Receptor Binding Profiles of Antipsychotic Medications and Glucose Dysregulation: an Acute Animal Model

by

Melanie Dawn Guenette

A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

© Copyright by Melanie Dawn Guenette (2012)
Receptor Binding Profiles of Antipsychotic Medications and Glucose Dysregulation: an Acute Animal Model

Melanie Dawn Guenette

Master of Science

Institute of Medical Science
University of Toronto

2012

Abstract

Atypical antipsychotics (AAPs) are associated with several metabolic sequelae including increased risk of type 2 diabetes. Growing evidence points to a direct drug effect of these compounds on glucose homeostasis, independent of weight gain. While the responsible mechanisms have yet to be elucidated, the heterogeneous binding profiles of AAPs likely include receptors involved in glucose metabolism. This study aimed to clarify weight-gain independent mechanisms of AAP-induced alterations in insulin secretion. Deconstruction of the receptor binding profiles of these agents was done using representative antagonists and the application of a hyperglycemic clamp. We assessed the acute effects of several selective receptor antagonists on glucose metabolism, namely prazosin, idazoxan, MDL100907, SB242084 and WAY100635. Treatment with prazosin and MDL100907, selective $\alpha_1$ and 5HT$_{2A}$ antagonists, respectively, resulted in significant decreases in both insulin and C-peptide secretion. These findings were corroborated with decreased glucose infusion rate and disposition index in the prazosin group. Results suggest that $\alpha_1$ and 5HT$_{2A}$ receptor antagonism may be involved in glucose dysregulation with AAP treatment, however, the exact mechanisms involved remain unknown.
Acknowledgments

I have been the fortunate recipient of an Institute of Medical Science Open Fellowship Award and for this I would like to extend my gratitude to the Faculty of Medicine at the University of Toronto.

Celine Teo has been my right hand throughout this rollercoaster of an adventure. Her consistency in helping me run my experiments and being my sounding board in times of stress have proven pivotal to the completion of this project. Although I initially trained her, I believe she has taught me a lot about myself and has made me a better researcher and person. I am thankful for her impeccable work ethic and willingness to help during clamps.

Drs. Araba Chintoh and Margaret Hahn have given many, many hours to my training and for this I am eternally grateful. Thank you so much for helping me gain my footing in both surgical and clamping techniques, in addition to data analysis. You are great teachers, showing me incredible kindness and compassion, and I consider myself fortunate to have learned the ropes from such fantastic scientists.

Many thanks to my project advisory committee members Drs. Adria Giacca and Roger McIntyre for their continued input and guidance. I am grateful to Dr. Giacca for allowing me to use her laboratory, in addition to the expertise of her two extraordinary laboratory technicians; Loretta Lam, who provided constant technical support during my experiments and Elena Burdett, always present and willing to offer words of encouragement. I would also like to extend my gratitude to the students of the Giacca
laboratory for their friendship, which made this experience a better one than I could have ever imagined.

Thank you to Connie Bartha and Steve Mann for their patience in helping me navigate the intricacies of completing a graduate degree. Thank you for your selflessness and understanding natures, which made me feel at home and supported. Your contribution in this journey will not soon be forgotten.

I have made so many true and great friends over the last two years because of my involvement in the Institute of Medical Science Students’ Association (IMSSA). Joining IMSSA was one of the best decisions I have made and I want to offer thanks to the phenomenal students who have become an important part of this experience (you know who you are!). The trifecta was pivotal in all things related to life; Melinalie will forever hold a special place in my heart. A specific mention goes to NB for being my library buddy and motivating me day after day to write this thesis. I also acknowledge the sustained support of many life long friends who came before the IMS; words cannot express my gratitude and love for you. Friendships are what make getting up every day worthwhile. Please know how much I appreciate your presence in my life.

Thank you to my mother who raised me to be persistent, sensitive and strong. I could not have gotten through this without your love and support. I hope to have made you proud. Pour mon grand-père qui décès avant la complétion de cette maîtrise, je t’aime de tout mon coeur. Que tu retrouves la paix qui t’as tant manquée.

Finally I would like to offer a heartfelt thank you to my wonderful supervisor, Dr. Gary Remington. I consider myself immensely fortunate that Dr. Remington agreed to take
me on as a graduate student. I could not have asked for a more knowledgeable and supportive mentor to guide me along this journey. Dr. Remington possesses a wealth of wisdom and is always receptive and patient with my numerous questions. His unwavering optimism and commitment to my research provided a solid base for my graduate experience and for this I will always be thankful.
Contributions

Tamara Arenovich (Centre for Addiction and Mental Health) conducted all statistical analyses.

Dr. Margaret Hahn (Centre for Addiction and Mental Health) aided in the collection of data during hyperglycemic clamps and contributed to the manuscripts in chapters 2 and 3 of this thesis.

Loretta Lam (Department of Physiology, University of Toronto) analyzed and generated reports for all samples collected during the hyperglycemic clamps.

Celine Teo (Centre for Addiction and Mental Health) aided in the collection of data during hyperglycemic clamps.
Table of Contents

Acknowledgments ........................................................................................................ iii

Contributions ............................................................................................................... vi

List of Tables ................................................................................................................. x

List of Figures ................................................................................................................. xi

Chapter 1 Introduction and Literature Review ............................................................. 1

1 Introduction and Literature Review ......................................................................... 2

1.1 Schizophrenia ......................................................................................................... 2

1.1.1 Pharmacotherapy and Antipsychotics ............................................................... 5

1.1.2 The Dopamine Hypothesis of Schizophrenia .................................................... 5

1.1.3 Side Effects of First-Generation Antipsychotics .............................................. 7

1.1.4 Clozapine and “Atypicality” .............................................................................. 8

1.1.5 Side Effects of Second-Generation (Atypical) Antipsychotics ....................... 9

1.1.6 Summary .......................................................................................................... 10

1.2 Energy Homeostasis and Glucose Metabolism ....................................................... 11

1.2.1 Abnormal Glucose Metabolism in Type 1 Diabetes ....................................... 16

1.2.2 Abnormal Glucose Metabolism in Type 2 Diabetes ....................................... 16

1.2.3 Diabetic Ketoacidosis .................................................................................... 17

1.3 Antipsychotic-Induced Weight Gain ..................................................................... 19

1.3.1 Atypical Antipsychotics and Glucose Dysregulation ...................................... 21

1.3.2 Atypical Antipsychotics and Insulin Resistance .............................................. 22

1.3.3 Atypical Antipsychotics and Type 2 Diabetes ................................................. 24

1.3.4 Atypical Antipsychotics and the Metabolic Syndrome ................................... 24

1.3.5 Atypical Antipsychotics and Metabolic Side Effects ..................................... 26

1.3.6 Summary ........................................................................................................ 28

1.4 Pre-Clinical Models of Atypical Antipsychotic-Induced Weight Gain .................. 28

1.4.1 Investigating Glucose Dysregulation .............................................................. 29

1.4.2 The Hyperinsulinemic-Euglycemic Clamp ...................................................... 29

1.4.3 The Hyperglycemic Clamp ............................................................................ 30

1.4.4 Pre-Clinical Models for Atypical Antipsychotic-Induced Glucose Dysregulation . 30

1.4.5 Summary ........................................................................................................ 33
Chapter 4 ............................................................................................................................... 74

4 General Discussion .............................................................................................................. 75
  4.1 Summary of Work ........................................................................................................... 75
  4.2 Discussion ....................................................................................................................... 75
    4.2.1 Prazosin, a selective α₁ antagonist ........................................................................... 77
    4.2.2 Idazoxan, a selective α₂ antagonist .......................................................................... 77
    4.2.3 WAY100635, a selective 5HT₁A antagonist ............................................................... 77
    4.2.4 SB242084, a selective 5HT₂C antagonist .................................................................. 77
    4.2.5 MDL100907, a selective 5HT₂A antagonist ............................................................... 77
  4.3 Caveats ............................................................................................................................ 78
    4.3.1 Diabetic Ketoacidosis with Atypical Antipsychotic Treatment ............................... 78
    4.3.2 Deconstruction of Atypical Antipsychotic Binding Profiles .................................... 78
  4.4 Relevance of Atypical Antipsychotic-Induced Glucose Dysregulation ............................. 81
    4.4.1 Clinical ...................................................................................................................... 81
    4.4.2 Economic ............................................................................................................... 83
  4.5 Implications ..................................................................................................................... 84
  4.6 Future Directions ............................................................................................................ 88
  4.7 Conclusions ..................................................................................................................... 91

References ............................................................................................................................ 92
List of Tables

Table 1. The DSM-IV diagnostic criteria for schizophrenia.

Table 2. Case reports of diabetic ketoacidosis with atypical antipsychotic treatment.

Table 3. Demographic summary of diabetic ketoacidosis cases with atypical antipsychotic treatment.

Table 4. Post-diabetic ketoacidosis antipsychotic treatment.

Table 5. Signs and symptoms of diabetic ketoacidosis.

Table 6. Factors included in monitoring protocols for patients prescribed atypical antipsychotics.

Table 7. Factors included in long-term monitoring of patients prescribed atypical antipsychotics.
List of Figures

Figure 1. Relationship between doses of antipsychotics and their respective dopamine receptor affinities.

Figure 2. Involuntary facial spasms characteristic of tardive dyskinesia.

Figure 3. Basic mechanisms of glucose stimulation of insulin secretion by beta cells of the pancreas.

Figure 4. Biphasic insulin secretion after a sudden rise in blood glucose.

