality of clinical data, use of insensitive surrogate markers for diabetes, variable comparison /control for risk factors of diabetes, and inability to prove causality (Scheen & De Hert, 2007). As noted in the preceding section, the usefulness of comparing agents by “generation” or “atypicality” may also be less helpful than comparisons based on individual drugs. From this perspective, olanzapine and clozapine appear to have the strongest association with development of diabetes (Newcomer, 2004).

Given that weight gain associated with antipsychotic medications remains one of the best established risk factors for diabetes, Henderson and colleagues, in a cross-sectional study design, used the FSIGTT to compare glucose metabolism in non-obese patients with schizophrenia who were matched for body mass index (BMI). Results indicated a differential effect for clozapine and olanzapine, as compared to risperidone, on measures of insulin resistance as well as insulin-independent glucose disposition (i.e. glucose effectiveness) (Henderson et al., 2005). Similarly, Newcomer and colleagues, matching patients and controls for BMI and age, found elevated serum glucose at baseline, and following an OGTT in patients treated with clozapine and olanzapine as compared to those on conventional antipsychotics or matched healthy controls (Newcomer et al., 2002). Contrary to these findings, Haupt and colleagues employed the FSIGTT in chronically treated patients with schizophrenia, reporting a strong relationship between adiposity and insulin resistance but failing to detect “adiposity-independent effects” (i.e. controlling for the antipsychotic used) (Haupt, 2006).

Several prospective trials in patients with schizophrenia have attempted to tease apart weight-gain independent effects of antipsychotic medications. A double-blind, randomized trial comparing treatment with clozapine, olanzapine, risperidone or haloperidol in patients refractory to prior treatment found increases in glucose levels in
those treated with olanzapine, clozapine and haloperidol, independent of changes in weight or BMI (Lindenmayer et al., 2003). Similarly, a recent trial randomizing patients with chronic schizophrenia to olanzapine or risperidone, demonstrated that treatment with olanzapine was associated with increases in fasting measures of insulin resistance and greater decreases in glucose tolerance during an OGTT (R. C. Smith et al., 2009).

Changes in insulin resistance were not strongly related to changes in waist circumference or BMI during the 5-month treatment period (R. C. Smith et al., 2009). Van Winkel and colleagues conducted a naturalistic study in individuals with schizophrenia starting treatment or being switched to specific AAPs. Patients were evaluated at baseline and three months post-treatment with an OGTT; findings indicated differential effects between agents studied, with the most pronounced deterioration in glucose metabolism noted in those patients started on clozapine, olanzapine, and quetiapine versus aripiprazole, independent of changes in BMI (van Winkel, De Hert, Wampers et al., 2008). They also reported a 3-month incidence of new onset diabetes of 4.2%, as compared to a 0.72% incidence rate estimated in a general population sample (Bonora et al., 2004). Interestingly, none of the known risk factors for diabetes present in the study subjects prior to treatment predicted changes in glucose homeostasis, supporting the notion that medication-related glucose dysregulation may represent a different phenomenon than adiposity associated changes (van Winkel, De Hert, Van Eyck et al., 2008). In a prospective design, Howes and colleagues examined the pre- and post- treatment effects of clozapine during a 2.5 month follow-up interval, noting no effects on the HOMA IR but, conversely, reporting increases in fasting and 2-hour OGTT, with 11/20 patients developing abnormal glucose tolerance independent of weight gain (Howes et al., 2004). Similarly, a double-blind randomized study employing the euglycemic clamp technique pre- and post-treatment with risperidone or olanzapine suggested decrements in insulin sensitivity correlating with changes in adiposity, also noting significant increases in fasting glucose which appeared to occur independent of changes in insulin sensitivity or fat mass (Hardy et al., 2011). On the other hand, a recent prospective 6-month study of olanzapine and risperidone treatment in chronic schizophrenia, using fasting measures and the FSIGTT, found no significant between group differences in measures of insulin sensitivity or β-cell function. However, they did note a decrease in the ability of the β-
cell to compensate for changes in insulin sensitivity with olanzapine in a subset of non-Caucasian patients (Ader et al., 2008).

Taken together, there are inconsistencies between studies, but in general the literature involving patients with schizophrenia suggests that weight-gain independent antipsychotic-induced effects may contribute, at least in part, to the increased rates of diabetes observed in this population, with as of yet unknown factors mediating this risk. Unfortunately, the complex relationship between illness, medication-associated changes in adiposity, and other factors which can add to the risk of glucose dysregulation, makes it extremely difficult to tease apart the roles of each. In this regard, use of animal models affords opportunities to a) control the environment in terms of potentially modifying factors, b) examine drug effects independent of the illness itself, and c) perform invasive procedures and/or harvest tissues to elucidate mechanisms of action. Further, if medications are dosed acutely possible confounders of early changes in adiposity (which can precede absolute weight gain) can be avoided.

4.0 Preclinical models of acute and chronic AAP-induced metabolic dysregulation

Researchers have turned to in vitro work and/or in vivo work in animals to model what is observed clinically and elucidate possible underlying mechanisms of antipsychotic-induced metabolic disturbances. Accordingly, the following section attempts to summarize existing preclinical studies, focusing on antipsychotic-related perturbations of glucose metabolism.

4.1 Weight gain and adiposity

As weight gain remains one of the most consistently recognized metabolic side effects of antipsychotic medications, it is not surprising that efforts have been undertaken to model this effect in rodents. Unfortunately, reproducing the weight gain effects seen in humans has proven more challenging than initially foreseen. As reviewed in detail by Boyda and colleagues, it has been observed that studies often do not demonstrate weight gain, and that when weight gain does occur it is confined to females (Boyda, Tse, Procyslyn,
Honer, & Barr, 2010). Furthermore, the differential weight gain liability seen between the various antipsychotic drugs in the clinical scenario is not always captured in the female rodent model (Kalinichev et al., 2005; Kalinichev, Rourke, & Jones, 2006). Speaking to the gender issue, there are clinical data suggesting that females may be more susceptible to AAP-associated weight gain (Aichhorn, Whitworth, Weiss, Hinterhuber, & Marksteiner, 2007; Gebhardt et al., 2009), although others have failed to demonstrate this (Basson et al., 2001; Ratzoni et al., 2002). Regardless of whether gender is a risk factor for AAP-associated weight gain, males indisputably also gain weight on these medications in the clinical setting.

Examining factors which may explain the inconsistencies described between rats and humans, several potential issues have been identified. First, there are significant interspecies differences in metabolism of antipsychotic medications. Rats metabolize these drugs 4-6 times more rapidly than humans (Aravagiri, Teper, & Marder, 1999) which, unless the drug is administered multiple times per day through a long-acting formulation or via continuous minipump infusion, would be expected to result in a failure to achieve comparable levels to humans. This may be particularly relevant given that rats feed throughout the dark cycle, and studies failing to dose antipsychotic medications close to feeding time may underestimate effects on food intake and weight changes. Although speculative, based on data in humans suggesting that males may have a more rapid drug clearance (Callaghan, Bergstrom, Ptak, & Beasley, 1999), it is also possible that male rats, as compared to females, may require higher doses to achieve comparable effects. However, this hypothesis remains to be tested in rodents. Similarly, dose itself may be an issue; in the animal literature doses are frequently chosen without outlining a rationale (Kapur, Wadenberg, & Remington, 2000). As reviewed by Kapur et al., D₂ receptor occupancy may be the most relevant method to approximate therapeutic dosing in humans (Kapur, Wadenberg et al., 2000). Other considerations that might account for the discrepancies observed between species include food palatability and fat content. This may be relevant given that patients with serious mental illness lead less healthy lifestyles, and may consume more fat than controls (Brown et al., 1999; Roick et al., 2007). Interestingly, in male rats the majority of studies that have reported either absolute weight
gain (Minet-Ringuet, Even, Lacroix, Tome, & de Beaurepaire, 2006; Park, Hong, Lee, & Sung, 2007; Shobo et al., 2011) or hyperphagia (Guesdon, Denis, & Richard, 2010; Hartfield, Moore, & Clifton, 2003) used diets higher in fat.

The identified limitations have led at least some researchers to conclude that a rodent model of antipsychotic-induced weight gain may not be tenable (Boyda, Tse, Procysyn, Honer et al., 2010; Pouzet, Mow, Kreilgaard, & Velschow, 2003). However, this conclusion may be premature given emerging data suggesting that increases in adipose tissue in association with antipsychotic treatment are adequately modeled and, furthermore, occur irrespective of gender. Indeed, changes in adiposity are consistently reported in both in males (Albaugh, Judson et al., 2011; Cooper et al., 2007; Guesdon et al., 2010; Minet-Ringuet, Even, Goubern, Tome, & de Beaurepaire, 2006; Minet-Ringuet, Even, Lacroix et al., 2006; Muller et al., 2010; Victiriano et al., 2009) and females (Albaugh et al., 2006; Cooper, Pickavance, Wilding, Halford, & Goudie, 2005; Fell, Marshall, Williams, & Neill, 2004; Guesdon et al., 2010; Han, Deng, Burne, Newell, & Huang, 2008; Kalinichev et al., 2006; Lykkegaard et al., 2008). In keeping with antipsychotic-induced changes in adiposity, in vitro studies in adipocytes have also consistently shown olanzapine and/or clozapine-associated increases in lipogenesis, antilipolysis (Albaugh, Judson et al., 2011; Minet-Ringuet et al., 2007; Vestri, Maianu, Moellering, & Garvey, 2007), and pre-adipocyte differentiation (Hemmrich, Gummersbach, Pallua, Luckhaus, & Fehsel, 2006; L. H. Yang, Chen, Yu, & Chen, 2007).

Summarizing, changes in adiposity, in particular in visceral adiposity, may be a more sensitive indicator of antipsychotic-induced changes than weight gain per se. Supporting this position, clinical data have suggested that weight increases with drugs like olanzapine are attributable to increases in body fat (Bergman & Ader, 2005; Eder et al., 2001; Faulkner, Cohn, Remington, & Irving, 2007; Gilles et al., 2010). Furthermore, evidence is emerging to suggest that changes in visceral adiposity may be one of the better predictors of CVD (Despres, 2007), hence representing a side effect which not only can be modeled in a more accurate manner than absolute weight gain, but which also is
highly relevant when translating knowledge to a population burdened by increased cardiovascular mortality.

4.2 Dyslipidemia
In the clinical setting, lipid-associated adverse effects occur routinely in association with drugs like clozapine and olanzapine; there is also data suggesting effects for risperidone and quetiapine, most commonly as elevations of serum triglycerides and total cholesterol (Atmaca, Kuloglu, Tezcan, & Ustundag, 2003; J. M. Meyer & Koro, 2004; Wirshing et al., 2002). Similar to the story involving antipsychotic-induced glucose dysregulation, there is some evidence that antipsychotics may induce dyslipidemia independent of changes in body mass (Birkenaes et al., 2008). Unfortunately, rodents do not appear to be a good model for serum lipid alterations, with most available studies failing to find effects on the lipid profile of rodents treated subchronically with olanzapine (Fell et al., 2007; Kalinichev et al., 2005; Minet-Ringuet, Even, Guesdon, Tome, & de Beaurepaire, 2005; Victoriano et al., 2009), at odds with what is observed clinically. Various reasons may account for this observed lack of effect. First, the majority of the studies may have under-dosed the animals based on frequency of drug administration, or dose selection (Fell et al., 2007; Minet-Ringuet et al., 2005; Patil, Kulkarni, & Unger, 2006; Victoriano et al., 2009). Second, in non-psychiatric models of dyslipidemias and their treatment, rats in general have been found to display responses at variance with those seen in humans (Krause & Princen, 1998).

4.3 Glucose metabolism

4.3.1 In vivo single dose studies
Several studies have now investigated a possible direct antipsychotic effect in rodents applying a single dose paradigm. Assessments of glucose metabolism have ranged from simple measurements of glucose and insulin only to calculations of HOMA-IR, the insulin tolerance test, the glucose tolerance test, and use of gold-standard HIEC and hyperglycemic clamps. Studies examining effects only on insulin and glucose have consistently found increases in both glucose and insulin measurements with clozapine.
(Assie et al., 2008; Murashita, Kusumi, Hosoda, Kangawa, & Koyama, 2007; Tulipano et al., 2007), olanzapine, haloperidol, risperidone, aripiprazole (Assie et al., 2008), with no effects noted with sulpride (Lacruz, Baptista, de Mendoza, Mendoza-Guillen, & Hernandez, 2000), or ziprasidone (Assie et al., 2008). Dosing and route of administration varied widely between studies.

Boyda et al. calculated the HOMA-IR in fasted female rats and also examined glucose clearance during a intraperitoneal glucose tolerance test, reporting a dose and time dependent effect of all the tested antipsychotic medications including clozapine (2mg/kg; 20mg/kg), olanzapine (1.5mg/kg; 15mg/kg), risperidone (0.5mg/kg; 2.5mg/kg), haloperidol(0.1mg/kg; 1.0mg/kg), with the most pronounced effect occurring following clozapine and olanzapine in terms of both insulin resistance and glucose tolerance (Boyda, Tse, Procyshyn, Wong et al., 2010).

Houseknecht et al., using the HIEC procedure in male rats, reported acute insulin resistance in a dose dependent fashion within 40-60 minutes following acute injections of olanzapine (1.0, 3.2, 10mg/kg), clozapine (1.0, 3.2, 10mg/kg), and risperidone (2mg/kg). Tracer studies identified the liver as the main target tissue for insulin resistance, as demonstrated by increases in glucose production (Houseknecht et al., 2007). Smith et al. examined effects of subcutaneous injections of clozapine (10mg/kg), haloperidol (0.25mg/kg), and quetiapine (10mg/kg), employing separate glucose tolerance and insulin tolerance tests (Smith GC 2008) 1 hour post-drug administration. Further, the OGTT was accompanied by a pyruvate and glycerol challenge. Pyruvate and glycerol are two major substrates for gluconeogenesis, and the finding of an increase in area under the curve (AUC) following administration of either substrate in rats exposed to antipsychotics versus vehicle suggested hepatic gluconeogenesis as a contributing factor to the observed glucose dysregulation. Interestingly, the ITT indicated no effect on peripheral insulin sensitivity. Results also indicated increased glucagon levels in association with clozapine despite the concurrent increases in insulin and glucagon, which should have suppressed this insulin-counter-regulatory hormone (G. C. Smith, Chaussade, Vickers, Jensen, & Shepherd, 2008).
Albaugh and colleagues examined two doses of olanzapine (4mg/kg and 10mg/kg), administered in food over a 2-day period, with 2/3 of the final dose administered by gavage one hour prior to assessment of glucose homeostasis. They employed the OGTT, ITT, and HIEC, reporting in all 3 methods dose dependent olanzapine-induced derangements in glucose metabolism, including glucose intolerance and impaired glucose disposal when compared to vehicle. Interestingly, in the HIEC, which employed a “low-dose insulin protocol” to estimate insulin levels occurring in a “fed state”, tracer studies suggested no drug-associated effects on HGP (Albaugh, Judson et al., 2011).

Our own laboratory has also examined the effects of acute, single dose administration of various antipsychotics to male rats, establishing doses based on 70% D2 occupancy, the level of occupancy associated with optimal response clinically (Kapur, VanderSpek, Brownlee, et al., 2003): clozapine 10mg/kg; olanzapine 3mg/kg; risperidone 1mg/kg; ziprasidone 3mg/kg; and, haloperidol 0.25mg/kg. Employing the HIEC technique, it was noted that clozapine and olanzapine had a rapid and potent effect on insulin sensitivity by increasing HGP. Further, both clozapine and olanzapine, in addition to risperidone, also decreased peripheral glucose utilization. Neither haloperidol nor ziprasidone had significant effects on insulin sensitivity. In the hyperglycemic clamp technique, both clozapine and olanzapine impaired β-cell function (Chintoh, Mann, Lam, Lam et al., 2008).