Figure 5. Synthesis of the active insulin hormone from pro-insulin.

Figure 6. The glycogen molecule.

Figure 7. Mechanisms of glucose homeostasis.

Figure 8. Mechanisms of diabetic ketoacidosis.

Figure 9. The propensity of conventional and atypical antipsychotics to induce changes in weight.

Figure 10. Breakdown of literature search to capture cases of diabetic ketoacidosis with atypical antipsychotic treatment.

Figure 11. Experimental protocol describing the hyperglycemic clamp technique.

Figure 12. Effect of a single subcutaneous dose of representative antagonist on plasma glucose levels during the hyperglycemic clamp in male Sprague-Dawley rats.

Figure 13. Effect of a single subcutaneous dose of representative antagonist on plasma insulin levels during the hyperglycemic clamp in male Sprague-Dawley rats.

Figure 14. Effect of a single subcutaneous dose of representative antagonist on plasma C-peptide levels during the hyperglycemic clamp in male Sprague-Dawley rats.
Figure 15. Effect of a single subcutaneous dose of representative antagonist on plasma glucose infusion rates (GINF) during the hyperglycemic clamp in male Sprague-Dawley rats.

Figure 16. Effect of a single subcutaneous dose of representative antagonist on the sensitivity index (SI), calculated during the last 30 minutes of hyperglycemic clamp in male Sprague-Dawley rats.

Figure 17. Effect of a single subcutaneous dose of representative antagonist on the disposition index (DI), calculated during the last 30 minutes of hyperglycemic clamp in male Sprague-Dawley rats.
Chapter 1
Introduction and Literature Review
1 Introduction and Literature Review

1.1 Schizophrenia

Schizophrenia represents one of the most debilitating and stigmatized of all psychiatric illnesses. It affects approximately 1% of the population in all cultures (1), and the diversity of its onset, clinical symptoms, and outcome have led to the current position that schizophrenia is not a single entity, but instead a heterogeneous group of disorders that may be mediated by different pathophysiologic mechanisms (2).

Although the current conceptualization of schizophrenia is relatively new, subtyping patients according to clinical presentation (catatonic, paranoid, hebephrenic) dates back as far as the 19th century (3). It was Emil Kraepelin (4), however, who first categorized these symptoms as manifestations of a single disease, one that he called ‘dementia praecox’, i.e. early dementia. Kraepelin chose these terms because of the relatively young age of onset and the progressive functional deterioration of patients with this disorder.

It was not long after Kraepelin’s work that Eugene Bleuler (5) introduced the term ‘schizophrenia’ to describe the same population of patients. Bleuler’s terminology stemmed from his less pessimistic view regarding disease outcome and his belief that symptoms were due to a splitting or fragmentation (schizo) of the mind (phren). In addition, Bleuler generated a symptom classification system that comprised fundamental and accessory symptoms in schizophrenia. Fundamental symptoms included ambivalence, alterations in association, and changes in affect as well as
attention. Accessory symptoms were derived from the fundamental symptoms and included hallucinations, delusions, and abnormalities in behaviour and speech.

The advent of chlorpromazine in the 1950s would mark the birth of modern psychopharmacology and revolutionize the treatment of schizophrenia (see 1.1.1 Pharmacotherapy and Antipsychotics). At the same time, a new classification system was introduced with the publication of the first Diagnostic and Statistical Manual of Mental Disorders (DSM) in 1952. The DSM offered, for the first time, a field-specific and operationalized approach to psychiatric diagnosis as an alternative to the International Classification of Diseases (ICD), which was and is still used for the classification of all medical diseases.

It is generally agreed upon that there are three major symptom domains in schizophrenia: positive, negative and cognitive (6). Historically, the bulk of attention has been focused on positive symptoms, defined as certain phenomena present in affected individuals but absent in the normal population, e.g. delusions, hallucinations, disorganized speech and behaviour (6). Negative symptoms are defined as the absence of certain abilities and emotions normally seen in healthy individuals, including blunted or flat affect, poverty of speech, lack of interest in social activities, recreation and goal-directed behaviour, catatonia, inability to experience pleasure and lack of interest in social interactions (7). Lastly, the cognitive deficits in schizophrenia pertain to disturbances in attention and concentration (8). Both negative and cognitive symptoms have been noted in the illness' prodrome, predating the onset of positive symptoms (9). Furthermore, evidence suggests that it is not the positive, but the negative and cognitive
symptoms, that constitute the rate-limiting aspects of the disease with regard to functional recovery (10).

The considerable change in conceptualization of schizophrenia is reflected in the current DSM-IV-TR criteria of the illness (see Table 1), which include a broader clinical definition, one that addresses social and functional measures over time.

Table 1. The DSM-IV diagnostic criteria for schizophrenia. (Source: DSM-IV, American Psychiatric Association, 1994)
1.1.1 Pharmacotherapy and Antipsychotics

The discovery of chlorpromazine’s antipsychotic activity in the 1950s was serendipitous, as the drug was being studied for its pre-anesthetic properties (11). The identification of effective pharmacotherapy, at least with respect to psychotic symptoms, offered convincing evidence regarding schizophrenia’s biological underpinnings, profoundly changing our understanding and approach to the disease. Chlorpromazine became the prototype for a new class of drugs called ‘major tranquilizers’ (for their sedating properties), later termed ‘neuroleptics’ (literally, to take the neuron) and, finally, ‘antipsychotics’ (the symptom domain that most effectively responds to pharmacological treatment) (12).

1.1.2 The Dopamine Hypothesis of Schizophrenia

Neuroleptics readily supplanted previously used somatic therapies (i.e. insulin coma, cold water baths, electroshock) for schizophrenia, and soon became the first line of treatment (13) for the disorder. Because of the unexpected discovery of chlorpromazine’s antipsychotic activity, and the rapid development of other neuroleptics immediately thereafter, an understanding of these drugs’ mechanism of action lagged in comparison to the rapid changes in clinical practice. It was Carlsson (14) who first suggested a possible role for dopamine in the pathogenesis of schizophrenia, postulating that hyperdopaminergic activity was responsible for the positive symptoms of the disease. He added that neuroleptics, via their dopamine antagonism, could mitigate psychotic symptoms.

Studies using dopamine agonists provide indirect support for the dopamine hypothesis of schizophrenia. For example, positive symptoms indistinguishable from those seen in
frank schizophrenia can be precipitated in healthy controls given amphetamine, a
dopamine agonist (15,16). Furthermore, psychotic symptoms can, under analogous
circumstances, be exacerbated in patients with an established diagnosis of
schizophrenia (16,17).

The understanding of antipsychotic mechanism of action improved considerably with the
characterization of the dopamine receptor family, e.g. D$_1$-D$_5$ (18). Specifically, the
discovery of the correlation between D$_2$ antagonism of antipsychotics and therapeutic
response (19) set the stage for a transition from more pleiotropic, low-potency
compounds (e.g. chlorpromazine and thioridazine) to a group of highly potent, selective
D$_2$ antagonists (e.g. haloperidol and pimozide); Figure 1 depicts the strong correlation
between therapeutic doses of antipsychotics and affinity for the D$_2$ receptor. The shift to
D$_2$ selective compounds also affected dosing regimens; more specifically, the older,
heterogeneous binding compounds required much higher doses to achieve therapeutic
effects (e.g. 300-1000mg/day) compared to their more D$_2$-selective counterparts (e.g.
haloperidol at 2-10mg/day).

*Figure 1. Relationship between doses of antipsychotics and their respective dopamine
receptor affinities. (Source: Seeman et al., 1976)*
1.1.3 Side Effects of First-Generation Antipsychotics

While \( D_2 \) antagonism is credited with the effectiveness of first generation neuroleptics, it is also linked to some of these compounds’ most serious side effects. Extrapyramidal symptoms (EPS) are caused by \( D_2 \) antagonism in the nigrostriatal dopamine pathway (one of four dopaminergic pathways in the brain, and responsible for the coordination of muscle movement), resulting in unwanted and/or uncoordinated muscle movement (e.g., acute dystonia, parkinsonism) (20).

While these movements can be seen from the outset of antipsychotic treatment, over the longer term the risk shifts to tardive dyskinesia (TD), which involves involuntary, repetitive and potentially irreversible movements most commonly affecting the face, lips and tongue, but also seen in the trunk, limbs and extremities (21). Characteristic presentations of TD include protrusion of the tongue, smacking, puckering and pursing of the lips, in addition to rapid eye blinking (see Figure 2).

The incidence of TD with chronic administration of first-generation antipsychotics is in the 20-25% range, a figure that increases in the geriatric population (22). The high rate of such devastating side effects propelled the search for compounds with diminished risk, clozapine representing the first agent to distinguish itself in this regard (see 1.1.4 Clozapine), and the prototype for a new class of drugs called second-generation or atypical antipsychotics.
1.1.4 Clozapine and “Atypicality”

Clozapine was first synthesized in 1959 and almost immediately distinguished itself clinically through its lack of EPS when compared to other antipsychotics of the era (23). The excitement over clozapine’s unique clinical profile was tempered, however, after reports surfaced of deaths associated with its use after its release in the early 1970s; these were later determined to be caused by agranulocytosis, a life-threatening condition characterized by abnormally low levels of white blood cells, affecting the immune response (24). In most countries, clozapine was withdrawn thereafter, only to be reintroduced in the early 1990s after evidence established its clinical superiority in refractory schizophrenia (25). At this point, however, mandated guidelines were implemented requiring regular monitoring of patients’ blood-work.
The uniqueness of clozapine’s clinical profile is paralleled in its pharmacology. Clozapine is a highly pleiotropic compound, reminiscent of earlier neuroleptics (e.g. chlorpromazine) and binds to the D₂ receptor with less affinity than its predecessors (26). Ultimately, these findings called into question the necessity of D₂ antagonism for antipsychotic activity (27), an appealing option given the undesired side effects associated with D₂ selective antagonism; however, this has since been challenged (28).