To summarize, acute studies in rodents have consistently shown a rapid and pronounced effect of antipsychotic medications on glucose metabolism. Studies using more sophisticated methods of assessment of glucose homeostasis, with the exception of one (Albaugh, Judson et al., 2011), have consistently shown the liver to be the target organ for the drug-induced dysregulation. The data also suggest that the drug-induced disturbances in glucose metabolism parallel the clinical propensity for weight gain induction, suggesting overlapping mechanisms between the two phenomena. Unlike the rodent antipsychotic-induced weight gain model, sex differences do not appear to mediate the observed effects.
4.3.2 Repeated dose *in vivo* studies:

While it has been assumed that the “non-tenability” of the rodent weight gain model in male rats could confer the advantage of studying direct medication effects independent of adiposity on glucose metabolism, this may not be the case given the consistent changes in visceral adiposity reported. Dosing paradigms also become an issue in repeated or chronic dose studies, as again the rapid metabolism of these drugs precludes maintenance of levels comparable to humans unless the drug is administered in a continuous fashion, and further is dosed in a manner linked to clinical therapeutic dose (e.g. D2 occupancy). Discussion not turns to studies addressing some of these issues, in addition to the specifically examination of glucose metabolism using methods more telling of glucose regulation than stand-alone glucose or insulin levels.

In the same set of experiments described in section 4.3.1., Houseknecht *et al.* examined clozapine administration (10mg/kg) over 4 days, albeit in a once daily dosing paradigm likely precluding sustained systemic drug levels (Kapur, VanderSpek, Brownlee, & Nobrega, 2003). The insulin resistance noted in the acute study was replicated with a 5th day of dosing, suggesting animals did not develop tolerance to the acutely observed effects. Interestingly, it was also noted that during the steady-state period of the clamp and preceding clozapine injection, there was no difference in the glucose infusion rate between drug and vehicle, suggesting rapid reversibility of the acute effect and indirectly supporting the relevance of pharmacokinetics and having the drug in the system to detect changes in insulin sensitivity. In this series of experiments, changes in weight or adiposity were not reported (Houseknecht *et al.*, 2007).

In the aforementioned study involving single dosing of haloperidol, quetiapine, or clozapine by Smith *et al.*, the group administered these drugs for 28 days, again within the limitation of single daily dosing. They found that the impairment in glucose tolerance persisted for clozapine and quetiapine, but not for haloperidol. Like Houseknecht *et al.*, they suggested reversibility of the drug effect, noting that the defect in glucose metabolism was almost reversed 48 hours post-drug administration and completely
abolished 7 days after the final drug dose. This study examined changes in adiposity, finding no changes with drug administration as compared to vehicle using DEXA scans, suggesting effects on glucose homeostasis occurred directly as a consequence of the medications (G. C. Smith et al., 2008).

Using a rat model of DM2 (a 90% pancreateomized rat, which represents non-obese diabetes with insulin resistance and deficiency) (Hosokawa, Hosokawa, Chen, & Leahy, 1996), once daily administration of chlorpromazine (5mg/kg and 50mg/kg) was compared to vehicle in male rats. Employing the HIEC procedure, it was reported that 8 weeks of high dose chlorpromazine administration resulted in increased HGP, but no changes in whole body glucose uptake. The chlorpromazine treated animals (both low and high dose) gained weight as compared to vehicle treated animals, with the high dose group also gaining more than low dose animals. The study could therefore not preclude that impairment in hepatic insulin sensitivity reflected body mass increase rather than direct medication effects (Park et al., 2007).

The same group also investigated effects of the above doses of chlorpromazine as compared to vehicle in partially pancreateomized rats, this time examining β-cell function. In a hyperglycemic clamp procedure, they identified that high dose chlorpromazine impaired both the acute and second phase of insulin secretion, also noting increased apoptosis of pancreatic β-cells (Park, Hong, Lee, Sung, & Kim, 2008). Again, the high dose chlorpromazine-treated rats gained more weight when compared to their low dose or vehicle counterparts, possibly accounting for the decline in β-cell function; for example, fatty acids associated with increased adiposity have been described to mediate apoptosis of the β-cell (Eitel et al., 2003).

In a dog model, animals were given oral risperidone (5 mg daily) or olanzapine (15 mg daily) over a 4-6 week study period. Using the HIEC technique, no effects on peripheral insulin sensitivity were found although hepatic insulin resistance was noted with olanzapine. While the dogs did not gain weight, their visceral fat depot increased;
moreover, an abolished compensatory β-cell response to the adiposity-induced insulin resistance was reported (Ader et al., 2005).

We also examined chronic administration of olanzapine (2 mg/kg or 7.5 mg/kg) in female rats. Olanzapine was administered via osmotic mini-pump for 4 weeks, followed by the HIEC and hyperglycemic clamp procedures to assess insulin sensitivity and secretion, respectively. In this series of experiments, both low dose and high dose olanzapine increased hepatic glucose production, while only the high dose olanzapine group exhibited decreased peripheral glucose utilization. Conversely to acute olanzapine administration, no between treatment group differences in β-cell function were noted during the hyperglycemic clamp. While there was no change in absolute body weight, high dose olanzapine increased visceral adiposity. As such, increases in HGP were likely attributable to direct, adiposity independent effects, whereas it could not be ruled out that changes in peripheral glucose uptake, noted only in the high dose olanzapine group alongside increases in adiposity, could be due to increased fat mass (Chintoh, Mann, Lam, Giacca, & Remington, 2008).

Taken together, repeated dose studies in animals again offer the most consistent evidence for impairment of hepatic mechanisms of glucose regulation, suggesting that weight gain independent or direct effects of these agents can be found not only acutely, but also chronically via mechanisms that remain to be determined.

### 4.3.3 In vitro/ Ex-vivo studies

Within the conceptualization framework that antipsychotic effects on glucose metabolism could occur in part through direct effects on peripheral insulin target tissues or the pancreatic β-cells, there has been a growing literature examining in vitro or ex vivo exposure to these drugs. Moreover, the potential role of various neurotransmitters, given the heterogeneous receptor binding profile of theses agents (Table 4), which share receptor effects implicated in weight/glucose regulation has garnered growing interest. However, we leave discussion of studies specifically addressing receptor binding profiles and possible implications in glucose dysregulation to chapter 2.
In general, there has been noted variability between studies examining antipsychotic associated effects on glucose uptake and utilization, with some of the variance possibly accounted for by differences in tissue types/species, culture conditions, duration of drug-incubation, and presence or absence of insulin or glucose in media. Many studies suggesting that various SGAs can induce insulin resistance have been criticized for using the drugs at supra-therapeutic concentrations (Ardizzone, Bradley, Freeman, & Dwyer, 2001; Dwyer, Pinkofsky, Liu, & Bradley, 1999; Engl et al., 2005; Lu, Bradley, & Dwyer, 2004; Vestri et al., 2007). Studies addressing this dosing concern have generally shown no effect with “therapeutic” doses. For example, Robinson et al. incubated 3T3-L1 adipocytes with an olanzapine concentration chosen to encompass and exceed estimated steady-state concentrations in humans approximating a 20mg daily dose. They found that 1 and 20 hours following treatment, neither dose affected basal or insulin stimulated glucose transport, at varying insulin concentrations (K. A. Robinson, Yacoub Wasef, & Buse, 2006). Another study examined olanzapine concentrations both approximating and exceeding Cmax by 27 fold, reporting that in maximally insulin-stimulated states there was no effect for the lower concentrations, whereas glucose transport was decreased in the supratherapeutic conditions after 72 hours of incubation (Vestri et al., 2007).

Similarly, at therapeutic concentrations, in vitro incubation of 3T3-L1 cells with olanzapine, clozapine and ziprasidone as compared to vehicle did not affect basal or insulin stimulated glucose transport, whereas supratherapeutic olanzapine concentrations were associated with a small decrement in uptake. Isolated soleus muscle however showed no effect on insulin-stimulated glucose uptake following any concentrations of the above noted drugs (G. C. Smith et al., 2008).

Houseknecht et al., at the culmination of the above-mentioned HIEC studies (acute dose and after 5 days of once daily 10mg/kg olanzapine administration), conducted an ex vivo analysis of glucose uptake. They suggested that acute clozapine but not olanzapine or ziprasidone caused increased uptake into adipose tissue and liver, and conversely a reduction in skeletal muscle. However, this effect was lost following 5 days of treatment. (Houseknecht et al., 2007). Albaugh et al. also examined glucose uptake following
olanzapine administration (10mg/kg divided in twice daily doses for 2 days, with 2/3 of second dose given 1 hour before the experiments) on glucose disposal under insulin conditions, and fasting conditions. Interestingly, they found significant decreases in glucose disposal in muscle, but increases in glucose disposal in adipose tissue (epidermal, renal, subcutaneous) under conditions of insulin. In the fasting condition (basal insulin levels), olanzapine had no effect on glucose clearance in muscle and adipose tissue, with the exception of subcutaneous adipose tissue, where an increase in glucose uptake persisted (Albaugh, Judson et al., 2011). In a follow-up study, the same group examined AKT phosphorylation and activation, employing the same experimental paradigm (fasting, or insulinenic states), finding no effects of olanzapine, suggesting the major insulin signaling pathway in muscle was not perturbed by olanzapine, despite the previously noted decrease in glucose disposal. In an elegant series of experiments that followed, the same study also suggested that invivo lipolysis (i.e. break down of adipose tissue) in the fasting state was impaired by olanzapine, with a concomitant increase in lipid disposal, and a corresponding inappropriate shift from carbohydrate metabolism to fat oxidation, given the impairment in mobilization of adipose tissue stores. Based on these findings, the authors questioned whether substrate competition by fat oxidation to preclusion of use of glucose sources in mitochondria could contribute to the acute glucose dysregulation, at least in part through insulin-independent pathways (Albaugh et al., 2012).

Given the importance of the liver in maintaining glucose homeostasis, several investigations have examined the effects of various antipsychotic medications on enzymes implicated in gluconeogenesis, glycogen break down, and fat storage. Consistent with findings of antipsychotic induced HGP in rats, upregulation of gluconeogenic genes has been reported in association with clozapine and olanzapine, as well as chlorpromazine (Jassim et al., 2011; Jassim et al., 2012; Park et al., 2007). Similarly, lipid related gene or enzyme expression of sterol regulatory element-binding protein (SREBP) transcription factors and related pathways, which have linked to hepatic steatosis and insulin resistance (Qi et al., 2005; Shimano et al., 1997), have been reported to be upregulated in association with clozapine, and olanzapine as compared to vehicle
Interestingly, in a study comparing various antipsychotics including clozapine, olanzapine, quetiapine, aripiprazole, haloperidol and quetiapine, found upregulation of SREBP proteins with clozapine and olanzapine, but not the other agents which were examined as compared to vehicle (Lauresergues et al., 2010). Notably, all the above-mentioned studies would be considered to have administered the drugs in supratherapeutic concentrations, possibly complicating relevance to clinical situations.

Examination of insulin secretion pathways in vitro has yielded some conflicting results. Older investigations using isolated pancreatic islets have shown that in vitro exposure to chlorpromazine decreases glucose-mediated insulin release (Ammon, Orci, & Steinke, 1973), a finding also replicated more recently, with high, but not low dose chlorpromazine following 8 weeks of in vivo administration and pancreatic isolation (Park et al., 2008). A series of separate studies by Melkersson and colleagues demonstrated increases in basal insulin release in isolated β-islets following exposure to clozapine, variably to olanzapine, with no effect for risperidone, haloperidol, or ziprasidone (Melkersson, 2004; Melkersson & Jansson, 2005, 2007; Melkersson, Khan, Hilding, & Hulting, 2001); in the case of glucose stimulated insulin release, haloperidol was noted to decrease glucose stimulated insulin response (Melkersson et al., 2001). These studies again employed drug concentrations which would be considered supratherapeutic. A separate study employing lower drug concentrations, albeit still at supratherapeutic levels, demonstrated acute inhibition of glucose stimulated insulin release with clozapine, but not haloperidol as compared to vehicle. Clozapine was found to hyperpolarize cell membrane potential, inhibiting electrical activity. Conversely, haloperidol decreased K+ permeability, which might be expected to result in enhancement of insulin release (L. Best, Yates, & Reynolds, 2005). The acute effects of haloperidol on blockade of K+ channels was also replicated in isolated mouse β-cells, and cloned K+ channels, but found to occur at 100 fold higher concentrations than would be expected as therapeutic levels (S. B. Yang, Proks, Ashcroft, & Rupnik, 2004). Other recent studies have indicated that in vitro exposure to therapeutically relevant concentrations of clozapine and olanzapine, but not risperidone or ziprasidone, inhibits
carbachol plus glucose stimulated insulin secretion, an effect attributed to muscarinic antagonism, and which did not occur with glucose stimulation alone (Johnson et al., 2005). Similarly, in another study, 3 days incubation with therapeutic concentrations of clozapine inhibited carbachol-induced insulin secretion, but had no effect if cells were stimulated only with glucose. Interestingly, following 7 days of culture, clozapine inhibited glucose stimulated insulin secretion, even in absence of carbachol (Sasaki et al., 2006).

4.3.4 Summary

Taken together, the studies do suggest that antipsychotics could affect insulin response and sensitivity by direct action on pancreas and insulin target tissues. In general, studies examining what could be considered “therapeutic” concentrations of antipsychotic drugs have not found consistent effects on insulin-mediated glucose transport in muscle or adipocyte cells. Studies in liver tissue have suggested more reliable drug-induced perturbations with respect to gluconeogenesis, and steatosis, within the limitation that most available studies could be argued to have employed supratherapeutic drug concentrations. Although studies examining AP-induced effects on β-cell response have been at variance with each other, those employing therapeutic concentrations of clozapine and olanzapine have strongly implicated cholinergic mechanisms leading to impairment of glucose stimulated insulin secretion. However, it still remains to be demonstrated conclusively that these drug-induced direct effects occur in vivo, at therapeutic doses.
Table 4: Receptor affinities of various antipsychotic drugs relative to dopamine D\(_2\) binding

<table>
<thead>
<tr>
<th>Dopamine D(_2) K(_i) (nM)</th>
<th>Haloperidol</th>
<th>Clozapine</th>
<th>Olanzapine</th>
<th>Risperidone</th>
<th>Quetiapine</th>
<th>Ziprasidone</th>
<th>Aripiprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine 431</td>
<td>2.0</td>
<td>431</td>
<td>72</td>
<td>4.9</td>
<td>567</td>
<td>4.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Olanzapine 72</td>
<td>270</td>
<td>0.66</td>
<td>0.98</td>
<td>25</td>
<td>0.22</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Risperidone 4.9</td>
<td>4.9</td>
<td>0.24</td>
<td>0.032</td>
<td>20.16</td>
<td>0.025</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Quetiapine 567</td>
<td>567</td>
<td>&lt;10-2</td>
<td>15</td>
<td>0.96</td>
<td>76</td>
<td>0.031</td>
<td>0.032</td>
</tr>
<tr>
<td>Ziprasidone 4.0</td>
<td>4.0</td>
<td>&lt;10-2</td>
<td>0.41</td>
<td>0.22</td>
<td>0.31</td>
<td>0.043 (a)</td>
<td></td>
</tr>
<tr>
<td>Aripiprazole 0.95</td>
<td>0.95</td>
<td>&lt;10-2</td>
<td>0.41</td>
<td>0.22</td>
<td>0.31</td>
<td>0.043 (a)</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from (Reynolds & Kirk, 2010). Values indicate drug affinity for identified receptor relative to dopamine D\(_2\) receptor affinity, calculated (D\(_2\)K\(_i\) / receptor K\(_i\)). Known partial agonism of receptor is identified by (a).

5.0 Comments/Summary:
Schizophrenia remains one of the most debilitating and costly chronic illnesses, and furthermore is associated with premature mortality with lifespan shortened by as much as 25%, a finding largely attributable to cardiovascular mortality. Antipsychotic medications remain the cornerstone of treatment, with established benefits with respect to decreased relapse rates and improved outcomes (Csernansky & Schuchart, 2002). While the introduction of the newer, so called second generation, or atypical antipsychotics garnered hope for improved tolerability as compared to the earlier, ‘typical’ agents, unfortunately significant metabolic disturbances have replaced concerns of tarditive dyskinesia associated with the earlier drugs, forcing psychiatry to align with more unfamiliar fields of endocrinology and physiology.
This current work, while acknowledging the complex relationship between illness and metabolic risk, focuses specifically on antipsychotic-induced glucose dysregulation, first building upon our established single-dose paradigm rodent model to investigate mechanisms, then moving to humans, to test the translational value of some of the animal work done to date. In both models, we use an acute dosing paradigm as to avoid changes in adiposity which have been described to occur rapidly with these medications.

6.0 Aims/Hypotheses:
Within the context of 2 acute dosing paradigms (in rodents, and healthy volunteers) designed to avoid confounding effects of psychiatric illness and antipsychotic-induced adiposity, we set out to address the following hypotheses:

- **Aim 1**: To utilize selective antagonists in order to deconstruct the heterogeneous receptor binding profile of antipsychotic medications to better understand the mechanisms by which a direct effect occurs.

- **Hypothesis 1**: Weight-gain independent effects of antipsychotics are likely due to interactions between different receptor systems, although there will be differential effects based on previously identified roles of neurotransmitter systems in metabolism and glucose homeostasis (acetylcholine >histamine>serotonin>dopamine).

- **Aim 2**: To employ invasive mechanistic techniques to determine the role of a central effect in modulation of insulin sensitivity and secretion

- **Hypothesis 2**: Antipsychotic-induced derangements in insulin sensitivity and secretion are mediated at least in part through central mechanisms, and intracranial injection of olanzapine will produce similar effects to those observed with peripheral drug administration
- **Aim 3**: To translate the direct effect of antipsychotics on glucose homeostasis from rodents to humans

- **Hypothesis 3**: Single dose administration of olanzapine to healthy lean human volunteers will result in acute changes in measures of insulin sensitivity and secretion independently of illness or adiposity changes, analogous to what is observed in rodents.
Atypical antipsychotics and effects of muscarinic, serotonergic, dopaminergic and histaminergic receptor binding on insulin secretion in vivo: an animal model

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1. Introduction:

Increasing evidence suggests that ‘atypical’ antipsychotics (AAPs), as a class, are associated with increased liability for weight gain, lipid abnormalities, and development of type 2 diabetes (Newcomer, 2005). Clinical evidence suggests considerable variability in individual risk of metabolic aberrations, with clozapine and olanzapine conferring the greatest risk. Weight gain, a well-documented side effect of these agents (Pi-Sunyer, 1993), is a leading risk factor for glucose dysregulation, suggesting that weight gain could account for the observed metabolic disturbances.

However, consistent evidence of a) acute metabolic effects of certain atypical agents on glucose homeostasis in animal models (Chintoh et al., 2009; Chintoh, Mann, Lam, Lam et al., 2008; Houseknecht et al., 2007), b) impaired glucose regulation in patients with schizophrenia independent of adiposity (Newcomer et al., 2002) and c) numerous reports of diabetic ketoacidosis (DKA) occurring early in treatment and without weight gain (Jin et al., 2002), have suggested that direct, weight-independent mechanisms may be involved. Further, the reports of DKA imply acute effects on insulin secretory mechanisms, which may also share common pathophysiologic pathways with failure of β-cell compensation implicated in longer-term risk of diabetes associated with these agents. Our group recently demonstrated that acute effects on insulin sensitivity appear to mirror risk of weight gain, suggesting some common pharmacological mechanisms between the 2 processes (Chintoh et al., 2009). Our findings also highlighted clozapine and olanzapine-induced deficits in β-cell functioning. This is in keeping with data suggesting that the normal compensatory increase in insulin secretion elicited in obesity-induced insulin resistance may be prevented by olanzapine in dogs (Ader et al., 2005), as well as the observation that olanzapine induces impaired insulin secretion in atypical antipsychotic-naïve patients with schizophrenia (Chiu et al., 2006).

Although the mechanisms underlying antipsychotic-induced glucose regulation remain poorly understood, it has been proposed that the heterogenous receptor binding pharmacology differentiating the atypical agents not only from each other, but also from their more selective high-potency “conventional” counterparts, may be implicated. As
reviewed elsewhere, antipsychotic binding to dopaminergic, serotonergic, adrenergic, and cholinergic sites is understood to influence receptors and transporters in essential body tissues implicated in glucose metabolism (Richelson, 1999; Starrenburg & Bogers, 2009). To this point, correlation studies, within the limitation of not always controlling for weight gain, have implicated affinity for histaminergic, muscarinic, and serotonergic binding sites in the increased risk of diabetes associated with these agents (Matsui-Sakata, Ohtani, & Sawada, 2005; Silvestre & Prous, 2005).

The aim of the present study was to ‘deconstruct’ the pharmacological binding profile of high liability AAPs (e.g. clozapine, olanzapine) and examine acute mechanisms underlying AAP-induced glucose dysregulation through single dosing of various representative antagonists. The *in vivo* effect of the antagonists on secretory capacity of pancreatic β-cells was assessed using hyperglycemic clamps, which also allows for estimation of peripheral sensitivity (Elahi, 1996).

2. Materials & Methods:

2.1 Animals
Healthy male, Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300-325g were singly housed and maintained on 12 hour light-dark cycle; food (standard chow, Harlan Teklad) and water were available ad libitum. Animals were treated in compliance with the guidelines of the Canadian Council on Animal Care.

2.2 Surgical Procedures
Rats were anaesthetized using inhaled isoflurane, following which polyethylene catheters (PE-50, Cay Adams. Boston, MA) with tips covered by 2.5 cm silastic tubing (Dow corning Corp., Midland, MI) were introduced in the jugular vein and carotid artery, respectively, and advanced to the right atrium and aortic arch. The catheter lines were externalized dorsally and blocked with a pin. For analgesia, buprenorphine (0.3mg/kg)
was administered after surgery. The animal was allowed at least 2-3 days of recovery before the hyperglycemic clamp experiment, which was performed in conscious rats.

2.3 Drug and Dose Selection
Following an overnight fast, rats were randomly assigned to one of the following treatment groups: vehicle (DMSO or 0.9% normal saline), darifenacin (6mg/kg), ketanserin (2mg/kg), raclopride (0.3mg/kg) and terfenadine (20mg/kg,), all delivered by subcutaneous (s.c.) injection. Terfenadine and raclopride were dissolved in 100% DMSO, darifenacin was dissolved in a 2:1 ratio of 0.9% normal saline to DMSO, and ketansarin was dissolved in 0.9% normal saline. All treatments were administered in a volume of 1ml/kg. To establish dosing, the literature was reviewed for each compound to screen for doses that produced physiological and/or behavioral changes. Ketanserine dose was chosen on the basis of inhibition of DOI-induced disruption of prepulse inhibition, a validated measure of sensorimotor gating mechanisms implicated in the psychopathology of schizophrenia (Geyer & Krebs, 1994). Terfenadine dosing was based on ability to mediate selective blockade of H1 receptor-mediated histamine-induced itch in a rodent model (Bell, McQueen, & Rees, 2004). The dose of raclopride was chosen to approximate 70% D2 occupancy, which in humans has been associated with optimal chance of antipsychotic clinical response(Wadenberg, Kapur, Soliman, Jones, & Vaccarino, 2000). All drugs were obtained from Toronto Research Chemicals (Toronto, Canada).

2.4 Clamp Procedure
Following overnight fast, vehicle (DMSO; n=6 and 0.9% normal saline; n=5), darifenacin (n=10), ketanserin (n=10), raclopride (n=11) or terfenadine (n=9) were injected s.c. 90 minutes before the hyperglycaemic clamp, as detailed in previous work (Chintoh, Mann, Lam, Lam et al., 2008). After 90 minutes, a glucose bolus (50% Dextrose 1ml/kg, Abbott Lab) was injected into the jugular vein at a dose of 500mg/kg. Immediately following the glucose bolus, the jugular catheter was connected to the pump for glucose infusion. Variable infusion of exogenous glucose maintained plasma glucose levels at approximately 300mg/dL (or 17 mM), and plasma samples were collected throughout the
clamp for insulin and C-peptide assay.

2.5 Laboratory Methods
Plasma glucose was measured by a Beckman Glucose Analyzer II (Beckman, Fullerton, CA). Insulin and C-peptide levels in plasma were determined by immunoassay using kits (Linco Research, St. Charles, MO) specific for rat insulin and C-peptide.

2.6 Calculations
Glucose infusion (GINF) rate was derived based on each animal's weight and GINF pump rate. An index of insulin sensitivity (SI) was calculated by dividing GINF by a product of insulin levels and glucose concentrations during the final 30 minutes of the clamp. In normal subjects, the relationship between insulin sensitivity and secretion is hyperbolic, i.e. the product of insulin sensitivity and secretion is a constant defined as disposition index (DI) and represents a measure of the adequacy of β-cell response to changes in insulin resistance, also considered a predictor of incipient impairments in glucose tolerance (Bergman, Finegood, & Kahn, 2002). The DI was calculated as the product of insulin sensitivity × C-peptide during the last 30 minutes of the clamp.

A series of mixed models repeated-measures (MMRM) analyses were conducted in order to determine whether a) insulin, C-peptide, GINF, glucose, SI, and DI values changed over the course of the experiment, b) these values differed across the five treatment groups, and c) the magnitude of any group differences present varied over the course of the experiment. Significant findings were further investigated through a series of Bonferroni-adjusted pairwise comparisons (SI, DI, and GINF) and Bonferroni-adjusted linear contrasts (glucose, insulin, and C-peptide). Linear contrasts were constructed to determine whether glucose, insulin, and C-peptide values differed between the control group and each of the remaining four study groups during the baseline phase (80 and 90 minutes), during the initial secretion phase (92, 97, and 102 minutes), and during the glucose-challenge phase (92, 97, 102, 120, 150, 170, and 180 minutes). Due to a heavily right-skewed distribution of model residuals, a natural-logarithm transformation was applied to insulin and DI data. For these two parameters, an estimate of the ratio between
Least-squares (LS) geometric means of treatment and control was provided. Significance was accepted at p<0.05, and all p-values provided were adjusted for multiple comparisons.

3. Results
The MMRM results demonstrated a significant time x treatment group interaction for glucose, insulin, and C-peptide, suggesting that differences observed across treatment groups with respect to these parameters varied over the clamp period (data not shown). Average basal glucose levels were similar for all groups (fig. 2A). Accordingly, no differences in least square means were observed for baseline insulin and C-peptide levels between treatment groups and controls. During the clamp, however, glucose levels during the entire glucose-challenge phase were significantly higher in the darifenacin group as compared to controls (5.8±0.6mM; p=0.0408) (fig. 2A). Despite the higher glucose levels, darifenacin significantly decreased plasma insulin (Geometric LS means ratio 0.03; p=0.0012) and C-peptide levels (6.95±2.25nM; p=0.0336) (fig. 2C and D, respectively) during the glucose challenge. These effects were mirrored by a significant decrease in the GINF required to maintain hyperglycemia (97.20±25.09μmol/kg×min; p=0.016) (fig. 2B), as well as significantly lower DI (Geometric LS-means ratio 0.588; p=0.0272) versus control animals (fig. 3A). Similar to darifenacin, ketansarin decreased plasma insulin levels during the glucose challenge (Geometric LS means ratio 0.06; p=0.0084), although this was not replicated with C-peptide secretion (p=0.1632). GINF levels required to maintain hyperglycemia were marginally lower (59.28±24.3 μmol/kg×min; p=0.0780) in the ketansarin group as compared to controls, while the SI was significantly increased (0.014±0.004; p=0.0056) (fig. 3B). Raclopride significantly increased insulin secretion (Geometric LS means ratio 18.99; p=0.006) and marginally increased C-peptide secretion (7.46±2.5 nM; p=0.0516) during the glucose challenge. Raclopride also marginally raised the GINF required to maintain hyperglycemia (59.24±24.31; p=0.0756), and marginally decreased SI (0.010±0.004; p=0.0660) relative to control animals. Terfenadine did not significantly affect any parameters of interest; it appeared to show a tendency to decrease the first phase of insulin secretion (p=0.0768), although interpretation of this effect was inconclusive as glucose levels were significantly higher
(p=0.0432) when compared to controls during this phase of the clamp procedure.

**Fig 2.** Effect of a single s.c. dose of representative antagonists on plasma glucose levels (A), glucose infusion rate (GIR) (B), plasma insulin (C) and C-peptide levels (D) during the hyperglycemic clamp in male Sprague Dawley rats. †p < 0.05 vs. control; ‡p < 0.01 vs. control. Raclopride marginally increased C-peptide secretion (p=0.0516) during the glucose challenge, and also marginally raised GIR required to maintain hyperglycemia (p=0.066). There were no differences in baseline values of glucose, insulin, or C-peptide levels between the respective treatment groups and controls.

**Fig 3.** Effect of a single s.c. dose of representative antagonists on the Disposition Index (DI) (A), Sensitivity Index (SI) (B) calculated during the last 30 minutes of hyperglycemic clamp in male Sprague Dawley rats. †p < 0.05 vs. control; ‡p < 0.01 vs. control. Raclopride marginally decreased the SI relative to control animals (p=0.066).
4. Discussion:
Mechanisms underlying the metabolic disturbances in schizophrenia are likely complex and multifaceted. Although weight gain and type-2 diabetes represent chronic complications, recent evidence has pointed to acute effects of atypical agents on insulin secretion. The activity of pancreatic β-cells is regulated by multiple neurotransmitters and hormones, (Satin & Kinard, 1998; Starrenburg & Bogers, 2009) many of which are affected by the complex pharmacology of AAPs. In this study, we investigated mechanisms behind the immediate, weight-gain independent effects of AAPs on insulin secretion through the deconstruction of their heterogenous receptor binding profile. Using representative antagonists and the hyperglycemic clamping technique, we examined the potential impact of D₂, 5HT₂A, H₁, and M₃ receptors on pancreatic β-cell function.

In general, the M₃ and H₁ binding affinity of the AAPs have been most closely linked to the diabetogenic potential of these drugs (Matsui-Sakata et al., 2005; Silvestre & Prous, 2005). Olanzapine and clozapine, which possess the highest M₃ and H₁ binding of the atypical agents (Roth et al., 2004), also appear to be associated most consistently with risk of glucose dysregulation in acute preclinical models (Chintoh et al., 2009). Further, evidence suggests they are associated with the greatest clinical risk of diabetes (Leslie & Rosenheck, 2004), as well as the greatest number of case reports of antipsychotic-associated DKA (Jin et al., 2002) (although, at least in the case of olanzapine, this may reflect its widespread use).

Thus, our findings suggesting a robust effect of acute M₃ blockade on insulin and C-peptide response to glucose challenge, as well a decrease in DI as compared to vehicle treated animals are perhaps not unexpected. From a mechanistic standpoint, M₃ receptors are highly expressed by pancreatic β-cells where they play a central role in glucose-dependent acetylcholine modulation of insulin secretion (Gilon & Henquin, 2001). In support of the critical role of the M₃ receptor in regulation of insulin secretion, pancreatic M₃ receptor knock-out mice demonstrate impaired glucose tolerance and significantly reduced insulin secretion (Gautam et al., 2007). Also converging with our findings,
**invitro** experiments have shown that olanzapine and clozapine (unlike risperidone, ziprasidone, or haldoperidol) can impair cholinergic-stimulated insulin secretion (Johnson et al., 2005). Interestingly, healthy subjects exposed to prolonged mild hyperglycemia compensate by an increase in β-cell function that can be attenuated with muscarinic blockade (Teff & Townsend, 2004). These findings raise the possibility that in the context of peripheral insulin resistance, predisposed individuals exposed to AAP-induced M₃ antagonism may develop an impaired compensatory insulin response, resulting in DM2.