The greater binding of clozapine to the serotonin (5HT₂) receptor has been implicated as one explanation for its superior clinical efficacy (29), and became the impetus for a new class of ‘serotonin-dopamine antagonists’ (i.e. second-generation or atypical antipsychotics), which includes risperidone, olanzapine, quetiapine and ziprasidone. It is worth noting, however, that alternative models to account for atypicality have been posited, including the fast dissociation model (30-32). While the pharmacological mechanisms that define atypicality remain open to question, the purest definition of atypicality entails antipsychotic activity in the absence of notable EPS across therapeutic doses (33). Clozapine remains unique among its atypical counterparts due to its clinical superiority in refractory schizophrenia; its use is currently restricted to this population (i.e. when trials of two other antipsychotics have been ineffective) because of the risk of agranulocytosis. The other atypical antipsychotics, however, now represent first line treatment in schizophrenia (34).

1.1.5 Side Effects of Second-Generation (Atypical) Antipsychotics
The development of atypical antipsychotics provided an advantage over older compounds through their reduced EPS profile. In addition, it was thought that these drugs had better overall tolerability (35), and for a period it was held that these drugs
were superior in the treatment of negative and cognitive symptoms (36-38), although these claims have since been challenged (39).

Within a decade of their introduction, it became increasingly apparent that atypical antipsychotics were not devoid of undesirable side effects. The most noted of these included an increased risk of weight gain and other metabolic disturbances (40). Recent estimates put the incidence of type 2 diabetes with atypical antipsychotic treatment at a staggering 20% (41). Adding to the concern was the increasing number of reports of diabetic ketoacidosis, some occurring immediately after treatment initiation (see Chapter 2) and a fraction of which resulted in death (42-47).

Although a number of studies have since shown that atypicals differ in their risk of metabolic sequelae (48,49) – with clozapine and olanzapine displaying greatest risk, and ziprasidone and aripiprazole at lowest risk – the mounting evidence was enough to impel the action of regulatory bodies. In 2001 and 2002, Canada, Japan and Britain each issued warnings regarding reports of diabetes, ketoacidosis and death in conjunction with atypical antipsychotic use. By 2003 the United Stated Food and Drug Administration (FDA) demanded a monograph warning regarding the relationship between atypical antipsychotics as a class and risk of glucose abnormalities. Canada followed suit, and its product monographs were also required to acknowledge this risk.

1.1.6  Summary
Antipsychotics are pivotal in the treatment of psychosis, providing better patient outcome and reduced relapse rates (50). First generation compounds, while effective, cause disturbing side effects such as EPS and TD, the latter of which can be
irreversible. Second generation or atypical antipsychotics have an advantage in this regard, but as a class they are burdened by a risk of weight gain and a constellation of metabolic side effects that cannot be ignored. As atypicals now represent the first line of treatment in schizophrenia (34), and are being used more broadly, e.g. in bipolar disorder, dementia and refractory depression (51,52), it is important that efforts now shift to better understanding the mechanisms responsible for their undesired side effects.

1.2 Energy Homeostasis and Glucose Metabolism

The maintenance of normal blood glucose levels in the healthy human is a tightly regulated process that is accomplished via several mechanisms. While numerous hormones are involved (e.g. insulin, glucagon, cortisol), insulin plays a central role by stimulating the uptake of glucose from the blood and absorption into peripheral tissues, i.e. skeletal and adipose cells (53). In addition, insulin also promotes the storage of excess energetic molecules (e.g. monosaccharides and fatty acids) into forms readily available for future use (i.e. glycogen and triglycerides) (53). The level of circulating insulin varies based on the energetic state of the organism. For example, insulin secretion is minimal under fasting conditions (i.e. 80-90/100 mg/dL). After a meal, however, large quantities of glucose enter the bloodstream, having been absorbed from the digestive system, and stimulate the secretion of insulin. This incoming glucose is sensed by beta cells in the islets of Langerhans of the pancreas. Glucose enters these cells via the GLUT-2 transporter (a transmembranous protein of the beta cell) and undergoes glycolysis, resulting in the generation of adenosine tri-phosphate (ATP). This increase of intracellular ATP triggers a cascade that culminates in the release of vesicles containing insulin from beta cell into the extracellular space (Figure 3) (53).
Insulin secretion is a bi-phasic process. The first phase is characterized by a rapid (10 fold) increase in insulin, occurring 3-5 minutes after a positive change in blood glucose levels. This initial rise is caused by the release of pre-formed vesicles containing insulin, which are docked at the beta cell membrane (54). Within another 5-10 minutes, however, insulin levels begin to decrease toward baseline levels (Figure 4). The second phase of insulin release will result in levels surpassing that of the initial phase, although this rise will be more gradual in nature (55). Occurring about 15 minutes after the initial glucose stimulus, the second rise in insulin concentration is a result of both pre-formed granule secretion and de-novo synthesis within the beta cell.
The synthesis of insulin begins with pre-pro-insulin, the translational product of the insulin gene. This 110 amino acid peptide is processed into pro-insulin by the removal of its signal peptide by cleaving enzymes. The resulting pro-insulin contains three chains; the A and B chains are held together via a disulfide bond, while the C chain is cleaved and stands alone (53). The A-B complex, making up the insulin hormone, and C chain are packaged together into vesicles and are stored in preparation for secretion (Figure 5). Upon exocytosis of the insulin granules, both insulin and C-peptide are released; it is for this reason that C-peptide levels are often used as an index of beta cell secretory capacity (57).
As noted, glucose is transported into peripheral tissue either for immediate catabolism (i.e. ATP generation) or for storage as fat or glycogen. The binding of insulin to its transmembranous receptor causes the upregulation of GLUT-4 transporters, which are responsible for the movement of glucose from the blood into peripheral tissues (i.e. skeletal or adipose cells) (53). This process results in the eventual decrease of plasma glucose levels and the formation of energetic polymers that can be broken down for later use.

The liver plays an important role in the maintenance of optimal plasma glucose levels. Excess glucose molecules that are not immediately needed are joined through phosphorylation to form glycogen (Figure 6), a process accomplished by hepatic enzymes. Insulin promotes glycogen synthesis by activating these enzymes and inhibiting others that are responsible for glycogen breakdown (i.e. glycogenolysis) (53).
During periods of fasting, the maintenance of optimal glucose levels is accomplished via two complementary mechanisms: glycogenolysis and gluconeogenesis. When plasma glucose falls below a physiologically acceptable level, glucagon, the hormonal counterpart to insulin, is released from the alpha cells of the islet of Langerhans. Glucagon promotes the catabolism of glycogen to release glucose molecules (i.e. glycogenolysis, through activation of hepatic enzymes) and also increases the rate of amino acid uptake into hepatocytes for transformation into glucose (i.e. gluconeogenesis) (54). The end result is the opposite of that accomplished by insulin (i.e. increased plasma glucose concentration) (Figure 7).

Figure 6. The glycogen molecule. (Source: www.themedicalbiochemistrypage.org)

Figure 7. Mechanisms of glucose homeostasis. (Source: http://www.apsu.edu)
1.2.1 Abnormal Glucose Metabolism in Type 1 Diabetes

Type 1 diabetes (DM1) is characterized by a severe deficiency or total absence of insulin secretion. While the exact cause remains unknown, it is generally thought that DM1 is due to autoimmunity against the insulin producing beta cells of the pancreas. The result is an absence of insulin and an inability to clear glucose from the blood, causing hyperglycemia (i.e. high glucose content in blood). Classic symptoms of DM1 include glucosuria (i.e. glucose in the urine), polydipsia (i.e. excessive thirst), polyphagia (i.e. increased hunger), and weight loss (56). Commonly diagnosed in childhood, DM1 is fatal unless treated with insulin therapy.

1.2.2 Abnormal Glucose Metabolism in Type 2 Diabetes

According to the National Diabetes Surveillance System, 6.2% of Canadians over 20 years of age had diabetes of some form in 2006-2007 (59). Best estimates suggest about 10% of people with diabetes present with type 1 and about 90% with type 2 (DM2) (59). Individuals with DM2 suffer a progressive decline in insulin sensitivity due to increased insulin resistance (i.e. lack of proper insulin signaling). Insulin resistance develops over time and is most often associated with a history of increased central adiposity and obesity.

In insulin resistance, the decreased sensitivity to insulin impairs the uptake of glucose into the cells of the body (e.g. skeletal muscle, adipose). In both early DM2 and insulin resistance, the beta cells of the pancreas will compensate for the resistance by secreting a surplus of insulin, resulting in hyperinsulinemia. Over time, the beta cells become damaged from chronic hyperglycemia (i.e. glucotoxicity) and eventually become unable to secrete insulin (53). Individuals with this degree of insulin resistance
require exogenous insulin therapy in addition to the traditionally used oral hypoglycemic agents (OHAs), used to sensitize cells to insulin. Poorly managed DM2 leads to many dangerous sequelae including nephropathy, retinopathy, neuropathy and cardiovascular disease (e.g. atherosclerosis, stroke) (53).