While the H₁ receptor has been linked to glucose metabolism (Wang et al., 2010), the lack of effect of terfenadine, a selective H₁ antagonist, on C-peptide, insulin levels and glucose infusion rate does not support acute peripheral effects via this receptor on β-cell function. At present, the H₁ receptor is best understood to function through central mechanisms affecting food intake, energy expenditure, adiposity, and leptin signaling (Masaki, Yoshimatsu, Chiba, Watanabe, & Sakata, 2001; Sakata et al., 1988). Given limited brain distribution of peripherally administered terfenadine (Mahar Doan et al., 2004), our experiments were unable to exclude central H₁ effects on glucose regulation. However, the observation that H₁ knock-out mice demonstrate an obese phenotype, but comparable insulin and glucose levels post-glucose challenge to control animals (Wang et al., 2010), would argue against a significant direct effect of the H₁ receptor on glucose homeostasis. Accordingly, glucose dysregulation remains best elucidated as a secondary phenomenon although the central effects of the H₁ receptor on peripheral pancreatic function and insulin sensitivity remain to be clarified.

Concomitant 5-HT₂ antagonism has been argued to distinguish most AAPs from the older first generation drugs (Meltzer, Matsubara, & Lee, 1989). Here, we have demonstrated that pretreatment of animals with ketansarin, a 5HT₂A antagonist, results in decreased insulin secretion although the strength of this effect was not as pronounced as that of darifenacin, as reflected by a lack of significant effect on C-peptide secretion or the DI. In keeping with our findings, recent data suggest that intracellular 5HT is crucial for insulin secretion (Paulmann et al., 2009). However, other than reported **in vitro** up-
regulation of 5HT$_2$ receptor mRNA following glucose stimulation in pancreatic islet cells (MacDonald, 1996), this is the first report to our knowledge implicating the 5HT$_{2A}$ receptor in insulin secretion. Overall, 5HT$_{2A}$ has been more thoroughly investigated in the context of insulin sensitivity rather than secretion, although the results have been inconsistent (Chaouloff, Laude, & Baudrie, 1990; Gilles et al., 2005; Hajduch et al., 1999; McCall & Harris, 1988; Rattigan, Clark, & Barrett, 1999). The noted inconsistencies in the literature may in part be related to the ability of ketanserin to cross the blood-brain barrier, where central and peripheral effects can have opposing effects. For example, central antagonism of the 5HT$_{2A}$ receptor may reduce sympathetic tone, improving sensitivity (McCall & Harris, 1988); conversely, peripheral blockade may impede skeletal muscle uptake of glucose (Hajduch et al., 1999), reducing peripheral insulin sensitivity.

Our findings also provide evidence that selective blockade of D$_2$/D$_3$ with raclopride enhances insulin secretion, also marginally decreasing insulin sensitivity. C-peptide levels paralleled that of insulin, suggesting that elevated insulin levels were not only secondary to decreased liver clearance, which can reflect increased peripheral resistance. Increased insulin secretion secondary to D$_2$ blockade is consistent with the expression of D$_2$-like receptors in pancreatic β-cells, which mediate inhibition of insulin secretion (Rubi et al., 2005). Recent in vivo data support D$_2$-mediated inhibition of glucose-stimulated insulin secretion, but also suggest that permanent loss of D$_2$ receptors eventually results in glucose intolerance (Garcia-Tornadu et al., 2010). Taken together, prolonged D$_2$ blockade with antipsychotics may predispose to depletion of insulin granule stores and a defect in pancreatic compensation, and the observation that bromocriptine, a D$_2$ agonist, improves insulin sensitivity (Pijl et al., 2000) provides indirect support to our observation of a tendency towards decreased peripheral sensitivity with D$_2$ antagonism. At the same time, the fact that D$_2$ receptor blockade remains a ubiquitous property by which antipsychotics are thought to exert their therapeutic effect (Kapur, Zipursky, Jones, Remington, & Houle, 2000) makes it unlikely that D$_2$ antagonism on its own explains the differences which exist among antipsychotics with respect to metabolic risk. It is more plausible that the apparent physiologic role of
dopamine in β-cell function, much like the muscarinic story, may tip the balance towards β-cell failure in certain predisposed individuals. Interestingly, it has been found that D₁, D₄, and D₅ receptors are also expressed in human islet cells (Rubi et al., 2005). To our knowledge the in vitro and in vivo roles of the D₄ receptor have not been examined in the context of insulin secretion; however, the fact that agents at increased risk of metabolic side effects i.e., clozapine, olanzapine, demonstrate a high affinity for the D₄ relative to the D₂ receptor (Seeman & Van Tol, 1994), supports an examination of D₄ antagonism on glucose homeostasis.

The current work must be considered in view of several limitations. First, the drugs employed are not entirely receptor specific. Ketanserin, in particular, shares some affinity with α₁ and H₁ receptors, and our data will benefit from replication with a more specific 5HT₂A antagonist. Second, euglycemic clamps, considered the gold standard to assess insulin sensitivity (DeFronzo et al., 1979), are needed to follow-up on the suggested acute changes in the SI observed with 5HT₂A and D₂ antagonism. Finally, we were unable to examine additive or synergistic receptor interactions, a phenomenon which cannot be ignored in accounting for the metabolic sequelae of these compounds. This study was not meant to be exhaustive, and other receptor subtypes characterizing the pharmacological profile of newer antipsychotics (e.g. 5HT₂C, 5HT₁A, α₁, α₂, D₄), are also potentially implicated in glucose regulation (Cheng, Liu, Yen, & Chen, 2000; Devedjian et al., 2000; Rubi et al., 2005; Starrenburg & Bogers, 2009) and merit investigation. Finally, both central and peripheral pathways are likely to play an important role in the diabetic side effects of certain antipsychotic agents, and our study design was not designed to separate these effects. We are currently in the process of examining central administration of olanzapine on insulin secretion, which if promising, will be extended to examine central administration of selective antagonists.

These limitations aside, our findings lend support to antipsychotic-induced, direct effects on β-cell function implicating muscarinic, dopaminergic, and serotonergic mechanisms. These data provide additional information as to potential pharmacological receptor mechanisms contributing to acute effects on glucose dysregulation; however, it remains
that at this point we still have a limited understanding regarding the precise mechanisms that distinguish certain agents and at-risk individuals, as well as the relationship between acute effects and antipsychotic-induced weight gain over the longer term.
CHAPTER 3
Effects of intracerebroventricular (ICV) olanzapine on insulin sensitivity and secretion in vivo: an animal model

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1. Introduction

Efforts to improve upon tolerability issues of the earlier “typical” agents led to the development of “atypical” antipsychotics (AAPs). Unfortunately, despite some purported advantages over the “typical” agents, there has been increasing concern regarding treatment-associated metabolic side effects, including dyslipidemia, weight gain and glucose dysregulation (Newcomer, 2005). While weight gain, which is associated with certain AAPs, remains a major risk factor for type 2 diabetes (Pi-Sunyer, 1993), there is growing evidence, both from clinical and animal models, to support increased liability for glucose dysregulation independent of changes in weight (Chintoh et al., 2009; Houseknecht et al., 2007; Newcomer et al., 2002; Vidarsdottir, de Leeuw van Weenen et al., 2010). Furthermore, clinical reports of diabetic ketoacidosis (DKA) occurring early in treatment prior to occurrence of any weight gain (Jin et al., 2002) have underscored the ability of these agents to directly induce β-cell failure in certain predisposed individuals. Our own work in a peripheral model of single dose administration has confirmed other evidence suggesting that certain agents (i.e. risperidone, olanzapine or clozapine, but not haloperidol or ziprasidone), can impair insulin sensitivity, with findings also highlighting a clozapine and olanzapine-induced deficit in β-cell functioning (Chintoh et al., 2009).

However, a limitation of existing experimental design investigating direct effects on glucose metabolism has been the inability to differentiate peripheral from central drug effects. In fact, the primary action of antipsychotics is central and atypical agents implicate numerous neurotransmitters and receptors in their mechanism of action as well as adverse side effects. At the same time, CNS control of glucose metabolism has been highlighted by the discovery of glucose sensing neurons prevalent in areas known to play key roles in metabolism and appetite control, including the hypothalamus (lateral hypothalamic area (LH), and the ventro-medial hypothalamus (VMH)) and hindbrain (area postrema, nucleus of the solitary tract, and the dorsal motor nucleus of the vagus nerve) (Marty, Dallaporta, & Thorens, 2007).
Despite accruing evidence for the importance of central regulation in glucose metabolism, surprisingly little work has been conducted in the area of central effects of AAPs and associated effects on glucose homeostasis. Martins and colleagues examined the effects of acute, continuous central administration of olanzapine during a hyperinsulinemic clamp, suggesting an olanzapine-induced defect in hepatic insulin sensitivity, implicating AMPK activation (Martins, Haas, & Obici, 2010). To our knowledge, the effects of central antipsychotic administration have not been examined in the context of insulin secretion.

In the current study, like Martins and colleagues, we set out to determine whether olanzapine could acutely impair insulin sensitivity during a hyperinsulinemic clamp. Further, to elucidate the central effects of olanzapine on β-cell function, we carried out hyperglycemic clamps immediately following ICV olanzapine or vehicle injection. Given a lack of data on clinically relevant ICV dosing of olanzapine, we also set out to determine a single dose model of olanzapine administration that would a) be well tolerated by the animals and, b) significantly decrease locomotion induced by d-amphetamine, an established pharmacological model of clinical antipsychotic efficacy (Arnt, 1995). Employing the gold-standard hyperglycemic clamp procedure, we report novel findings suggesting that intracranial administration of olanzapine acutely suppresses β-cell function.

2. Methods

The following protocol was approved by the Center for Addiction and Mental Health and University of Toronto Animal Care Committee. Healthy, male Sprague-Dawley rats (300-325g, Harlan) were singly housed, in a room kept on a 12-hour light/dark cycle with access to food and water ad libitum. Testing was conducted during the light cycle, with all experimental protocols initiated between 0800-0900 hours.

2.1 Drug and Dose Selection

Olanzapine (Toronto Research Chemicals, Ontario) was dissolved in 1% acetic acid buffered with 1N NaOH to a pH >5.5. Intracerebralventricular (ICV) dosing of
olanzapine was established based on inhibition of amphetamine-induced locomotion. Control animals received an equal volume of 1% acetic acid as olanzapine-treated animals, also adjusted to a pH between 5.5-6.0.

2.2 Locomotion Assessment
Animals were administered a single ICV dose of olanzapine or vehicle followed by intraperitoneal injection of 1.0mg/kg of d-amphetamine (Sigma-Aldrich, St. Louis, MO) dissolved in 0.9% saline. Locomotion was assessed over a 120-minute period in specialized activity monitoring boxes which were similar in size as the housing cages and were equipped with 6 photocell beams located 3cm above the floor of the box. Recordings were made every 5 minutes. Prior to tests with olanzapine, we controlled for exposure to a novel environment by three 120-minute habituation sessions.

2.3 Surgeries
The animals were given a 7-day adaptation period prior to ICV cannula implantation. Animals were anaesthetized with a ketamine (75mg/kg) and xylazine (10mg/kg) cocktail. A single 3.0mg/kg dose of ketolorac (Sandoz, Princeton, NJ) was administered for post-operative analgesia. A stainless steel guide (HRS Scientific, Montreal, Canada) was embedded into the third cerebral ventricle using a stereotaxic frame (Kopf, Tujunga, California) at the coordinates: A/P = -2.5mm; M/L = 0.0mm; D/V-8.0mm. Cannula was secured using four stainless steel screws (Lomat, Montreal, Quebec) fixed to the skull and held down using dental cement (Jet Repair, Niagara Falls, Ontario). The cannula was kept patent with a stainless steel obturator (HRS Scientific, Montreal, Canada).

After a 1-week recovery period from the ICV surgery, the animals underwent a vascular catheterization surgery. Using the same anesthetic, polyethylene catheters (PE-50, Cay Adams, Boston, MA) with 2.5 cm of silastic tubing were advanced into the right atrium and aortic arch through the jugular vein and carotid artery, respectively. The catheter lines were externalized dorsally. Buprenorphine (0.3mg/kg) was administered for post-operative analgesia. The animal was allowed 3-5 days of recovery before the clamping experiments, which were performed in conscious, fasted rats.
2.4 Hyperglycemic Clamp
Following collection of baseline samples, the animals received a single bolus (over 60 sec) ICV injection of either olanzapine (75µg) or vehicle. The hyperglycemic clamp was initiated immediately post injection. As outlined in our previous work (Chintoh, Mann, Lam, Lam et al., 2008), a glucose bolus (50% Dextrose 1ml/kg, Abbott Lab) was injected into the jugular vein at a dose of 500mg/kg. Immediately following the glucose bolus, the jugular catheter was connected to a pump allowing for variable infusion of exogenous glucose to maintain plasma glucose levels at approximately 300mg/dL (or 17 mM). Plasma samples were collected throughout the clamp for insulin and C-peptide, analyzed by radioimmunoassay specific for rat insulin and C-peptide (Linco Research, St. Charles, MO).

2.5 Hyperinsulinemic-Euglycemic Clamp
After extending catheter lines to infusion pumps, animals received a primed, continuous infusion of [3-3H]-glucose, maintained for the duration of the clamp. Basal samples were collected at 10 min intervals between 60 and 90 min. At time = 90 min, animals received a single bolus (over 60 s) ICV injection of either olanzapine (75µg, n = 10) or vehicle (n = 10). The injector was left in the cannula for approximately 5 min post injection. After ICV injection, the insulin clamp was initiated; the insulin infusion contained somatostatin (3µg/kg/min; Bachem, California) and porcine insulin (3mU/kg/min; Sigma, St. Louis, MO). At the time of insulin infusion, a 20µL/min bolus of glucose (25% dextrose) was infused for 10s. The infusion rate was set at 10µL/min and subsequently adjusted accordingly to clamp each animal’s plasma glucose concentration at approximately 120mg/dL. Thirty minutes after the insulin infusion was initiated (i.e., time = 120 min), plasma glucose was sampled continuously at 10 min intervals for the remainder of the clamp (150-210min). Samples for insulin were collected at 150, 180, 200, and 210min time points of the clamp. Due to missing samples following a lab move, only 6 olanzapine animals, and 7 vehicle animals had insulin levels available for analysis.
Plasma glucose was measured with a Beckman Glucose Analyzer II (Beckman, Fullerton, CA). Plasma radioactivity from [3-\(^{3}\text{H}\)] glucose was determined after deproteinization with \(\text{Ba(OH)}_2\) and \(\text{ZnSO}_4\) and subsequent evaporation to remove tritiated water.

### 2.6 Calculations

For both hyperglycemic and euglycemic procedures, glucose infusion (GINF) rate was derived based on each animal's weight and GINF pump rate. In the euglycemic clamp protocol, glucose turnover (rate of appearance, \(R_A\), of glucose determined with [3-\(^{3}\text{H}\)] glucose) was calculated using steady state formulae (Stetten, Welt, Ingle, & Morley, 1951). In the basal state the total rate of glucose appearance corresponds to the endogenous glucose production. During the clamps, endogenous glucose production was calculated by subtracting the exogenous glucose infusion rate from the total rate of glucose appearance. At steady state, glucose disappearance, \(R_D\), corresponds to the rate of glucose appearance, and at euglycemia, glucose disappearance corresponds to tissue glucose utilization because renal glucose clearance is zero.

For the hyperglycemic clamps, an index of insulin sensitivity (SI) was calculated by dividing GINF by a product of insulin and glucose concentrations during the final 30 minutes of the clamp, representing steady state. In normal subjects, the relationship between insulin sensitivity and secretion is hyperbolic, i.e. their product is a constant defined as disposition index (DI) and represents a measure of the adequacy of \(\beta\) -cell response to changes in insulin resistance (Bergman et al., 2002). The DI was calculated as the product of insulin sensitivity \(\times\) C-peptide during the last 30 minutes of the clamp.

ANOVA for repeated measures was performed using Statistica software (StatSoft Inc, Tulsa, OK) to determine ICV olanzapine dosing based on inhibition of amphetamine-induced locomotion.

For the hyperglycemic clamp procedures, a series of mixed models repeated-measures (MMRM) analyses were conducted in order to determine whether a) insulin, C-peptide, GINF, glucose, SI and DI values changed over the course of the experiment, b) these
values differed across treatment groups. Significant findings (time x group interactions) were further investigated through pairwise comparisons (SI, DI and GINF) and linear contrasts (glucose, insulin, and C-peptide). Linear contrasts, and associated differences in Least-Squares group means (LS-means), were constructed to determine whether glucose, insulin, and C-peptide values differed between the control and olanzapine group during the baseline phase (-10 and 0 minutes), and during the glucose-challenge phase (2, 7, 12, 30, 60, 80, and 90 minutes). Significance was accepted at \( p<0.05 \).

Similarly, for the euglycemic-hyperinsulinemic clamps, MMRS analyses were conducted to determine whether glucose, insulin, GINF, Ra and Rd values changed overtime, and whether these values differed across olanzapine or vehicle. Any significant findings (time x group) were further investigated as indicated by a series of linear contrasts, constructed to determine whether glucose, insulin, Ra, and Rd values differed between treatment groups during the baseline phase (60-90 minutes for Ra and Rd; 0-90 minutes for glucose and insulin) and during the post-ICV injection clamp phase (150-210 minutes). A single linear contrast if indicated was constructed to examine group differences in GINF values during the steady-state clamp phase only (150-210 minutes).

3. Results

3.1 Inhibition of Amphetamine-induced Locomotion:

A standard 10μg dose of olanzapine was initially chosen, but concomitant behavioral observations failed to note an appreciable effect on behavior. This was followed by a ten-fold increase in the dose; the 100μg dose caused an observable decrease in movement, but also caused seizure activity post injection. As a result, a 75μg ICV dose of olanzapine was tested on animals to quantify inhibition of amphetamine-induced locomotion assessed by the number horizontal beam breaks as compared to vehicle (figure 4). Based on lack of seizure activity and a significant decrease in horizontal beam breaks \( (F_{1,10}=5.87, p=0.036) \), the 75μg dose of olanzapine was administered to animals during the hyperglycemic clamp experiments.
3.2 Hyperglycemic clamps

Average basal plasma glucose levels were similar for olanzapine and control groups (figure 5A). Similarly, no differences were observed for baseline insulin and C-peptide levels between the two treatment groups (figure 5B and 5C, respectively). During the glucose challenge phase, plasma glucose was raised to hyperglycemic levels (~17mM), with no difference noted between olanzapine and control groups (figure 5A). However, both plasma insulin levels (LS-mean difference = 1541.5 ±457pmol/L; p=0.0042), and C-peptide levels (LS-mean difference = 8.63 ± 2.52nM; p=0.0038) (figure 5B and 5C, respectively) were significantly lower in the olanzapine group than the control group (LS-mean difference = 1541.5 ±457pmol/L; p=0.0042). These effects, suggesting a decrease in insulin secretion (C-peptide and insulin are co-secreted by the β-cell cell but cleared from plasma by different mechanisms), were mirrored by a significant decrease in the steady-state GINF required to maintain hyperglycemia between the olanzapine and control groups (F_1,15=13.96 p=0.002) (figure 6A), as well as a significantly lower DI (figure 6C). Given that the calculated insulin sensitivity index was not affected by
olanzapine (figure 6B), the decrease in GINF was likely attributable to a defect in β-cell function.

**Figure 5**

![Graphs A, B, and C](image)

**Fig 5.** Effect of a single ICV dose of olanzapine on plasma glucose levels (A), insulin (B), and C-peptide (C) during the hyperglycemic clamp †p < 0.05 vs. control; ‡ p < 0.01 vs. control.
3.3 Euglycemic hyperinsulinaemic clamps:

In all outcome measures of interest, the time x group interaction was not found to be significant, indicating that the magnitude of group differences did not vary over time. Given lack of an interaction term, the main treatment effects were interpreted directly. There were no differences between olanzapine and vehicle for glucose, insulin, Ra, Rd, or GINF (figure 7 A, B, C, D, E). Mean basal levels for glucose production were $11.86 \pm 0.73$ mg/kg/min for olanzapine, and $10.43 \pm 0.76$ mg/kg/min for vehicle animals. Post treatment/ clamp mean values for glucose production were $3.81 \pm 0.62$ mg/kg/min for olanzapine, and $2.33 \pm 0.86$ mg/kg/min for vehicle animals. Mean basal glucose disposal rates were $11.91 \pm 0.86$ mg/kg/min and $10.94 \pm 0.98$ mg/kg/min, for olanzapine and vehicle
respectively. During the clamp period, post ICV injection, mean glucose disposal rates were $18.94\pm1.13$ mg/kg/min under the olanzapine condition, and $16.92\pm0.93$ mg/kg/min with vehicle.
Fig. 7: Effect of a single ICV dose of olanzapine or vehicle on plasma glucose (A), insulin (B), rate of glucose appearance (Ra) production (C), rate of glucose disappearance (Rd) / utilization (D) and glucose infusion rate (GINF) (E) during a euglycemic hyperinsulinaemic clamp procedure. All graphs include data from 10 animals in each treatment group, with the exception of insulin, which included 6 olanzapine animals, and 7 vehicle animals. There was no statistically significant difference noted in any outcomes between the two treatment groups.

4. Discussion
Several avenues have been explored in an effort to uncover mechanisms responsible for the metabolic abnormalities induced by atypical antipsychotic agents. However, a paucity of studies have examined central brain mechanisms, leading us to examine the in vivo effects of a single intracranial dose of olanzapine on insulin sensitivity and secretion.

We established tolerability and concomitant suppression of amphetamine-induced locomotion to occur with a 75μg single dose of centrally administered olanzapine. Antipsychotic inhibition of amphetamine-induced locomotion remains a viable pharmacological model of clinical efficacy, and is likely to approximate therapeutic doses (Arnt, 1995; Ljungberg & Ungerstedt, 1985). Further, a strong correlation between the affinity of antipsychotic compounds at D₂ striatal receptors and their ability to block amphetamine-induced behavioral effects in rodents has been demonstrated, although the precise threshold of D₂ occupancy at which these effects occur has not been reported (Lang et al., 1994). Under these conditions, we proceeded to administer a single 75μg dose of olanzapine proximally to the hypothalamus. Immediately following olanzapine injection, we report a significantly decreased insulin and C-peptide response during the glucose challenge, suggesting centrally drug-mediated impairment of β-cell function. Although we did not directly compare peripheral to central administration in this study, an analogous impairment in secretion with peripheral olanzapine administration previously noted by our group (Chintoh, Mann, Lam, Lam et al., 2008) would suggest that olanzapine associated inhibitory effects on insulin response occur at least in part through centrally-mediated mechanisms. The lack of an observed effect of centrally administered olanzapine on insulin sensitivity and HGO might suggest that at least in the acute single dose paradigm, avoiding contribution of adiposity-related mechanisms, central effects play a less significant role in the profound effects on peripheral and hepatic insulin sensitivity observed with systemic olanzapine administration. These negative findings would also argue against the likelihood of non-specific toxic effects on glucose metabolism, suggesting that the observed olanzapine-associated decrement in insulin response was due to direct drug effects on underlying central biological pathways of glucose homeostasis.
The mechanisms through which olanzapine may acutely exert central effects leading to the observed peripheral impairment of β-cell function are unknown, but are likely to involve increased sympathetic activity, and/or decreased vagal parasympathetic activity. For example, it has been noted that acute ICV administration of chlorpromazine results in a hyperglycemic response, which can be blocked by adrenal-demедullation, central ganglionic blockade with hexamethonium, or peripheral adrenergic blockade (Fujimori & Iwamoto, 1974). Savoy and colleagues similarly demonstrated reversal of hyperglycemia induced by clozapine and chlorpromazine, either by pretreatment with hexamethonium or a peripheral α2-adrenergic antagonist (Savoy et al., 2010). The observation that M₃ receptors are highly expressed by β-cells where they possess a well-established role in glucose-dependent acetylcholine modulation of insulin secretion (Gilon & Henquin, 2001), alongside data suggesting that M₃ affinities of the AAPs follow the diabetogenic risk profile of these agents (Silvestre & Prous, 2005) could suggest involvement of cholinergic parasympathetic vagal pathways in antipsychotic-induced glucose derangements. Further, the presence of cholinergic neurons adjacent to the 3rd ventricle, including the VMH, LH and PVN (Hoover, Muth, & Jacobowitz, 1978; Tago, McGeer, Bruce, & Hersh, 1987), areas linked to glucose metabolism, might additionally implicate central mechanisms of cholinergic parasympathetic suppression by olanzapine. Previous data have in fact shown that enhancement of glucose-induced insulin secretion involves the hepatic afferent and pancreatic efferent vagal pathways (K. C. Lee & Miller, 1985; Ohnuma et al., 1996). This circuitry can be disrupted by ICV administration of atropine or methyl-atropine into the 3rd ventricle or nucleus ambiguous (Bereiter, Berthoud, Brunsmann, & Jeanrenaud, 1981; Ohnuma et al., 1996) supporting the notion of central, cholinergic control of insulin response.

It should be said, however, that very little is known as to how the complex receptor binding pharmacology of AAPs, may influence central signaling related to metabolism, in particular relating to acute effects on glucose homeostasis and insulin secretion. Data suggest that adrenergic, serotonergic and possibly dopaminergic cell groups project from specific brain regions to pancreatic parasympathetic pre-ganglionic neurons although their function with respect to insulin responses remains undetermined (Loewy, Franklin,
& Haxhiu, 1994). Bromocriptine, a dopamine agonist, has been proposed as an emerging anti-diabetic agent with potential central mechanisms of action on insulin sensitivity (Belavic, 2010), supporting a role for dopamine in glucose homeostasis. However, inconsistencies in data have also been noted, with findings suggesting, for example, that short-term administration of bromocriptine can also impair glucose tolerance (de Leeuw van Weenen et al., 2010). Acute central dopaminergic regulation of insulin secretion is also unclear. For example, mice lacking neuronal D2 receptors show acute insulin responses to glucose similar to wild type animals (Risso GS, personal communication). Implicating 5HT2C receptor in central glucose regulation, Zhou et al. demonstrated that acute 5HT2C agonism improved glucose tolerance via downstream activation of melanocortin-4 receptors in hyperinsulinemic diet-induced obese mice (Zhou et al., 2007). It remains to be shown however, whether 5HT2c antagonism could be associated with detrimental effects on glucose homeostasis. The association in literature between H1 affinities of antipsychotic agents and diabetogenic risk (Matsui-Sakata et al., 2005) might question whether this could in part be explained by central mechanisms. This is supported by the in vitro observation that the H1 receptor mediates central AMPK activation by orexigenic antipsychotics (S. F. Kim, Huang, Snowman, Teuscher, & Snyder, 2007). Linking this finding to acute effects on peripheral glucose homeostasis, Martins et al. demonstrated that ICV olanzapine acutely disrupted hepatic insulin sensitivity, likely through increased hypothalamic NPY and AgRP activity (Martins et al., 2010), neurons also linked to AMPK activation (Konner et al., 2007). AMPK activation in the VMH has been shown to amplify counter-regulatory responses to hypoglycemia (McCrinnmon et al., 2006), suggesting this may be a mechanism of antipsychotic-induced central activation of the sympathetic nervous system. The proposed mechanism is however at variance with a recently published study examining acute in vivo central administration of olanzapine and clozapine on activation of AMPK, finding only a trend towards increased levels of phosphorylated AMPK (pAMPK) (Ferno et al., 2011).

Contrary to Martins and colleagues, in the present study, we could not detect any effect of ICV olanzapine on insulin sensitivity. Differences in dosing of ICV olanzapine may also help to explain the discrepancies between studies. For example, Martins et al. used a
cumulative dose of 330μg over approximately 2 hours, including an initial bolus of 110μg over 5 minutes (Martins et al., 2010), as compared to the single 75 μg bolus administered over 60 sec in our experimental paradigm. Conversely, the study examining hypothalamic AMPK activation applied a single acute central bolus of 50μg, measuring pAMPK levels 15 and 30 min post-injection (Ferno et al., 2011). To our knowledge, among the existing studies examining in vivo ICV antipsychotic administration (Ferno et al., 2011), our study remains the only one to use an experimental paradigm with a quantifiable measure of antipsychotic effect to determine dose (i.e. inhibition of amphetamine-induced locomotion)(Arnt, 1995). Having said that, the present findings would benefit from determination of dose-response curves and D2 occupancy kinetics, and similarly such data might also help to clarify the noted variability between existing studies. While neither our study nor the study by Martin’s and colleagues examined peripheral olanzapine levels precluding that leakage into periphery could account for the observed effects, our dosing would argue against this. We administered 75μg to rats weighing between 0.300 -0.325 kg. Even if the full dose were to leak into the periphery, in a 0.300kg rat, this would equate to a maximal peripheral dose equal to 0.25mg/kg. The cumulative exposure in the Martins study would maximally approximate a dose equivalent to 1.2mg/kg, as given through a peripheral route. The current literature however suggests dose-dependent effects of acute olanzapine systemic administration on glucose metabolism, with no olanzapine-associated effects noted at doses of 1.0mg/kg or 1.5mg/kg, respectively, in two independent studies (Boyda, Tse, Procyslyn, Wong, et al., 2010; Houseknecht, et al., 2007). This would indirectly argue against peripheral effects of the centrally administered dose in the current study.

In conclusion, we show that a single centrally administered dose of olanzapine impairs β-cell secretory function in response to glucose challenge. This effect was analogous to our previous findings of impaired insulin secretion following acute peripheral olanzapine administration, suggesting that the CNS may be an important site in mediating drug-related effects on insulin response. Interestingly, the same dose administered centrally appeared to have no effect on peripheral insulin sensitivity, a finding in contrast to the pronounced effects of peripheral administration observed by ours laboratory and other
groups. A lack of perturbation on insulin sensitivity with central administration of therapeutically relevant doses of olanzapine could have interesting implications for drug delivery (i.e. intra nasal) to minimize some adverse effects. As it stands however, the relative role of central vs. peripheral mechanisms remains an interesting unanswered question. Similarly, the mechanisms underlying the central effect we observed on insulin response remain obscure, but may involve faulty central activation of sympathetic pathways and/or disruption of vagal insulino trophic mechanisms. The longer-term consequences of the observed acute effects on β-cell function, for example in the context of weight gain-related insulin resistance and β-cell decompensation leading to diabetes, also require elucidation. In this preliminary study, however, our aim was to assess the adiposity independent, or direct drug effects on central pathways of glucose metabolism, paving the way for future work employing chronic paradigms, and more precise approximation of therapeutic drug levels in the CNS through D₂ occupancy studies. Despite the many unanswered questions, we present novel findings supporting the notion of centrally mediated, antipsychotic-induced derangements in the regulation of insulin response. Future studies clarifying mechanisms and clinical implications of these direct central effects on metabolism are required.
CHAPTER 4
Acute effects of single dose olanzapine on metabolic, endocrine and inflammatory markers in healthy controls

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1. Introduction:
Use of the older “typical” antipsychotics has been largely supplanted by newer “atypical” agents (AAPs), although their purported advantage in terms of diminished risk of extrapyramidal symptoms (Emsley & Oosthuizen, 2004) has been replaced by concerns regarding AAP-induced metabolic changes, including significant weight gain, dyslipidemia, and glucose dysregulation (Newcomer, 2005). These side effects are particularly disconcerting given that increased overall morbidity and mortality from cardiovascular (CV) disease is an already well-established phenomenon in schizophrenia (Hennekens et al., 2005).

It has been widely assumed that glucose dysregulation associated with AAPs reflects the related weight gain observed with these medications; however, the picture appears more complex. Reports predating the introduction of AAPs identified a link between schizophrenia itself and diabetes (Kohen, 2004). In addition, there are case reports of diabetic ketoacidosis (DKA) occurring early in treatment with various AAPs, and in the absence of weight gain (Jin et al., 2002). Animal data indicate that AAPs, even following a single dose, produce an acute and pronounced effect on insulin sensitivity, and in the case of olanzapine and clozapine also on insulin secretion (6,7).