Insulin resistance is one of several risk factors of the ‘metabolic syndrome’, a concept developed by the National Cholesterol Education Program (NCEP). An individual is classified as having metabolic syndrome if he/she presents with any three of the following: elevated fasting glucose levels; increased waist circumference (i.e., abdominal obesity, often reflected by an increased BMI); hypertension; increased triglyceride levels; or reduced HDL cholesterol. The metabolic syndrome increases one’s risk of developing atherosclerotic cardiovascular disease, even in the absence of diabetes (60).

1.2.3 Diabetic Ketoacidosis
When there is a prolonged lack of insulin effect, most commonly due to insulin resistance, the body begins to ‘starve’ and resorts to using fat as a source of energy (i.e. lipolysis). This process involves the mobilization of triglycerides from adipocytes and their subsequent hydrolysis into free fatty acids, followed by degradation into ketone bodies (e.g. acetoacetate and β-hydroxybutyrate) by the liver (53).

The synthesis of ketone bodies (i.e. ketosis, a bi-product of lipid metabolism) is a normal physiologic mechanism in place to provide alternative sources of energy during starvation (i.e. when glycogen stores are depleted). Diabetic ketoacidosis (DKA), however, is a metabolic crisis that primarily affects individuals with DM1, but can also
occur in DM2 (61). DKA is most often caused by poorly managed diabetes, infection, or alcohol abuse (62,63).

In DKA there is an absolute shortage of insulin and, as noted, the body resorts to using fat as a source of energy. Ketone bodies, however, are acidic and pose a problem in the maintenance of the acid-base homeostasis of the blood. When normal compensatory mechanisms (e.g. bicarbonate buffering, hydrogen ion absorption by protein and bone, increased renal excretion) fail, blood pH falls below 7.35 and frank metabolic acidosis ensues (53).

DKA may be the first sign of undiagnosed diabetes, or be precipitated by an added physiological stressor (e.g. infection, tissue infarct) in known diabetics. Under normal conditions, insulin works not only to promote glucose uptake but, in much smaller quantities, suppresses ketogenesis. In addition, insulin suppresses glycogenolysis (i.e. breakdown of glycogen) and gluconeogenesis (i.e. de-novo synthesis of glucose from amino acids) by acting on hepatocytes (53). In DM2 there is generally enough insulin to suppress ketogenesis; however, increasing insulin resistance impairs glucose uptake. Poorly managed type 2 diabetics therefore are likely to present develop hyperglycemia without ketoacids (53).

When frank DKA occurs in DM2, the physiologic picture changes. An acute shortage of insulin not only fails to suppress gluconeogenesis and glycogenolysis, but also tips the balance to increase glucagon secretion, further promoting the aforementioned glucose-producing mechanisms. As a result, the blood of a DKA patient becomes flooded with unwanted glucose, causing the symptoms mentioned previously.
DKA is a potentially life-threatening event that requires immediate medical attention. Treatment typically involves hydration and the re-establishment of metabolic equilibrium through electrolyte administration. Intensive insulin therapy is also required, but rendered more difficult with the insulin resistance seen in DM2. Typically, this is circumvented with concomitant treatment with OHAs (64).

Figure 8. Mechanisms of DKA. (Source: http://dtc.ucsf.edu)

1.3 Antipsychotic-Induced Weight Gain

It is well established that atypical antipsychotics cause weight gain, although liability varies between individual compounds (48). Furthermore, the clinical picture is
complicated by the high incidence of polypharmacy and previous antipsychotic treatment in the schizophrenia population (65). Generally speaking, patients begin gaining weight soon after treatment initiation and maintain the gain throughout their course of treatment (66-68). Henderson et al., (69) conducted a naturalistic study and found significant weight gain with clozapine administration during the first year of treatment; these patients continued to gain weight until the 46th month of follow-up. Interestingly, the patients in this study continued to gain weight despite diet and lifestyle interventions. Another study examined first episode, drug-naïve patients who were randomized to a clozapine, olanzapine, risperidone or sulpiride (i.e. another atypical antipsychotic drug) treatment group for an eight-week period (70). An increase in both body mass index (BMI) and waist-to-hip ration (WHR) was found in all groups, however the change was significant in the clozapine, olanzapine and sulpiride groups. Comparing clozapine (i.e. the antipsychotic with highest metabolic liability) and amisulpride (a very specific D2 dopamine antagonist), Rettenbacher et al. (71) found a significant increase in BMI in the clozapine group within the first four weeks of the study, and a further significant increase over four months, whereas no changes were noted in the amisulpride group. Defining significant weight gain as a ‘categorical increase of greater than 7% of a patient’s baseline weight’, McIntyre et al. (72) found that 55.6% of patients in their cohort met this criterion when treated with quetiapine, 24.1% with olanzapine and 23.7% with risperidone.

As noted, both the United States Food and Drug Association (FDA) and the Health Canada’s Therapeutic Product Division (TPD) acknowledged the mounting evidence linking atypical antipsychotics and weight gain by issuing a monograph warning on all
compounds belonging to this class of drugs. Not all atypical antipsychotics, however, carry the same level of risk for significant weight gain (see Figure 9); clozapine and olanzapine are considered high risk, risperidone and quetiapine, low to moderate risk, and ziprasidone and aripiprazole, minimal risk (49). Of note, it is not only atypical compounds that carry a risk of weight gain; it appears that first generation compounds also induce significant changes in weight, as illustrated by a comprehensive meta-analysis reviewing weight change over the course of 10 weeks of treatment with various antipsychotic drugs (48).

Figure 9. The propensity of conventional and atypical antipsychotics to induce changes in weight. (Source: Allison et al., 1999)

1.3.1 Atypical Antipsychotics and Glucose Dysregulation
The oral glucose tolerance test (OGTT) is a diagnostic tool used to screen for dysfunction in glucose metabolism. Briefly, patients are given a glucose tablet and blood samples are taken at regular intervals to assess the speed with which glucose is cleared from the blood (73). Several studies have used the OGTT to evaluate the diabetogenic effects of various antipsychotic compounds. In an open label study, Chae and Kang (74) used the OGTT to assess blood glucose levels during eight weeks of
treatment with clozapine or haloperidol in patients with psychotic disorders. Thirty-five percent of patients in the clozapine group were found to have impaired glucose tolerance (IGT) while none of the haloperidol treated patients showed this effect. Using a frequently sampled modified OGTT, Newcomer et al. (75) compared non-diabetic patients with schizophrenia treated with first- or second-generation (clozapine, olanzapine or risperidone) antipsychotics and healthy volunteers. Significant increases in fasting plasma glucose levels, and at 75 minutes post glucose load were found in the second-generation group when compared to both the first-generation and control subjects.

In many cases the impairment in glucose tolerance occurs early in treatment (76-79). In some cases hyperglycemia is coupled with beta cell dysfunction, resulting in diabetic ketoacidosis (i.e. acute absence of insulin and metabolic crisis), and requiring immediate hospitalization with intensive treatment (see Chapter 2 for review). A number of these cases involve patients who did not experience extensive weight gain or have a positive personal or family history of glucose tolerance abnormalities. Support of beta cell impairment with atypical antipsychotic was demonstrated in drug-naïve patients taking olanzapine or risperidone for a mere two weeks; these patients showed attenuated insulin secretion in response to glucose challenge using an intravenous glucose tolerance test (IVGTT) (80).

1.3.2 Atypical Antipsychotics and Insulin Resistance
As noted, abnormalities in plasma glucose clearance are related to changes in insulin sensitivity. Additionally, early insulin resistance is typically accompanied by beta cell compensation resulting in hyperinsulinemia. There is also evidence to suggest that
insulin resistance is caused by atypical antipsychotic use, however, paralleling the findings for weight gain (48,81), it appears different compounds have varying degrees of impact in this regard. Henderson et al. (82) examined several measures of glucose metabolism in patients treated with clozapine, olanzapine or risperidone; they found significant differences in insulin sensitivity (decrease), insulin resistance (increase) and glucose effectiveness (decrease) for both the clozapine and olanzapine groups, compared to risperidone. These findings are in contrast to a series of studies performed by Sowell and colleagues (83,84) investigating insulin secretion and sensitivity in healthy volunteers given olanzapine, risperidone or placebo over three weeks. Using a euglycemic-hyperinsulinemic clamp (see section 1.4.2), volunteers were found to have no change in insulin sensitivity. While subjects did experience significant weight gain in the olanzapine and risperidone treatment groups, application of a hyperglycemic (see section 1.4.3) clamp revealed no significant change in beta cell secretory capacity.

The aforementioned study by Henderson (82) also found elevated fasting insulin levels in their participants, as have other authors studying schizophrenia patients taking atypical antipsychotics such as clozapine, olanzapine and risperidone (70,85,86). In a study by Melkersson and Dahl (85), findings of increased insulin levels were complemented with increased C-peptide levels in 72% and 44% of patients treated with clozapine and olanzapine, respectively. Because insulin and C-peptide are co-secreted but eliminated via different mechanisms by the kidneys, c-peptide levels can be used to characterize the occurrence of hyperinsulinemia. In other words, hyperinsulinemia can be due to a frank increase in insulin secretion (i.e. concomitant with increased C-peptide levels), or due to a change in insulin metabolism (i.e. concomitant with comparatively
lower C-peptide levels). If the levels of insulin and C-peptide are concurrently elevated, this confirms a hyper-secretion of insulin by the pancreatic beta cells, and insulin resistance is present.

1.3.3 Atypical Antipsychotics and Type 2 Diabetes

The literature is now replete with case reports indicating the development of type 2 diabetes (DM2) with atypical antipsychotic use (49). In a naturalistic study following patients on clozapine, Henderson (69) found that 36.6% of the cohort received a diagnosis of DM2 over the five-year period of study. One large-scale study (38, 632 patients) evaluated outpatients with schizophrenia in the Veterans Health Administration of the Department of Veterans Affairs taking typical and atypical antipsychotics over a four-month period (87). Patients taking atypical antipsychotics were 9% more likely to have DM2, and the prevalence was significantly higher in patients taking clozapine, olanzapine and quetiapine, but not risperidone. A trend analysis by Basu and Meltzer (88) evaluating three time periods (i.e. before, shortly after and long term) surrounding the introduction of atypical antipsychotics revealed an association between the introduction of these compounds and the development of DM2. Data from the Centre for Addiction and Mental Health (CAMH) reveals the incidence of DM2 among chronic schizophrenia patients treated in this hospital to be 18%, three times that found in a control population (89).

1.3.4 Atypical Antipsychotics and the Metabolic Syndrome

All five metabolic syndrome criteria put forth by the National Cholesterol Education Program (see section 1.2.2) (90) are established cardiovascular risk factors; furthermore, the presence of multiple factors further increases risk of coronary heart
disease (CHD) (91). Using the NCEP criteria, Heiskanen et al. (92) found the frequency of metabolic syndrome to be 2-4 times higher in schizophrenia patients taking both first- and second-generation antipsychotics, compared to a matched control population. A staggering 42.6% of males and 48.5% of females were found to meet criteria for metabolic syndrome in a large Canadian study (89) while data from the CATIE trial found 40.9% and 51.6% for the males and females, respectively (67). These data are all the more disconcerting when contrasted with the prevalence of metabolic syndrome in the general population, i.e. 24% in males and 23% in females (93).

It would be misleading, however, to suggest that treatment with atypical antipsychotics is the primary reason for an increased association between schizophrenia and the metabolic syndrome. In fact, the link between diabetes and schizophrenia has existed for decades (94,95) and continues to be supported (96-100). Such is the strength of this association that the American Diabetes Association (ADA) now considers a diagnosis of schizophrenia per se as a risk factor for the development of diabetes (101). Increases in weight (68) and occurrences of diabetes have also been reported with first-generation compounds (76), however, a fact that must not be discounted.

Individuals with schizophrenia have increased morbidity and mortality compared to the general population (1). Independent of medication, this population is more likely to develop cardiovascular disease (CVD), diabetes, and hypertension (102). In fact, schizophrenia patients have a 20% reduction in lifespan (103,104), with CVD being the most common natural cause of death, implicated in 34% of male and 31% of female deaths, values significantly higher than that of the general population (52,81,102). While disturbing, these findings are contextualized by data illustrating the increased propensity
for poor lifestyle habits in schizophrenia patients, i.e. higher rates of smoking (105) and inactive lifestyle with poor diet (106-108). Sadly, this population has been shown to have a decreased access to medical care (109) and documented underutilization of primary care services (110).

While the focus here is on atypical antipsychotic use and metabolic side effects, it is important to remain cognizant of the context in which these effects occur, i.e. under the influence of both significant biological and environmental factors, each adding to the complexity of the clinical picture.

1.3.5 Atypical Antipsychotics and Metabolic Side Effects

While the link between atypical antipsychotics and metabolic side effects continues to be strengthened with additional case reports (111), the mechanisms behind these sequelae remain poorly understood. The leading theory, however, attributes glucose dysregulation to the significant weight gain incurred by taking these compounds.

Several lines of evidence support the link between obesity and the development of DM2; specifically, it is the increase in plasma free-fatty acid (FFA) concentration that has garnered the most attention. Increased weight results in adipocyte proliferation and the breakdown of triglycerides (TG) (i.e. storage form of excess lipids), causing the formation of glycerol and FFAs (112). It is well known that most obese people have increased plasma FFA levels (113,114) and acute elevations of FFA cause insulin resistance (115-118). Studies have shown that FFAs inhibit glucose uptake, glycogen synthesis and glycolysis equally and to the same degree (116). FFAs also cause hepatic insulin resistance by inhibiting the suppression of insulin-mediated glycogenolysis (119).
Artificially increasing serum FFA in healthy humans increases muscle fat content and is associated with an acute development of insulin resistance (120). While this does not demonstrate causality, it has been inferred that an insulin resistance signal is generated during either the synthesis or breakdown of TG, ultimately inhibiting the action of insulin via a cascade modulating effect on the insulin receptor itself or its substrate (121-123). Given these findings, it is not unreasonable to attribute the glucose dysregulation associated with atypical antipsychotic treatment to a downstream effect initiated with increased adiposity caused by these same agents.

Further work in this vein has suggested that insufficient beta cell insulin secretion in DM2 is a result of both functional impairment and loss of beta cell mass (124-128) due to apoptosis (124,129,130). The high content of saturated fats in Western diets exposes beta cells to chronic increases in plasma FFA levels (131), which have been shown to increase apoptosis (132-134) via the induction of endoplasmic reticulum stress (135). It has been documented that patients taking atypical antipsychotics crave unhealthy foods (136) and make poor dietary selections (107), leading to increased adiposity and thus leaving them susceptible to DM2. The loss of beta cells by apoptosis would therefore explain the progressive nature of insulin resistance and the eventual requirement of exogenous insulin therapy in DM2.

There exist other studies, however, that point to the possibility of another explanation for the link between atypical antipsychotics and diabetes, one independent of weight gain and adiposity. For example, non-diabetic schizophrenia patients taking clozapine or olanzapine showed significant fasting and post-challenge glucose levels, compared to patients taking conventional antipsychotics or healthy controls matched for adiposity...
In another study, non-obese patients taking clozapine, olanzapine and risperidone, matched for age and BMI, showed increased insulin resistance with glucose challenge.

The growing number of case reports of DKA with atypical antipsychotic use also points to a direct, weight-independent effect on glucose homeostasis (see Chapter 2 for review); this is especially relevant in cases occurring soon after treatment initiation and in the absence of notable changes in adiposity.

1.3.6 Summary

Atypical antipsychotics have been associated with a constellation of metabolic side effects including increased adiposity and type 2 diabetes. The mechanisms behind these phenomena remain elusive, and it is likely that factors outside drug treatment alone contribute to these sequelae (i.e. diagnosis of schizophrenia itself, poor diet and lifestyle habits). Weight gain with atypical antipsychotic use is now an acknowledged class effect, however, the link to diabetes remains debated. While many studies support the possibility of pathological adiposity causing diabetes, strong evidence for a direct, weight-independent does exist, meriting future investigation.

1.4 Pre-Clinical Models of Atypical Antipsychotic-Induced Weight Gain

Many attempts have been made to model the weight gain seen in humans with antipsychotic treatment using a rodent model. These studies have inconsistently mirrored human data, leaving this approach vulnerable to significant criticism. Certain studies have been able to demonstrate significant weight gain in female rats, while other only modestly so. With regards to male rats, studies showing increased
weight are virtually non-existent. One group looked at the effect of olanzapine (high metabolic risk) and risperidone (moderate metabolic risk) on adiposity in a canine model (143). Although using male dogs, results showed significant increases in adiposity (total, central and subcutaneous) with olanzapine treated animals compared to controls; this was not found in the risperidone group. In humans, both males and females readily experience significant weight gain while taking atypical antipsychotic agents, albeit to differing degrees (48,99).

1.4.1 Investigating Glucose Dysregulation

The hyperinsulinemic-euglycemic and hyperglycemic clamp procedures provide a model to assess insulin sensitivity and secretion and can be employed in rodents. They are considered the ‘gold standard’ for assessing insulin sensitivity and the secretory function of the pancreatic beta cells in vivo (144).

1.4.2 The Hyperinsulinemic-Euglycemic Clamp

The hyperinsulinemic-euglycemic clamp enables the determination of whole body insulin sensitivity. Subjects are infused with a fixed rate of insulin as well as a variable rate of exogenous glucose to counteract the insulin-induced decline in plasma glucose. In this technique the goal is to maintain (i.e. ‘clamp’) the subject’s plasma glucose at pre-determined euglycemic levels. Under pathological conditions such as insulin resistance, the rate of exogenously infused glucose is decreased, i.e. cellular response to insulin is compromised, resulting in attenuated glucose uptake. Plasma glucose would tend to stay elevated and subjects would therefore require less exogenous glucose to maintain euglycemia. Radioactive glucose can be used in a modified version
of this technique to determine the rate of glucose production and utilization, i.e. the locus of insulin resistance can be ascertained.

1.4.3 The Hyperglycemic Clamp
The hyperglycemic clamp technique provides an index of the secretory capacity of pancreatic beta cells, and can assess the sensitivity of peripheral tissues (145). Subjects are given an initial bolus of glucose to elevate plasma glucose rapidly, and then a fixed degree of hyperglycemia is maintained through infusion of exogenous glucose via a pump. Insulin and C-peptide levels are measured via regular sampling throughout the experiment. The hallmark of this technique is that beta cells of subjects are stimulated with the same concentration of glucose that facilitates the evaluation of in vivo beta cell response to glucose.