However, clinical studies attempting to replicate findings suggestive of direct medication effects have proven more challenging. Examining patients with schizophrenia treated with AAPs is confounded by the illness itself, which may represent an interface of inherent biological and environmental risk factors (Kohen, 2004). Furthermore, even short-term treatment with AAPs can lead to changes in adiposity (Ader et al., 2005; Kinon, Kaiser, Ahmed, Rotelli, & Kollack-Walker, 2005), an established risk factor for insulin resistance. To circumvent this, trials have turned to healthy volunteers and subchronic antipsychotic administration, ranging between 8-21 days of medication exposure (Sacher et al., 2008; M. Sowell et al., 2003; M. O. Sowell et al., 2002; Vidarsdottir, de Leeuw van Weenen et al., 2010; Vidarsdottir, Vlug, Roelfsema, Frolich, & Pijl, 2010). Results have been inconsistent, with some suggesting changes in insulin sensitivity (Sacher et al., 2008; Vidarsdottir, de Leeuw van Weenen et al., 2010;
Vidarsdottir, Vlug et al., 2010) while others have failed to find an association (Fountaine et al., 2010; M. Sowell et al., 2003) and/or reported early increases in weight thought to account for the changes in glucose metabolism (M. O. Sowell et al., 2002). Further, these studies have not always accounted for changes in fat distribution, which can occur without significant weight changes.

To our knowledge, to date, there has been only one “acute dosing” study in healthy controls; it reported early perturbations in glucose metabolism, as reflected by worsening of oral glucose tolerance testing (OGTT), following 3 doses of OLA (Albaugh, Singareddy, Mauger, & Lynch, 2011). In the current study, we employed the frequently sampled intravenous glucose tolerance test (FSIGTT) to evaluate changes after a single dose of placebo or OLA (10mg) in healthy, normal weight volunteers, thereby also examining the translational value of the single dosing rodent model. Although hyperinsulinemic and hyperglycemic clamps are considered to be the gold-standard methods for the separate assessment of insulin sensitivity and insulin secretion (DeFronzo et al., 1979), we chose the FSIGTT procedure with minimal model analysis as it is a less invasive method of assessment of both parameters of insulin sensitivity and secretion simultaneously; moreover, it is also widely used and considered valid and comparable and to clamping techniques for the assessment of insulin sensitivity (Bergman et al., 1987).

To elucidate possible mechanisms of OLA-induced glucose dysregulation, we also examined other metabolic and endocrine markers (leptin, adiponectin, C-Reactive Protein (CRP), cortisol, interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNFα), prolactin, and free fatty acids (FFAs)) as secondary outcome measures. We hypothesized that OLA would acutely decrease parameters of insulin sensitivity and secretion.

2. Methods:

2.1 Subjects:
Fifteen healthy non-obese participants between the ages of 18 and 45 were recruited via advertisements posted at the University of Toronto. Exclusion criteria included: (i)
current DSM-IV diagnosis of substance use disorder, positive urine drug screen, or major mental illness; (ii) nicotine use within 12 months of enrolment; (iii) diabetes or impaired fasting glucose according to the American Diabetes Association criteria ("Diagnosis and classification of diabetes mellitus," 2005) (iv) first degree relative with a history of diabetes; (v) regular use of any medication two months prior to study enrolment; (vi) any medication use during study enrolment (vii) history of liver disease or AST > 2 times upper limit of normal; (viii) serum creatinine > 1.2 times upper limit of normal; (ix) major medical or surgical event within the last 6 months; (x) history of hypertension or current blood pressure > 130/80; and (Xie & Lautt) a body mass index (BMI) < 18 or ≥ 27 kg/m². Females were screened for pregnancy and had to be on an acceptable method of birth control other than a hormonal contraceptive. All participants completed the International Physical Activity Questionnaire (IPAQ), a validated activity questionnaire (Craig et al., 2003), as well as a three-day dietary recall at the baseline visit. Baseline characteristics of study subjects are summarized in Table 5. All participants provided voluntary, informed consent and the study was approved by the institutional Research Ethics Board.
2.2 Study design:

This was a randomized, double-blind, placebo controlled cross-over trial comparing a single 10 mg dose of OLA with placebo (PL) administration. Participants had 2 FSIGTT procedures conducted on separate days within a 2-week period (to limit major confounding effects such as change in weight or fitness). Each visit day was separated by a minimum 6-day washout period, and prior to each visit subjects were asked to fast for

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Table 5: Baseline Characteristics of all Subjects

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Legend: SE, standard error; BMI, Body mass index; IPAQ, International Physical Activity Questionnaire; M, male; F, female; HDL, high density lipoprotein. Daily caloric intake information is missing for subject 5, who did not hand in the food diary.
10-12 hours, and to avoid strenuous physical activity as well as deviations from their usual dietary habits for the preceding 24 hours. Subjects were admitted to hospital at 0700 h, at which time fasting baseline samples for glucose and lipids were collected and a 10mg dose of either OLA or PL administered.

2.3 FSIGTT:
We preformed a 3-h FSIGTT with Bergman’s minimal model analysis using an insulin-modified protocol (Finegood, Hramiak, & Dupre, 1990). Two intravenous catheters were inserted into each forearm for blood sampling and for glucose/insulin injection, respectively. OLA has a half-life of approximately 31 h, with peak plasma levels occurring in 4-5 h (Markowitz et al., 2006). Three basal samples were drawn at -20, -10, and -5 min (with -20 corresponding to 4 h post-OLA or PL administration) for basal serum glucose and insulin concentrations. To coincide with the Tmax of OLA, the FSIGTT was commenced 4 h 20 min post-PL or OLA administration with a bolus of 50% glucose (25.1ml/m² body surface area), corresponding to time = 0 min. Blood samples for measurement of glucose, insulin and c-peptide were drawn at 2, 3, 4, 6, 8, 10, 12, 14, 16 and 19 min. At 20 min, a bolus of insulin was given (1.6U/m²) and further blood samples for glucose and insulin drawn at 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160 and 180 min. Bergman’s minimal model was used to calculate the insulin sensitivity index (SI), the acute insulin response to glucose (AIRg), the Disposition index (DI), and glucose effectiveness (Boden, Cheung, Stein, Kresge, & Mozzoli) (24). SI is defined as the ability of insulin to enhance glucose disappearance and inhibit glucose production. SG represents net glucose disappearance independent of changes in insulin above basal levels. AIRg is a measure of suprabasal insulin secretion assessed as the incremental area under the acute (0-10min) plasma insulin response curve after the IV glucose challenge. DI is calculated by multiplying SI by AIRg, and takes into account the hyperbolic relationship between insulin sensitivity and secretion, representing a measure of the adequacy of ß-cell response to changes in insulin resistance (Bergman et al., 2002).

Blood samples taken at the -5 min time point (i.e. 4.25 h post OLA or PL administration, directly preceding the FSIGTT) were also used to measure fasting non-esterified fatty
acid (FFAs) and prolactin. To look at insulin-suppression of FFAs, FFA samples were collected at the 50 and 60 min time points of the FSIGTT (30 and 40 min post-insulin injection). Other biochemical markers of interest included cortisol, IL-6, leptin, TNFα, adiponectin, and CRP, and were measured at -5 min, directly preceding the FSIGTT. All blood samples taken at the single -5min time point, or in the case of insulin suppression of FFAs, averaged over the 50 and 60 min time point, were compared within subjects, across the two treatment conditions. The change in fasting glucose (d_glucose) over the course of 4.25 h post-PL or drug administration was calculated as the difference in plasma glucose between fasting (07:00hr) and the -5min blood sample, and also compared within-subjects. OLA plasma levels were measured 2 h, 4.25 h and 7.25 h post-drug/PL administration.

2.4 Laboratory Measurements:
Plasma glucose was determined using the Glucose hexokinase assay (Roche Diagnostics, USA). C-peptide, insulin, and cortisol were determined using the electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics, USA). Prolactin was measured from serum using a sequential two-step sandwich immunoassay (Roche Diagnostics). Meso Scale Discovery Multiplex Assay was used to analyze TNFα, and IL-6 (Merck pharmaceuticals). FFAs and leptin were measured using a sandwich Enzyme Linked Immunosorbent Assay (ELISA) (LINCO Research, MI, USA). Adiponectin was measured using a manual radioimmunoassay (LINCO Research, MI, USA). C-reactive protein was measured with a particle enhanced immunoturbidmetric assay (Roche Diagnostics, USA). For all markers, the interassay coefficient of variability was less than 10%. The minimum detection range for the hormones of interest were: insulin 1.39 pmol/L; c-peptide 3pmol/L; leptin 0.5ng/ml; adiponectin 1ng/ml; CRP 0.03mg/L; FFAs 0.01mEq/L; prolactin 0.0470µg/L; cortisol 1.0nmol/L; IL6 0.27 pg/mL; TNFα 0.50pg/mL. Following collection, samples were immediately stored on ice, and transferred to -80C within 3-4 h of collection.

2.5 Statistical Analysis:
To determine whether outcomes differ across study conditions (OLA vs. PL), a series of mixed models were constructed. Full maximum likelihood (FML) estimation was selected as the method of estimation for each of these analyses, and an unstructured covariance structure was used. In addition to examining outcome measures within subjects across treatments, predictors accounting for order effects were included in all analyses. Given the small sample size, additional covariates of interest (e.g. baseline BMI, ethnicity, activity levels, caloric intake, OLA levels, fasting glucose, FFAs, \(d_{\text{glucose}}\)) could not be included in the model. Accordingly, exploratory Pearson’s correlations (for normally distributed data: WC, BMI, fasting glucose, fasting lipids, difference between treatments in \(d_{\text{glucose}}\)) or Spearman’s correlations (for non-normally distributed data: IPAQ, daily caloric intake, OLA levels, differences between treatments in prolactin, cortisol, adipocytokines, fasting FFA levels) or independent \(t\)-tests (for nominal variables) were used to look for possible associations between treatment effect on the main outcomes of interest (SI, DI, AIRg, SG) and the variables noted above. Paired \(t\)-tests were used to compare weight, WC, glucose, lipids, and blood pressure (BP) between the 2 visits (prior to drug/PL administration).

3. Results:

3.1 Completion and adverse events:
All 15 subjects who consented for the study completed both visits. Subject 1 was excluded from the minimod analysis due to technical difficulty with the FSIGTTT procedure (visit 1), and poor venous access (visit 2). Subject 7, at visit 2 (OLA treatment), was excluded from minimod analysis due to loss of the IV site. Subject 8, at visit 2 (OLA treatment) was excluded from all analyses (minimod, \(d_{\text{glucose}}\), FFAs, cytokines) as the individual forgot to fast and presented with an elevated fasting glucose. Subject 1 and 7’s data for glucose, FFAs, prolactin, and cytokines were included in subsequent analyses. Accordingly, all statistical tests for the outcomes related to minimod output used a sample of 14 (excluding 1 subject entirely, and 1 visit each from subject 7 and 8). Outcomes for all other tests used an \(n = 15\) (excluding one visit from subject 8). Fourteen of the 15 volunteers experienced fatigue/sedation with OLA vs. 3 subjects on PL. Two subjects experienced symptomatic hypoglycemia after PL and one after OLA.
Hypoglycemia was monitored by frequent blood glucose accu-cheks (Roche Diagnostics, USA), and resolved without intervention. None of the subjects elected to drop out of the study due to adverse side effects.

3.2 Glucose metabolism, endocrine and inflammatory markers:
There were no differences in body weight, WC, BP, fasting glucose or lipids between the two treatment visits. Changes in plasma glucose, C-peptide and insulin during the FSIGTT did not differ significantly between treatments (data not shown).

Mixed model analysis for treatment effects is summarized in Table 6. None of the outcomes of interest demonstrated order effects, suggesting that treatment effects were not dependent upon sequence of administration. There was no significant difference in SI, AIRg or C-peptide area under the curve (AUC) (Figure 8, Table 6). DI tended to be higher after OLA than PL, but the difference just missed statistical significance (p=0.077). SG was significantly lower after OLA than PL. Change in glucose (from baseline to pre-FSIGTT) was significantly higher after OLA compared to PL (Figure 9, Table 6). OLA was also associated with significantly lower FFAs and cortisol levels, but higher prolactin (Figure 9, Table 6). Levels of adipocytokines (leptin, adiponectin, IL-6, TNFα, CRP) did not differ significantly across study conditions (Table 6). Of note, Subject 7 had unusually large IL-6 values under both treatment conditions (PL: 49.6pg/ml and OLA: 87.8pg/ml), and was therefore considered excluded from the analysis.

The difference between treatments (OLA-PL) in SI (ΔSI) was positively related to fasting glucose and plasma OLA at 4.25hr and negatively to recorded caloric intake and Δcortisol; ΔAIRg was positively related to fasting glucose (and negatively to IPAQ and Δadiponectin; ΔDI was positively related to fasting glucose and plasma OLA at 2hr and negatively to Δcortisol; and ΔSG was positively related to fasting prolactin and negatively to recorded caloric intake (Table 7). There was no significant noted effect of gender or ethnicity with respect to the outcomes of interest, except in the case of prolactin where the difference in prolactin levels between OLA and PL was significantly higher in
females \((t_{12}=4.6, \ p = 0.0006)\). Serum OLA increased after OLA administration (to 35.67 ± 5.75nM at 2 hrs post administration; 48.85 ± 2.72nM at 4.25 hrs post administration; 45.31 ± 2.87nM at 7.25 hrs post administration), but was undetectable after PL administration. Two hour OLA levels were unavailable for subjects 2 and 7 due to refusal to have additional venipuncture prior to insertion of intravenous catheters for the FSIGTT.
### Table 6: Treatment Effects

<table>
<thead>
<tr>
<th>Outcome</th>
<th>LS Means ± SE</th>
<th>95% CI</th>
<th>F, df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OLA</td>
<td>PL</td>
<td>OLA</td>
<td>PL</td>
</tr>
<tr>
<td>SI (x10^4) (min^-1 per μU/ml)</td>
<td>4.57±0.43</td>
<td>4.19±0.42</td>
<td>(3.63, 5.50)</td>
<td>(3.28,5.09)</td>
</tr>
<tr>
<td></td>
<td>510±74</td>
<td>485.8±73</td>
<td>(350,670)</td>
<td>(376,645.4)</td>
</tr>
<tr>
<td>DI</td>
<td>2060.6±233</td>
<td>1785.5±277.3</td>
<td>(1552.8, 2568.3)</td>
<td>(1290.4, 2280.7)</td>
</tr>
<tr>
<td>SG (min^-1)</td>
<td>0.018±0.001</td>
<td>0.021±0.001</td>
<td>(0.015,0.021)</td>
<td>(0.019,0.024)</td>
</tr>
<tr>
<td>C-peptide (AUC), pmol/L per 10min</td>
<td>3.0809±2555</td>
<td>2.9212±2510</td>
<td>(2.5243,36375)</td>
<td>(2.3742, 34681)</td>
</tr>
<tr>
<td>ΔGlucose (4.25 hour-baseline)</td>
<td>0.23±0.03</td>
<td>-0.14±0.03</td>
<td>(-0.03,0.49)</td>
<td>(0.22,0.36)</td>
</tr>
<tr>
<td>FFA (mEq/L)</td>
<td>0.23±0.02</td>
<td>0.31±0.02</td>
<td>(0.19,0.28)</td>
<td>(0.27,0.36)</td>
</tr>
<tr>
<td>FFA % suppression</td>
<td>88.3±3.8</td>
<td>82.6±3.6</td>
<td>(80.2,96.4)</td>
<td>(74.8,90.4)</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>204.6±40.9</td>
<td>318.6±41.8</td>
<td>(116.4,429.3)</td>
<td>(228,409)</td>
</tr>
<tr>
<td>Prolactin (ug/L)</td>
<td>39.9±4.3</td>
<td>7.8±4.2</td>
<td>(30.2,49.3)</td>
<td>(-1.3,17.0)</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>204.6±40.9</td>
<td>318.6±41.8</td>
<td>(116.4,429.3)</td>
<td>(228,409)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.13±0.76</td>
<td>1.54±0.76</td>
<td>(0.55,1.70)</td>
<td>(0.99,1.09)</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>10.24±0.38</td>
<td>10.26±0.38</td>
<td>(9.41,11.07)</td>
<td>(9.45,11.08)</td>
</tr>
<tr>
<td>Adiponectin (pmg/ml)</td>
<td>917.45±1257.5</td>
<td>9249.6±1261.7</td>
<td>(6458.6,11890.0)</td>
<td>(6523.8, 11975.0)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>5.08±1.47</td>
<td>5.57±1.47</td>
<td>(1.92,8.25)</td>
<td>(2.40,8.74)</td>
</tr>
<tr>
<td>CRP (pg/ml)</td>
<td>0.49±0.08</td>
<td>0.49±0.09</td>
<td>(0.31,0.66)</td>
<td>(0.31,0.68)</td>
</tr>
</tbody>
</table>