1.4.4 Pre-Clinical Models for Atypical Antipsychotic-Induced Glucose Dysregulation
The use of animal models to corroborate antipsychotic-induced weight gain has been largely inconsistent. Several important studies, however, have investigated the acute effects of these compounds on glucose metabolism, affirming the utility of this model in exploring the weight gain independent effects of these agents.

Assié et al. (146) showed that fasted rats, treated orally with clozapine and olanzapine displayed a significant, dose-dependent increase in plasma glucose concentration at one and two hours post drug administration. Haloperidol (i.e. a first-generation compound) and risperidone showed modest effects in this regard, while aripiprazole, bifeprunox and ziprasidone had little or no effect. Using a hyperinsulinemic-euglycemic clamp, Houseknecht et al., (147) found clozapine and olanzapine to acutely induce a
dose-dependent decrease in whole body insulin sensitivity, whereas ziprasidone and risperidone had no effect. These changes were attributed to increases in hepatic glucose production due to hepatic insulin resistance, as assessed with the use of radioactive tracers. The effect of clozapine and olanzapine on peripheral tissues (i.e. adipose and skeletal cells) was shown to be unaffected, thus re-affirming the locus of abnormality to the liver.

Boyda and colleagues (148) examined the effects of clozapine, olanzapine, risperidone and haloperidol on fasted rats that were given an intra-peritoneal glucose tolerance test. The atypical antipsychotics (i.e. clozapine, olanzapine and risperidone) all showed significant dose- and time-dependent effects on fasting plasma glucose and insulin concentrations, HOMA-IR values (i.e. a measure of fasting insulin resistance), insulin resistance and glucose tolerance. In a separate group of rats treated with olanzapine, a significant increase in plasma insulin concentration was found over the course of the glucose tolerance test. The effects of haloperidol on the aforementioned parameters were either attenuated or absent altogether.

Smith et al., (149) found increased plasma glucose and insulin levels in rats subjected to a glucose tolerance test one hour after acute treatment (i.e. single subcutaneous dose) with clozapine, quetiapine and haloperidol; these effects were also found in sub-chronic (i.e. 7 days) and chronic (i.e. 28 days) clozapine and quetiapine treatment groups. Similar to findings by Houseknecht et al., (147) no differences were found in insulin-stimulated glucose uptake in pre-incubated adipose or skeletal muscle cells. Findings of hyperinsulinemia, coupled with normal insulin-stimulated glucose uptake, prompted further investigation into the questioned presence of insulin resistance.
Findings of elevated biological markers (phosphorylated Akt/PKB), reflecting insulin action in the liver, concurrent with increases in plasma glucose levels with clozapine administration, indicated an absence of acute insulin resistance (149). It was later shown through pyruvate and glycerol (i.e. major substrates of gluconeogenesis) tolerance tests that clozapine, quetiapine and haloperidol acutely promote hepatic glucose production, an effect demonstrated as being due to increases in glucagon secretion.

In a series of experiments conducted by Chintoh et al., (150, 151), the effects of clozapine, olanzapine, risperidone, ziprasidone and haloperidol were investigated using both hyperinsulinemic-euglycemic and hyperglycemic clamps techniques. Clozapine and olanzapine were found to induce significant hepatic insulin resistance and reduced glucose utilization (i.e. reduced glucose uptake into peripheral tissues). This is in contrast to findings by Smith et al., (149) and Houseknecht et al., (147) who reported, using cultured adipocytes and skeletal muscle cells, a minimal effect for clozapine in this regard. Both clozapine and olanzapine were noted to induce a significant impairment in insulin secretion in response to hyperglycemia. Of note, ziprasidone (i.e. the atypical antipsychotic with least metabolic liability) was noted to cause decreased gluconeogenesis (i.e. compared to vehicle), and risperidone, while having no effect on glucose production, showed a significant decrease in glucose utilization.

Generally speaking, the metabolic liability seen with acute treatment of atypical antipsychotics mirrors that found for weight gain (48). In other words, clozapine and olanzapine, atypical with the greatest liability for weight gain, consistently induce acute
changes in glucose metabolism, whereas other agents of this class vary in the severity of their effects.

Whether or not the same mechanisms involved in weight gain underlie the acute effects outlined here remains to be seen. Atypical compounds bind various neurotransmitter systems involved in weight gain and metabolism, and to differing degrees. These include, but are not limited to, dopamine, serotonin, histamine, acetylcholine and noradrenergic (152,153).

1.4.5 Summary
The use of a rodent model to investigate atypical antipsychotic-induced weight gain has been criticized due to the difficulty in reliably replicating findings from clinical populations. Several studies, however, have proven that this model can be used to evaluate the acute effects of atypical antipsychotics on glucose metabolism, independent of weight gain. The ability to study the effects of these compounds without the added confound of increased adiposity is clinically relevant, as many patients experience deleterious metabolic changes without alterations in weight. Further work is needed to deconstruct the pleiotropic binding profile of these compounds as it relates to acute dysregulation of glucose metabolism, which is the focus of the work presented here.

1.5 Research Aims and Hypothesis
1.5.1 Research Aims
The aim of the present study was to deconstruct the pharmacological binding profile of atypical antipsychotics within the context of acute model. To achieve this, we used a
single dose of selective receptor antagonists, assessing their *in vivo* effects on the secretory capacity of pancreatic beta cells using the hyperglycemic clamp, which allows the estimation of peripheral tissue insulin sensitivity (145).

### 1.5.2 Research Hypothesis

Within the context of a rodent (rat) model, we set out to address the following hypothesis:

- Selective antagonists of different receptors produce varied effects on insulin secretion after acute treatment, as measured using the hyperglycemic clamp procedure.
Chapter 2

Atypical Antipsychotics and Diabetic Ketoacidosis
2 Atypical Antipsychotics and Diabetic Ketoacidosis

2.1 Introduction

Atypical antipsychotics, with clozapine the prototype, now represent the treatment of choice in psychotic conditions such as schizophrenia (154-156). While their benefits have been challenged more recently (35,157), early evidence of clinical superiority, in combination with reports of improved tolerability (158,159), led these newer agents to rapidly supplant their conventional counterparts in clinical use. Clozapine specifically garners a unique position in treatment algorithms, as it stands alone as the antipsychotic of choice in refractory schizophrenia (160,161).

The newer antipsychotics are not without side effects, however, and it has been weight gain and related metabolic sequelae that have drawn the greatest attention and concern. Despite differences between agents (162,163), this liability has been identified as a class effect and related warnings are now embedded in product monographs (164). The risk and magnitude of weight gain associated with these drugs has, in turn, provided a strong rationale for the increased risk of type 2 diabetes, dyslipidemia, and metabolic syndrome also associated with the use of these medications (163).

Reports of diabetic ketoacidosis (DKA), albeit uncommon (165), argue against the position that glucose dysregulation associated with atypical antipsychotic use is related to weight gain alone. While excessive adiposity represents a significant risk factor for type 2 diabetes (166), this is not the case with DKA, as it is linked to type 1 diabetes and/or physical illness (64,167,168). That it has been noted in conjunction with atypical antipsychotic use has important implications, both clinically and mechanistically. For
example, DKA has been reported soon after the initiation of atypical antipsychotic treatment and in individuals who experience no significant change in weight (169), emphasizing that weight gain cannot be used as the sole proxy for concerns regarding possible glucose abnormalities. In terms of mechanism(s) of action, DKA also raises issues as to whether these agents impact insulin and glucose metabolism via two distinct mechanisms (i.e., one through antipsychotic-induced weight gain and one that is independent and more acute in nature).

Despite its high risk of mortality (167), very little attention has been given to DKA linked to atypical antipsychotic use. A previous review examining new onset diabetes associated with atypical antipsychotic use included 35 cases involving DKA; however, the time range spanned only 1966-2001 (170).

2.1.1 Aims of Study
The present investigation represents an update specific to DKA, with the aims of a) providing a summary that could shed light on the current scope of the problem, b) better understanding its presentation in the context of existing antipsychotic treatments, c) examining the possible role of established risk factors (e.g., infection), d) reviewing outcome, and e) commenting on mechanisms of action.

2.2 Materials and Methods
This review focused on reports of DKA in association with the atypical antipsychotics available for use in North America at the time the review was initiated, namely aripiprazole, clozapine, olanzapine, quetiapine, risperidone and ziprasidone. A Medline search was conducted via PubMed (http://www.ncbi.nlm.nih.gov/PubMed) using the
following: ‘neuroleptic’ or ‘antipsychotic’ in combination with ‘ketoacidosis’; individual antipsychotic names (aripiprazole, clozapine, olanzapine, quetiapine, risperidone, and ziprasidone) were also cross-referenced with ‘ketoacidosis’. The same search terms were used in the University of Toronto’s Scholar’s Portal Search in order to capture any missed reports (see Fig. 1). All relevant cases, where an abstract and/or text were reported in English, until March of 2011 were analyzed.

*Figure 10. Breakdown of literature search to capture cases of diabetic ketoacidosis with atypical antipsychotic treatment.*

2.3 Results

The search yielded 60 reports, with a total of 69 cases, and demographic details for each are detailed in Table 2 (below).

*Table 2 (below): Diabetic Ketoacidosis with Atypical Antipsychotic Use*
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Church et al., 2005) (171)</td>
<td>F</td>
<td>34</td>
<td>AA</td>
<td>S</td>
<td>A</td>
<td>30</td>
<td>N</td>
<td>N/S</td>
<td>Y</td>
<td>N/S</td>
<td>4 d</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>(Reddy et al., 2006) (172)</td>
<td>M</td>
<td>33</td>
<td>AA</td>
<td>S</td>
<td>A</td>
<td>N/S</td>
<td>N/S</td>
<td>Y</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>18 m</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>INS</td>
</tr>
<tr>
<td>(Dharni &amp; Verma, 2008) (173)</td>
<td>M</td>
<td>12</td>
<td>C</td>
<td>O</td>
<td>A</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>6 m</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>None</td>
</tr>
<tr>
<td>(Makhloufi et al., 2008) (174)</td>
<td>M</td>
<td>44</td>
<td>AA</td>
<td>SD</td>
<td>A</td>
<td>15, 30</td>
<td>N</td>
<td>Y</td>
<td>DEC</td>
<td>N</td>
<td>N</td>
<td>17 d</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>INS, OHA</td>
</tr>
<tr>
<td>(Babu et al., 2005) (175)</td>
<td>F</td>
<td>15</td>
<td>N/S</td>
<td>BD</td>
<td>A</td>
<td>N/S</td>
<td>Y</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>4 m</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>(Kibbey et al., 2010) (176)</td>
<td>M</td>
<td>29</td>
<td>Filipino</td>
<td>S</td>
<td>A</td>
<td>40</td>
<td>N/S</td>
<td>N/S</td>
<td>INC</td>
<td>N/S</td>
<td>N</td>
<td>12 m</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
</tr>
<tr>
<td>(Wilson et al., 2003) (177)</td>
<td>M</td>
<td>33</td>
<td>AA</td>
<td>SD</td>
<td>C</td>
<td>550</td>
<td>N</td>
<td>N/S</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>1 m</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
</tr>
<tr>
<td>(Lafayette et al., 2003) (178)</td>
<td>F</td>
<td>22</td>
<td>Hispanic</td>
<td>I</td>
<td>talian</td>
<td>S</td>
<td>C</td>
<td>150</td>
<td>Y</td>
<td>DEC</td>
<td>Y</td>
<td>10 w</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Diet</td>
</tr>
<tr>
<td>(Cho &amp; Lindenmayer, 2009) (179)</td>
<td>F</td>
<td>45</td>
<td>AA</td>
<td>S</td>
<td>C</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>N/S</td>
<td>N/S</td>
<td>2 m</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N/S</td>
<td></td>
</tr>
<tr>
<td>(Reis et al., 2007) (180)</td>
<td>M</td>
<td>28</td>
<td>Hispanic</td>
<td>S</td>
<td>C</td>
<td>150</td>
<td>N</td>
<td>N/S</td>
<td>DEC</td>
<td>N/S</td>
<td>N</td>
<td>1 m</td>
<td>Y</td>
<td>N</td>
<td>N/S</td>
<td>Diet</td>
</tr>
<tr>
<td>(Kristensen et al., 2003) (181)</td>
<td>F</td>
<td>54</td>
<td>C</td>
<td>S</td>
<td>C</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/A</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td></td>
</tr>
<tr>
<td>(Koval et al., 1994) (182)</td>
<td>F</td>
<td>34</td>
<td>AA</td>
<td>S</td>
<td>C</td>
<td>250</td>
<td>Y</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>6 w</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>(Koutkoglu et al., 1996) (183)</td>
<td>M</td>
<td>42</td>
<td>N/S</td>
<td>O</td>
<td>C</td>
<td>350</td>
<td>N</td>
<td>Y</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>4-5 w</td>
<td>N/S</td>
<td>Y</td>
<td>N</td>
<td>Diet</td>
</tr>
<tr>
<td>(Peterson &amp; Byrd, 1996) (184)</td>
<td>M</td>
<td>46</td>
<td>AA</td>
<td>S</td>
<td>C</td>
<td>500</td>
<td>N</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>5 w</td>
<td>Y</td>
<td>N</td>
<td>N/S</td>
<td>INS</td>
</tr>
<tr>
<td>(Pierides et al., 1997) (185)</td>
<td>M</td>
<td>50</td>
<td>N/S</td>
<td>S</td>
<td>C</td>
<td>300</td>
<td>N</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>6 d</td>
<td>N</td>
<td>N</td>
<td>N/S</td>
<td>N/S</td>
<td></td>
</tr>
<tr>
<td>(Ai et al., 1998) (186)</td>
<td>M</td>
<td>30</td>
<td>AC</td>
<td>S</td>
<td>C</td>
<td>300</td>
<td>N</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>5 m</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>OHA</td>
<td></td>
</tr>
<tr>
<td>(Wirshing et al., 1998) (187)</td>
<td>M</td>
<td>32</td>
<td>AA</td>
<td>SD</td>
<td>C</td>
<td>400</td>
<td>N/S</td>
<td>N</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>18 m</td>
<td>N/S</td>
<td>Y</td>
<td>N</td>
<td>OHA</td>
</tr>
<tr>
<td>(Colli et al., 1999) (188)</td>
<td>M</td>
<td>31</td>
<td>C</td>
<td>SD</td>
<td>C</td>
<td>200</td>
<td>N</td>
<td>Y</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>3 m</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>(Mohan et al., 1999) (189)</td>
<td>M</td>
<td>30</td>
<td>AA</td>
<td>S</td>
<td>C</td>
<td>325</td>
<td>N</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>3 m</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>OHA</td>
<td></td>
</tr>
<tr>
<td>(Smith et al., 1999) (190)</td>
<td>M</td>
<td>40</td>
<td>AC</td>
<td>S</td>
<td>C</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>N/S</td>
<td>N</td>
<td>16 d</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>(Avram et al., 2001) (191)</td>
<td>M</td>
<td>33</td>
<td>C</td>
<td>S</td>
<td>C</td>
<td>100</td>
<td>N</td>
<td>Y</td>
<td>INC/DEC</td>
<td>N</td>
<td>N</td>
<td>8 m</td>
<td>Y</td>
<td>N</td>
<td>N/S</td>
<td>None</td>
</tr>
<tr>
<td>(Nicola et al., 2001) (192)</td>
<td>M</td>
<td>33</td>
<td>Indian</td>
<td>S</td>
<td>C</td>
<td>450</td>
<td>Y</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>4 y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>(Rigalleau et al., 2000) (193)</td>
<td>M</td>
<td>38</td>
<td>C</td>
<td>S</td>
<td>C</td>
<td>N/S</td>
<td>N/S</td>
<td>Y</td>
<td>DEC</td>
<td>N/S</td>
<td>N</td>
<td>6 m</td>
<td>N/S</td>
<td>N/S</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(Maule et al., 1999) (194)</td>
<td>F</td>
<td>50</td>
<td>C</td>
<td>N/S</td>
<td>C</td>
<td>400</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>1 m</td>
<td>N/S</td>
<td>Y</td>
<td>N</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Gender</td>
<td>Ethnicity</td>
<td>Methodology</td>
<td>Follow-Up</td>
<td>Inc</td>
<td>Inc/Dec</td>
<td>N</td>
<td>N/S</td>
<td>10m</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------</td>
<td>--------</td>
<td>-----------</td>
<td>-------------</td>
<td>------------</td>
<td>-----</td>
<td>---------</td>
<td>---</td>
<td>-----</td>
<td>-----</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>-----</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Wilson et al., 2003)</td>
<td>1995</td>
<td>M</td>
<td>AA</td>
<td>OL</td>
<td>30</td>
<td>N</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>10m</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Torrey &amp; Swalwell, 2001)</td>
<td>2001</td>
<td>M</td>
<td>AA</td>
<td>BD</td>
<td>30</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N/S</td>
<td>10m</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Tavakoli et al., 2003)</td>
<td>1996</td>
<td>M</td>
<td>C</td>
<td>BD</td>
<td>5</td>
<td>N</td>
<td>Y</td>
<td>INC</td>
<td>N</td>
<td>Y</td>
<td>18m</td>
<td>Y</td>
<td>N</td>
<td>N/S</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>(Hoves &amp; Rifkin, 2004)</td>
<td>2004</td>
<td>F</td>
<td>N/S</td>
<td>SD</td>
<td>20</td>
<td>N</td>
<td>Y</td>
<td>INC</td>
<td>N</td>
<td>Y</td>
<td>3.5m</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>(Avella et al., 2004)</td>
<td>2004</td>
<td>M</td>
<td>N/S</td>
<td>BD</td>
<td>15</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>3m</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Avella et al., 2004)</td>
<td>2004</td>
<td>M</td>
<td>N/S</td>
<td>BD</td>
<td>15</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>3m</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Avella et al., 2004)</td>
<td>2004</td>
<td>M</td>
<td>N/S</td>
<td>S</td>
<td>10</td>
<td>N</td>
<td>Y</td>
<td>DEC</td>
<td>N/S</td>
<td>Y</td>
<td>1m</td>
<td>N/S</td>
<td>N</td>
<td>N</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(Kyrizas et al., 2006)</td>
<td>2006</td>
<td>M</td>
<td>C</td>
<td>O</td>
<td>20</td>
<td>Y</td>
<td>Y</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>4m</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Diet</td>