**Legend:** LS, least-squares; CI, confidence interval; df, degrees of freedom; SI, sensitivity index; AIRg, acute insulin response to glucose; AUC, area under curve; DI, disposition index; SG, glucose effectiveness; FFA, free fatty acids
<table>
<thead>
<tr>
<th></th>
<th>Δ SI</th>
<th>Δ AIRg</th>
<th>Δ DI</th>
<th>Δ SG</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>BMI</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>FBG</td>
<td>R= 0.62; p=0.011</td>
<td>R=0.67; p=0.016</td>
<td>R= 0.66; p=0.019</td>
<td>N/S</td>
</tr>
<tr>
<td>HDL</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Tgs</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>2hr [OLA]</td>
<td>N/S</td>
<td>N/S</td>
<td>R=−0.66, p=0.026</td>
<td>N/S</td>
</tr>
<tr>
<td>4.25 hr [OLA]</td>
<td>R=−0.62; p=0.024</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>7.25 hr [OLA]</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>IPAQ</td>
<td>N/S</td>
<td>N/S</td>
<td>R=−0.60, p=0.026</td>
<td>N/S</td>
</tr>
<tr>
<td>Caloric intake</td>
<td>R=−0.72; p=0.011</td>
<td>N/S</td>
<td>N/S</td>
<td>R=−0.81; p=0.003</td>
</tr>
<tr>
<td>prolactin</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>R=−0.71; p=0.010</td>
</tr>
<tr>
<td>glucose (change over 4.25 hrs)</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Δ FFA</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Δ cortisol</td>
<td>R=−0.65; p=0.022</td>
<td>N/S</td>
<td>R=−0.65; p=0.021</td>
<td>N/S</td>
</tr>
<tr>
<td>Δ adiponectin</td>
<td>N/S</td>
<td>R=−0.68; p=0.014</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Δ leptin</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Δ CRP</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Δ IL-6</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Δ TNFα</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
</tbody>
</table>
**Figure 8:**

- **A)** Sensitivity Index (SI)
- **B)** Disposition Index (DI)
- **C)** Acute Insulin Response to Glucose (AIRg)
- **D)** Glucose Effectiveness (SG)

*Fig. 8.* Individual (within subject) data points for (A) SI, (B) DI, (C) AIRg, and (D) SG for each participant, according to treatment received. Data excluded for subject 1 (OLA and PL), subject 7 (OLA), and subject 8 (OLA).
4. Discussion:
This is the first study to examine an AAP single dosing *in vivo* paradigm in healthy volunteers, thereby avoiding medication-induced changes in adiposity as well as inherent biological and/or lifestyle factors related to schizophrenia itself. In keeping with some (Fountaine et al., 2010; M. Sowell et al., 2003; M. O. Sowell et al., 2002), but not all
(Albaugh, Singareddy et al., 2011; Sacher et al., 2008; Vidarsdottir, de Leeuw van Weenen et al., 2010; Vidarsdottir, Vlug et al., 2010) subchronic dosing studies in healthy controls, we failed to demonstrate acute effects of OLA on insulin sensitivity. As reviewed elsewhere, the two earliest studies, employing either euglycemic or hyperglycemic clamps in healthy subjects (M. Sowell et al., 2003; M. O. Sowell et al., 2002), have been methodologically criticized, with one reanalysis suggesting a decrease in insulin sensitivity following OLA and risperidone vs. placebo (Bergman & Ader, 2005). Conversely, a second study comparing 10 days of ziprasidone or OLA administration found a significant decrease in whole body insulin sensitivity in the OLA arm from baseline. However, subjects in the OLA group in this study gained weight (BMI increase of 0.6 kg/m²) over the 10-day period, which may have contributed to the change in insulin sensitivity (Sacher et al., 2008). Two other studies in healthy, normal weight subjects treated with OLA for 8 days demonstrated decreased insulin sensitivity as measured by the euglycemic clamp or calculated homeostasis model assessment of insulin resistance (HOMA IR), respectively (Vidarsdottir, de Leeuw van Weenen et al., 2010; Vidarsdottir, Vlug et al., 2010). Both studies looked at body composition, concluding that observed changes in insulin sensitivity were not due to changes in adiposity (Vidarsdottir, de Leeuw van Weenen et al., 2010; Vidarsdottir, Vlug et al., 2010). In a study that most closely approximates our acute dosing paradigm, administration of OLA 10mg to healthy controls over 3 days reported an acute detrimental effect on glucose metabolism, reflected by an increase in the AUC during an OGTT as compared to placebo (Albaugh, Singareddy et al., 2011). The apparent discrepancy with the findings of our study may be accounted for by differences in duration of OLA administration, and possibly differences in the method of assessment of glucose metabolism. Muscarinic vagal pathways, which have an established role in glucose regulation (Gilon & Henquin, 2001), are acutely more responsive to portal or oral glucose load (Ohnuma et al., 1996); in addition, AAP risk of diabetes has been linked to muscarinic-M₃ affinity (Silvestre & Prous, 2005). Complex methods of assessing glucose metabolism, such as the FSIGTT or clamping techniques employing an intravenous glucose challenge, with the advantage of greater precision and decreased confounds of other processes that can influence derived values of insulin sensitivity and response (i.e.
glucose absorption rates, incretin responses and non-quantified insulin-independent effects on glucose metabolism) (Hucking et al., 2008), may minimize the contribution of AAP-related perturbations on the vagally mediated physiological responses.

It is also possible though that direct medication effects are more difficult to detect experimentally, and/or occur more variably in humans as compared to the impact of changes in adiposity on insulin sensitivity. Furthermore, if the reports of DKA in association with certain antipsychotics are thought to be representative of a causal effect on glucose homeostasis, the overall incidence rate, although 10-times higher than in the general population, remains low (e.g. 3.1 per 1000 patient years for clozapine) (Cohen & Correll, 2009). Arguably, the direct effects of acute administration of certain AAPs on glucose metabolism in healthy controls appear to be more variable than those observed in rodent models. Differences may speak to greater variability in antipsychotic specific effects in humans as compared to the bred strains of rodents used for experimental paradigms. The other point to consider is that the half-life of antipsychotic medications is 4-6 times shorter in rodents than humans, and while single dosing in rats can approximate clinical D₂ occupancy, peak plasma levels are multiple times higher than that seen in clinical scenarios (Kapur et al., 2003). Whether this could have differential pharmacodynamic effects through peripheral receptors linked to glucose metabolism remains an interesting unanswered question.

Our findings nonetheless support early, direct effects of OLA on several metabolic and endocrine parameters; specifically, we noted a rise in plasma glucose from baseline over 4.25 hours post-OLA administration, as well as a decrease in SG during the FSIGTT vs. PL. The ability of glucose to enhance its own disposal and suppress endogenous production independent of insulin effects remains an additional important factor that determines glucose tolerance (J. D. Best et al., 1996). In keeping with our data, Henderson and colleagues reported differential effects of higher liability antipsychotics on SG in non-obese patients with schizophrenia (Henderson et al., 2005). Similarly, a study in schizophrenia patients employing the euglycemic clamp pre- and post-treatment with olanzapine or risperidone suggested that while insulin sensitivity decreased with
olanzapine treatment, correlating with changes in adiposity, significant increases in fasting glucose occurred independently of changes in insulin sensitivity or fat mass (Hardy et al., 2011). Taken together, this could suggest that direct effects of antipsychotic medications on glucose metabolism may occur, at least in part, through insulin-independent pathways.

Consistent with other studies in AAP-treated healthy volunteers (Cohrs et al., 2006), our data suggest that OLA acutely decreases serum cortisol levels. Cortisol has established detrimental effects on glucose homeostasis, including suppression of insulin-mediated inhibition of hepatic glucose production, and reduced glucose utilization in muscle (G. Meyer & Badenhoop, 2003). Thus, the OLA-associated decrease in serum cortisol may serve to attenuate other deleterious effects on glucose metabolism. Indirect support for this hypothesis is reflected in the significant negative correlations we observed between Δcortisol and both the ΔSI and the ΔDI, suggesting that those individuals with a greater OLA-related cortisol suppression have higher SI and DI vs. PL.

With respect to lipid metabolism, our data replicate findings in healthy volunteers exposed to 3-8 days of OLA that demonstrate a decline in fasting FFA concentrations (Albaugh, Singareddy et al., 2011; Vidarsdottir, de Leeuw van Weenen et al., 2010; Vidarsdottir, Vlug et al., 2010). Although the mechanisms underlying this decrease are not known, one explanation could be the decrease in cortisol with OLA treatment, as cortisol is known to activate hepatic sensitive lipase (HSL), lipoprotein lipase (LPL), and adipose triglyceride lipase (ATGL) in adipocytes (Samra et al., 1998). It is alluring to speculate that impairment in the distribution of lipids away from adipose tissue in fasting states could result in inappropriate adipose tissue trapping of FFAs, predisposing to long-term weight gain. This is in keeping with a recent study in adipocytes demonstrating a differential ability of various antipsychotic drugs to increase basal rates of lipogenesis, as well as reduce rates of lipolysis in response to adrenergic stimulation (Vestri et al., 2007).

The growing conceptualization of type 2 diabetes mellitus (DM2) and metabolic syndrome (MS) as a proinflammatory state characterized by aberrant cytokine production
(Rabe, Lehrke, Parhofer, & Broedl, 2008), led us to examine key adipokines. Vidarsdottir and colleagues examined leptin and adiponectin levels following 8 days of OLA in healthy volunteers, reporting an increase in nocturnal adiponectin levels independent of weight changes (Vidarsdottir, de Leeuw van Weenen et al., 2010). Another study exposing healthy volunteers to 15 days of OLA or PL noted OLA-associated increases in leptin, TNFα, adiponectin, as well as a decrease in ghrelin. However, subjects receiving OLA gained weight as compared to PL, precluding the conclusion that OLA induces changes in these biomarkers independent of changes in adiposity (Fountaine et al., 2010). Our findings suggest that OLA, in a single dosing paradigm, does not induce changes in the examined biomarkers.

The current study must be considered within its own limitations, several of which may have resulted in an underestimation OLA’s effects on parameters of glucose regulation. We chose to examine OLA in healthy volunteers; however, data suggest that drug-naïve patients with schizophrenia may have an underlying biological predisposition to glucose dysregulation (T. A. Cohn et al., 2006; Fernandez-Egea et al., 2009; Spelman et al., 2007). A next logical step, therefore, would be to replicate this study in antipsychotic-naïve individuals with schizophrenia as this population may be more sensitive to single dose perturbations. Our sample represented a very active subset of the general population, reflected in the IPAQ scores (median score 4405 met min/week); minimal recommended health requirements are, in fact, 150 met min/week (Caspersen, Pereira, & Curran, 2000). As regular exercise is a protective factor for glucose tolerance (S. E. Kahn et al., 1990), the effects of OLA on parameters of glucose regulation noted in our sample may not be generalizable to persons with severe mental illness who are frequently less active (McCreadie, 2003). A crossover design was employed here to minimize inter-individual variability but we did not regulate amount of physical activity, food intake, or sleeping patterns on the day/night prior to hospital admission, all factors that can influence insulin sensitivity and cytokines between the 2 visits. We also chose a single 10 mg dose of OLA for reasons of practicality and safety, although this is not representative of how the drug is used clinically (i.e. daily administration, often at higher doses). Further, we examined a finite period to assess for changes (approximately 4.5-7.5 hr post-drug
administration), using peripheral kinetics and $T_{\text{max}}$ to guide our decision-making; however, changes in our identified outcome measures could occur outside this window.

In summary, we identified a small and immediate signal following a single 10 mg dose of OLA in parameters of glucose and lipid metabolism, an effect which may be more pronounced in the clinical setting where the population involves more metabolically vulnerable individuals with schizophrenia and treatment conditions differ. Taken together, these finding suggest that OLA does exert some early direct drug-related effects on metabolism that are independent of weight gain and psychiatric illness. Further studies of this sort are required to replicate and expand the present findings, and are warranted as we seek to develop newer medications devoid of these significant risks. That we presently have newer antipsychotics at decreased risk of weight gain (Newcomer, 2007) is tempered by the fact that all currently available antipsychotics have been linked to DKA, with the exception of ziprasidone which has however been associated with hyperglycemia and pancreatitis (Dhamija & Verma, 2008; Jin et al., 2002; Makhzoumi, McLean, Lee, & Ibe, 2008; S. H. Yang & McNeely, 2002); moreover, we are reminded that CLZ, linked as it is to diabetes, significant weight gain over time, and numerous reports of DKA (Henderson et al., 2000; Jin et al., 2002), represents the treatment of choice in refractory schizophrenia (Agid, Foussias, Singh, & Remington, 2010). It remains that the biological underpinnings of antipsychotic-related metabolic side-effects remain largely unknown, and similarly risk factors that may identify individuals who will develop these adverse effects are poorly characterized. While the current study focuses on illness- and adiposity-independent effects, future work should also examine how these factors interact with medication-induced perturbations on glucose metabolism.
CHAPTER 5
GENERAL DISCUSSION

1.0 Summary of Experiments
In this body of work, we aimed to further elucidate adiposity- and illness-independent effects of antipsychotic medications on glucose metabolism. In experiment 1, we employed hyperglycemic clamps following peripheral administration of selective antagonists to specifically examine effects of M₃, D₂, 5HT₂A, H₁, antagonism on pancreatic β-cell function, as well as a calculated index of insulin sensitivity. In keeping with our initial hypothesis, we identified acute and direct effects of M₃, D₂, 5HT₂A, but not H₁ antagonism on measures of β-cell function. Contrary to our expectations, there was also no effect of any of the antagonists on insulin sensitivity; furthermore, 5HT₂A antagonism appeared to improve this parameter. In experiment 2, we set out to examine the role of central mechanisms in the disruption of insulin secretion and sensitivity, predicting that an acute central injection of olanzapine would produce similar deficits to that observed with systemic treatment. Interestingly, we were able to replicate effects observed with respect to β-cell response in the hyperglycemic clamp, which however failed to demonstrate any changes in the hyper-insulinemic euglycemic clamp (HIEC) with respect to changes in insulin sensitivity. Finally, we moved to test the single dosing paradigm established in rodents using a human model. Contrary to our working hypothesis, we failed to observe effects on calculated measures of insulin sensitivity or secretion measured through the Frequently Sampled Glucose Tolerance Test (FSIGTT). Interestingly though, we observed effects on other measures of glucose and lipid metabolism, which may have implications with respect to underlying mechanisms of direct effects.

2.0 Discussion
Atypical antipsychotics (AAPs) currently represent the mainstay of therapy for psychotic disorders such as schizophrenia. Initial enthusiasm for their purported decreased association with extrapyramidal side effects (EPSE) has, however, been tempered by significant metabolic side effects, including in some cases the development of type 2
diabetes (DM2) (Newcomer, 2004). In turn, impaired glucose tolerance, insulin resistance, and hyperglycemia not only define DM2, but also contribute to microvascular disease and have been associated with cardiovascular mortality. Furthermore, increased rates of diabetes in patients with schizophrenia are a well-established phenomenon, and both the Canadian and American Diabetes Associations now identify schizophrenia per se as a risk factor for diabetes(ADA & APA, 2004; "Canadian Diabetes Association 2008 clinical practice guidelines for the prevention and management of diabetes in Canada," 2008). Initially, the contribution of antipsychotic medications to glucose dysregulation was thought to occur secondary to their strong association with weight gain liability; however, the relationship has turned out to be more complicated. As discussed in the introduction, both inherent biological factors, as well as other illness/lifestyle-related factors relevant to schizophrenia, contribute to this risk. Finally, a growing body of literature stemming from case reports of diabetic ketoacidosis (DKA) occurring without weight gain, in addition to animal work employing acute dosing paradigms, have suggested that weight gain independent or direct effects of these medications contribute to the risk of associated glucose dysregulation. In the current body of work, we set out to elucidate the biological underpinnings of direct, or adiposity and illness-independent effects of antipsychotic medications on glucose metabolism. For experiments 2 and 3, we chose to use olanzapine, as it along with clozapine represents one of the two antipsychotics with greatest metabolic liability (olanzapine also remains one of the most widely prescribed antipsychotic medications). Administration of clozapine to healthy volunteers in experiment 3 would have been difficult to justify given the associated risk between clozapine and agranulocytosis.

In an effort to better understand the mechanisms by which antipsychotic-mediated effects on glucose dysregulation occur, we first set out to deconstruct the heterogeneous receptor binding profile of the antipsychotics. Using selective antagonists, we examined effects of \( M_3 \), \( D_2 \), \( 5HT_{2A} \), \( H_1 \), antagonism on pancreatic \( \beta \)-cell function, as well as a calculated index of insulin sensitivity. In keeping with the critical role of the \( M_3 \) receptor in the physiological regulation of glucose stimulated insulin secretion, we found that \( M_3 \) antagonism impaired insulin and C-peptide response during the glucose challenge in the
hyperglycemic clamp. While it has been shown that intracellular serotonin is crucial to regulation of pancreatic insulin secretion, our findings further suggested that antagonism of the 5HT$_{2A}$ receptor may be associated with impairment of the insulin response during glucose challenge. Our results also indicated that D$_2$ antagonism resulted in an increase in insulin response, a finding in keeping with the physiological expression of D$_2$-like receptors in β-cells, which mediate inhibition of insulin secretion. Conversely, H$_1$ blockade did not result in acute effects on insulin or C-peptide secretion, suggesting that the association between H$_1$ receptor and glucose dysregulation noted in the literature is not related to acute peripheral effects on β-cell function, although it has been linked to control of energy metabolism and weight gain. Contrary to our working hypothesis that the analogous measure of insulin sensitivity provided by the hyperglycemic clamp would be impaired by blockade of all four examined receptor pathways, there was no effect of any of the antagonists on insulin sensitivity; further, 5HT$_{2A}$ antagonism appeared to increase this parameter. While we noted a trend for the D$_2$ antagonist to impair insulin sensitivity, this just fell short of significance, and as discussed in chapter 2, these data in general require replication with the HIEC, which is considered the gold standard to assess insulin sensitivity. Interestingly, some of our findings may be explained by differential effects of neurotransmitter systems with respect to glucose metabolism, depending on central vs. peripheral antagonism. For example, terfenadine, the drug we used to examine H$_1$ antagonism, has poor penetrance of the central nervous system (CNS) and, notably, histamine has most commonly been implicated in energy and glucose metabolism through central pathways. Conversely, ketansar, the 5HT$_{2A}$ antagonist employed here, readily crosses the blood brain barrier, where serotonin may have opposing effects as compared to the periphery. For example, central antagonism of the 5HT$_{2A}$ receptor may reduce sympathetic tone, improving sensitivity (McCall & Harris, 1988); conversely, peripheral blockade may impede skeletal muscle uptake of glucose (Hajduch et al., 1999), reducing peripheral insulin sensitivity. The work in experiment 1 must also be considered within the limitations of non-absolute specificity of antagonists (in particular ketansar), uncertain approximations of clinically relevant antagonist dosing as compared to antipsychotic administration, and, as discussed, the inability to tease out central vs. peripheral effects.
Given the increased awareness of the CNS’ role in both energy metabolism and glucose homeostasis, we set out to investigate whether the single dose effect on glucose metabolism observed with systemic administration could be replicated with central administration. Dosing of olanzapine was first established based on inhibition of amphetamine-induced locomotion, a previously validated model of clinical antipsychotic efficacy. A hyperglycemic clamp procedure was employed immediately following ICV injection, with findings suggesting analogous effects on insulin and C-peptide response to those seen in systemic olanzapine administration. Contrary to prior existing hypotheses, we failed to observe an effect on insulin sensitivity, in contrast to findings by an independent study which found that continuous, acute central administration of olanzapine caused impairment in hepatic insulin sensitivity. The discrepancy between our study and the latter could possibly be explained by differences in dosing. Despite the incongruence between the 2 existing studies, we nonetheless present novel findings which support the notion that central mechanisms contribute to impairment in insulin response observed in rodent models with systemic administration. These arguably represent important findings given that failure of β-cell compensation is understood to be the final determining step in the development of diabetes, as well as a critical mechanism implicated in the development of DKA.

In the final set of experiments, we set out to examine whether acute, direct effects of antipsychotic medications could be replicated in vivo in humans, independent of effects of psychiatric illness or adiposity, also testing the translational model of the single dose rodent model. We felt this work was important in addressing some of the inconsistencies in the schizophrenia literature that examine the direct associations between AAPs and glucose metabolism, which have been difficult to interpret precisely because of potential illness-related risk factors which overlap with diabetes risk determinants. At the time we were conducting our study, several sub-chronic antipsychotic dosing studies (8-21 days) in healthy volunteers examining effects on glucose regulation had been published, with some discrepancy noted between studies, possibly reflective of early concomitant changes in weight or adiposity, and/or failure to account for the same.
Contrary to our a priori hypotheses, we did not see an effect on insulin sensitivity or response, as measured via the FSIGTT, a method considered comparable to gold standard HIEC and hyperglycemic clamps. We did note effects of olanzapine on fasting glucose levels, which increased over approximately 4 hours of drug administration versus placebo, as well as an olanzapine-associated decrement in glucose effectiveness (Boden et al.), a measure representing the ability of glucose to enhance its own disposal and suppress endogenous production independently of insulin effects. We also noted that olanzapine was associated with significantly lower serum cortisol and fasting FFA. Interestingly, decreased FFAs represent a very consistent finding both in the schizophrenia literature, as well as in available data involving healthy controls (Albaugh, Singareddy et al., 2011; Vidarsdottir, de Leeuw van Weenen et al., 2010; Vidarsdottir, Vlug et al., 2010). Decreased fasting levels of FFAs are also consistent with some in vitro and in vivo animal data (Albaugh, Judson et al., 2011; Vestri et al., 2007), suggesting a shift to impaired adipolysis and fat storage under conditions where fat should be mobilized. While our small sample size precluded examination of potential covariates related to our outcomes of interest, exploratory correlations suggested that those individuals with greater olanzapine-related cortisol suppression had higher indices of insulin sensitivity, a finding in keeping with the established detrimental effects of cortisol on glucose metabolism (G. Meyer & Badenhoop, 2003). An interesting speculation stemming from this latter observation might be that relative non-suppression of cortisol levels following antipsychotic administration could serve as an early risk marker for development of glucose dysregulation attributable to direct medication effects.

While suggesting early effects of olanzapine on various markers of glucose and fat metabolism, the results in experiment 3 were arguably less pronounced than those seen in the single dose rodent model. This could be due to greater genetic heterogeneity in humans as compared to bred rodent strains. Differences in pharmacokinetics between species, with rats exhibiting faster metabolism but also higher peak serum levels following peripheral antipsychotic injections could account for more pronounced immediate effects on tissues involved in glucose homeostasis. That the majority of in
vitro studies employing “therapeutic serum” doses of antipsychotic medications fail to show consistent effects on insulin-mediated glucose transport in muscle or adipocyte cells offers indirect support for this position. Our findings of an olanzapine-associated increase in fasting blood glucose levels and impairment in SG make it tempting to speculate that insulin-independent pathways may be more sensitive to lower doses of olanzapine. In this regard, an additional caveat of our study was that the employed 10mg dose of olanzapine (for safety and practicality reasons) was neither representative of typical clinical dosing nor associated steady-state serum levels. The observation in animal models that antipsychotic-induced effects demonstrate the most robust effects on hepatic glucose output could also suggest that the FSIGTT, which cannot differentiate hepatic from peripheral insulin sensitivity, would have been less sensitive than a measure such as the HIEC to pick up effects on this parameter. Similarly, the route of administration of a glucose challenge may affect the strength of the olanzapine signal on glucose metabolism. Complex methods of assessing glucose homeostasis employing an acute intravenous glucose challenge may minimize cholinergic involvement in humans, which is more responsive to an oral challenge (Ohnuma et al., 1996). As demonstrated in experiment 2, these may play an important role in olanzapine-induced perturbations of insulin response; in fact, M3 antagonism of antipsychotic medications has been linked to risk of diabetes (Silvestre & Prous, 2005). This may also help to explain why the single other published acute dosing study in healthy controls (albeit administering 3 doses as compared to our single dose design) noted pronounced effects of olanzapine as compared to placebo during an OGTT(Albaugh, Singareddy et al., 2011). One of the future challenges in the field will be to identify the separate physiological pathways, as well as the extent they may contribute to the impairment of glucose metabolism by these medications (i.e. gut-hormone axis, insulin-independent pathways vs. insulin dependent pathways; including differential effects on specific insulin target tissues). At present, no single method of assessment of glucose metabolism can provide those answers. Finally, while our work here has specifically focused on direct drug effects on glucose pathways, these perturbations may be more prominent in patients with psychosis/schizophrenia due to their various underlying vulnerabilities, leading to an underestimation of olanzapine-related effects in experiment 3.
3.0 Clinical relevance/implications:
As reviewed in the introduction, diabetes and metabolic syndrome are far more common in patients with schizophrenia than compared to the general population. In keeping with this observation, insulin resistance is highly prevalent (30%) in patients on antipsychotics and often goes undiagnosed (Sernyak, Gulanski, & Rosenheck, 2005). The above, taken together with reports of DKA in association with these agents occurring independent of weight gain, suggest that clinicians should be aware of the direct diabetogenic effects of these drugs, as compared to weight gain, frequently considered to be both cause and proxy for metabolic abnormalities. Evidence for a direct and relatively acute effect should shift this focus, and underline the need for close monitoring from treatment onset. Similarly, elucidation of mechanisms underlying these direct effects are important in order to better determine patient risk, inform targeted interventions, and guide development of agents devoid of these risks.

It could perhaps be argued that availability of low liability AAPs such as ziprasidone or aripiprazole might avoid the metabolic issue. Similarly, clinical data suggesting there may be little difference between conventional and AAPs in overall effectiveness has raised the possibility of returning to FGAs with their lower overall metabolic risk, this time using more judicious dosing to minimize EPS (Jones et al., 2006). However, it remains that we cannot circumvent these issues. For example, it has been consistently demonstrated that patients early on in their illness are more prone to metabolic side effects, regardless of whether newer and older agents are utilized (D. G. Robinson et al., 2005). This is not so surprising individuals with first episode psychosis have also been identified as more sensitive to EPSE (Remington, 2007). Along these same lines, all currently available antipsychotic agents have been associated with DKA, with the exception of ziprasidone which has, though, been associated with hyeprglycemia and pancreatitis (Jin et al., 2002; S. H. Yang & McNeely, 2002). Further, clozapine, one of the agents with the highest liability of metabolic perturbations (Henderson et al., 2000; Jin et al., 2002), remains the only agent with proven superiority in the face of treatment refractory schizophrenia and is the only antipsychotic currently available that has been
approved for suicidality (Leucht, Komossa et al., 2009; Meltzer et al., 2003). Olanzapine, the other agent with comparable metabolic risk to clozapine, has also been associated with superiority claims (Johnsen & Jorgensen, 2008; Lieberman et al., 2005). As such, avoiding high metabolic liability agents, or in the case of emergence of serious metabolic side effect, switching to agents of lesser liability may not always be feasible.

Future drug development, as well as treatment of drug-related side effects can benefit greatly if we better understand which features of antipsychotic pharmacology account for the notable liability in terms of metabolic disturbances. Teasing out peripheral and central mechanisms with respect to these metabolic perturbations, alongside experiments assessing putative antipsychotic effects, could lead to not only novel agents but also the possibility of alternative methods of drug administration, for example directly into the CNS (i.e. intranasal), avoiding drug effects on peripheral tissues involved in metabolism. The animal model itself (both acute and subchronic dosing) may have utility for future work, both with respect to screening putative agents for side effects and testing putative treatments to counter antipsychotic-associated metabolic side effects.

4.0 Future directions:
Before turning to the various interesting applications of our work outlined, it is important to review our results from the standpoint of limitations and call for further data. More immediate work should include use of the HIEC to examine representative receptor binding profiles of these medications (experiment 1) to better assess effects on insulin sensitivity. As the work in experiment 1 was not meant to be exhaustive, future investigations should examine other receptors affected by antipsychotics which are also potentially implicated in glucose regulation (e.g. 5HT\textsubscript{2c}, 5HT\textsubscript{1A}, \(\alpha_1\), \(\alpha_2\), D\textsubscript{4}). Similarly, the findings in experiment 2 should be extended to examine centrally-mediated effects of other antipsychotic agents, followed thereafter by deconstruction of receptor binding profiles in an analogous manner to experiment 1. Given the paucity of data with respect to clinically relevant centrally administered doses, our current state of knowledge would also benefit greatly from direct determination of dose-response curves and D\textsubscript{2} occupancy kinetics of representative antipsychotics (in terms of both peripheral and central
administration). With respect to acute dose systemic drug administration, the observation that higher peak serum drug levels may be observed in rodents raises this as a possible explanation for the more pronounced effects on glucose metabolism observed in these animals. This question might be followed-up with a direct comparison of acute drug administration approximating $C_{\text{max}}$ serum levels to those approximating 60-70% $D_2$ occupancy on glucose metabolism. In this regard, it remains to be seen whether the $D_2$ occupancy story might be more relevant to central administration, whereas approximating therapeutic serum levels in the periphery might be more representative of drug effects on organs/tissues involved in glucose regulation.

The other interesting question that came up during our investigations is whether route of glucose challenge might impact the degree of olanzapine-associated perturbations on glucose homeostasis. In this respect, it would be interesting to conduct a pilot study directly comparing, for example, the OGTT to the HIEC/ hyperglycemic clamp procedure in rodents, possibly including various implicated hormones (i.e. glucagon, GLP-1) as covariates.

Moving forward, it is also important to address limitations related to the single dose paradigm. While such an approach is arguably of great value in examining direct medication-induced effects on glucose metabolism, these effects in isolation may not be representative of certain clinical realities. For example, we discussed the issue of confounds related to the effects of adiposity on glucose metabolism, which should also be examined in the context of a multiple dosing paradigm. As reviewed in the introduction, the majority of rat studies have focused on absolute weight gain when, in fact, changes in adiposity may be more closely modeled to what is observed in clinical settings both in terms of adiposity-associated diabetes and cardiovascular risk. Likewise, the underlying illness-associated vulnerability to metabolic risk that characterized schizophrenia should be examined in the context of the additional risk of medications. To this end, examining treatment-naive individuals early in the illness course may serve as a starting point to assess the acute effects of antipsychotic agents on glucose metabolism, moving also to examine longer-term effects of these agents on a variety of metabolic parameters.
5.0 Conclusions:
Taken together, our findings have begun to elucidate pathways involved in the adiposity-independent effects of these agents, identifying that cholinergic, serotonergic, and dopaminergic pathways may be involved. We have also suggested that such effects may occur in part through effects on the CNS. Although these ‘direct’ effects may be less pronounced in humans than in rodent models, we nonetheless show that even a single dose of olanzapine given to healthy control subjects may cause early perturbations in lipid and glucose metabolism. While the mechanisms underlying metabolic disturbances are likely very complex and multifaceted, we present novel work providing clues underlying antipsychotic-induced effects on glucose metabolism, along with several proposed avenues for future investigations.
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