<td></td>
</tr>
<tr>
<td>(Kahn &amp; Bourgeois, 2007)</td>
<td>2007</td>
<td>M</td>
<td>AA</td>
<td>S</td>
<td>30</td>
<td>Y</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>Y</td>
<td>5y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(Varma et al., 2007)</td>
<td>2007</td>
<td>F</td>
<td>N/S</td>
<td>BD</td>
<td>10</td>
<td>Y</td>
<td>Y</td>
<td>N/S</td>
<td>N</td>
<td>N</td>
<td>6w</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>(Wong et al., 2007)</td>
<td>2007</td>
<td>M</td>
<td>Chinese</td>
<td>S</td>
<td>10</td>
<td>N</td>
<td>N</td>
<td>INC/DEC</td>
<td>N</td>
<td>N</td>
<td>39m</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>(Niazry et al., 2009)</td>
<td>2009</td>
<td>M</td>
<td>Kuwaiti</td>
<td>S</td>
<td>10</td>
<td>N</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>18m</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Waldman &amp; Yaren, 2002)</td>
<td>2002</td>
<td>M</td>
<td>Aboriginal</td>
<td>S</td>
<td>30</td>
<td>N</td>
<td>N</td>
<td>INC</td>
<td>N/S</td>
<td>3m</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>OHA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Tsolaki et al., 2002)</td>
<td>2002</td>
<td>F</td>
<td>Greek</td>
<td>O</td>
<td>10</td>
<td>N</td>
<td>Y</td>
<td>DEC</td>
<td>N</td>
<td>N</td>
<td>3m</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(Saeverud et al., 2010)</td>
<td>2010</td>
<td>M</td>
<td>N/S</td>
<td>N/S</td>
<td>10</td>
<td>N</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>6m</td>
<td>N/S</td>
<td>N</td>
<td>N</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Fulbright et al., 2006)</td>
<td>2006</td>
<td>M</td>
<td>AA</td>
<td>S</td>
<td>40</td>
<td>N</td>
<td>Y</td>
<td>DEC</td>
<td>N</td>
<td>N</td>
<td>40d</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(Rigalleau et al., 2000)</td>
<td>2000</td>
<td>M</td>
<td>C</td>
<td>O</td>
<td>10</td>
<td>N</td>
<td>N/S</td>
<td>Y</td>
<td>DEC</td>
<td>N/S</td>
<td>3m</td>
<td>N/S</td>
<td>N/S</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Gatta et al., 1999)</td>
<td>1999</td>
<td>M</td>
<td>C</td>
<td>S</td>
<td>10</td>
<td>N</td>
<td>Y</td>
<td>DEC</td>
<td>N</td>
<td>N</td>
<td>3m</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Diet</td>
<td></td>
</tr>
<tr>
<td>(Goldstein et al., 1999)</td>
<td>1999</td>
<td>F</td>
<td>C</td>
<td>SD</td>
<td>10</td>
<td>N</td>
<td>N</td>
<td>INC</td>
<td>N</td>
<td>Y</td>
<td>6m</td>
<td>N/S</td>
<td>N</td>
<td>Y</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>(Goldstein et al., 1999)</td>
<td>1999</td>
<td>F</td>
<td>C</td>
<td>S</td>
<td>10</td>
<td>N</td>
<td>Y</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>17m</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(Lindenmayer &amp; Patel, 1999)</td>
<td>1999</td>
<td>M</td>
<td>AA</td>
<td>S</td>
<td>30</td>
<td>N</td>
<td>Y</td>
<td>INC/DEC</td>
<td>N</td>
<td>N</td>
<td>8m</td>
<td>Y</td>
<td>N</td>
<td>N/S</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(Muench &amp; Carey, 2001)</td>
<td>2001</td>
<td>M</td>
<td>C</td>
<td>S</td>
<td>20</td>
<td>N</td>
<td>Y</td>
<td>INC</td>
<td>Y</td>
<td>Y</td>
<td>12m</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>(Ragucci &amp; Wells, 2001)</td>
<td>2001</td>
<td>F</td>
<td>AA</td>
<td>BD</td>
<td>15</td>
<td>N</td>
<td>Y</td>
<td>INC</td>
<td>N</td>
<td>Y</td>
<td>14m</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>INS, OHA</td>
<td></td>
</tr>
<tr>
<td>Study (Year)</td>
<td>Type</td>
<td>Gender</td>
<td>Ethnicity</td>
<td>Sex</td>
<td>AAP</td>
<td>Dx</td>
<td>Hx Hgy</td>
<td>Wt change</td>
<td>OV/OB</td>
<td>Age (y)</td>
<td>Rx</td>
<td>Duration</td>
<td>tx</td>
<td>Wt change</td>
<td>Wt change</td>
<td>Nutritional Interventions</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>------</td>
<td>--------</td>
<td>-----------</td>
<td>-----</td>
<td>-----</td>
<td>----</td>
<td>--------</td>
<td>-----------</td>
<td>-------</td>
<td>---------</td>
<td>----</td>
<td>----------</td>
<td>----</td>
<td>-----------</td>
<td>-----------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Neuburg et al., 2001 (214)</td>
<td>M</td>
<td>27</td>
<td>AA</td>
<td>S</td>
<td>OL</td>
<td>10</td>
<td>N</td>
<td>N/S</td>
<td>DEC</td>
<td>N</td>
<td>N/S</td>
<td>29 m</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>OHA</td>
</tr>
<tr>
<td>Selva &amp; Scott, 2001 (43)</td>
<td>F</td>
<td>16</td>
<td>Hispanic</td>
<td>O</td>
<td>OL</td>
<td>10/15</td>
<td>N/S</td>
<td>INC</td>
<td>N</td>
<td>Y</td>
<td>6+ m</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>None</td>
</tr>
<tr>
<td>Johnson et al., 2002 (215)</td>
<td>M</td>
<td>49</td>
<td>C</td>
<td>S</td>
<td>OL</td>
<td>20</td>
<td>N</td>
<td>Y</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>11 m</td>
<td>N/S</td>
<td>Y</td>
<td>N</td>
<td>None</td>
</tr>
<tr>
<td>Straker et al., 2002 (216)</td>
<td>F</td>
<td>44</td>
<td>AA</td>
<td>S</td>
<td>OL</td>
<td>25</td>
<td>N/S</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>7 w</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Diet</td>
<td>INS</td>
</tr>
<tr>
<td>Wilson et al., 2003 (195)</td>
<td>M</td>
<td>64</td>
<td>C</td>
<td>S</td>
<td>Q</td>
<td>400</td>
<td>N</td>
<td>Y</td>
<td>INC</td>
<td>N/S</td>
<td>Y</td>
<td>2 m</td>
<td>N</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Dibben et al., 2005 (217)</td>
<td>F</td>
<td>51</td>
<td>C</td>
<td>S</td>
<td>Q</td>
<td>400</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>2 y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>None</td>
</tr>
<tr>
<td>Macfarlane &amp; Fisher, 2006 (218)</td>
<td>M</td>
<td>33</td>
<td>N/S</td>
<td>S</td>
<td>Q</td>
<td>600</td>
<td>N</td>
<td>N/S</td>
<td>DEC</td>
<td>N</td>
<td>N</td>
<td>4 w</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>INS</td>
</tr>
<tr>
<td>Marlowe et al., 2007 (219)</td>
<td>M</td>
<td>45</td>
<td>N/S</td>
<td>S</td>
<td>Q</td>
<td>800</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N/S</td>
<td>N/S</td>
<td>5 m</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>None</td>
</tr>
<tr>
<td>Sirois, 2008 (220)</td>
<td>F</td>
<td>41</td>
<td>AA</td>
<td>O</td>
<td>Q</td>
<td>400</td>
<td>N</td>
<td>Y</td>
<td>N/S</td>
<td>N</td>
<td>N</td>
<td>37 d</td>
<td>Y</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>Rashid et al., 2009 (221)</td>
<td>F</td>
<td>30</td>
<td>Bengali</td>
<td>S</td>
<td>Q</td>
<td>200</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>2 m</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>Takahashi et al., 2005 (222)</td>
<td>M</td>
<td>72</td>
<td>Asian</td>
<td>O</td>
<td>Q</td>
<td>50</td>
<td>Y</td>
<td>N</td>
<td>N/S</td>
<td>N</td>
<td>N</td>
<td>14 d</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>None</td>
</tr>
<tr>
<td>Wilson et al., 2003 (195)</td>
<td>F</td>
<td>26</td>
<td>AA</td>
<td>SD</td>
<td>R</td>
<td>3</td>
<td>N</td>
<td>Y</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>7 w</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Diet</td>
</tr>
<tr>
<td>Dibben et al., 2005 (217)</td>
<td>M</td>
<td>33</td>
<td>Chinese</td>
<td>S</td>
<td>R</td>
<td>depot</td>
<td>N/S</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N/S</td>
<td>8 m</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td></td>
</tr>
<tr>
<td>Mithat et al., 2005 (223)</td>
<td>M</td>
<td>37</td>
<td>N/S</td>
<td>BD</td>
<td>R</td>
<td>2-4</td>
<td>Y</td>
<td>Y</td>
<td>INC</td>
<td>N</td>
<td>N</td>
<td>6 m</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>None</td>
</tr>
<tr>
<td>Hamanaka &amp; Kamijo, 2007 (224)</td>
<td>M</td>
<td>32</td>
<td>Japanese</td>
<td>S</td>
<td>R</td>
<td>2-4</td>
<td>N/S</td>
<td>N/S</td>
<td>INC</td>
<td>N</td>
<td>Y</td>
<td>3 y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Diet</td>
</tr>
<tr>
<td>Sato et al., 2008 (225)</td>
<td>F</td>
<td>46</td>
<td>Japanese</td>
<td>S</td>
<td>R</td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>4 m</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>INS</td>
</tr>
<tr>
<td>Lu &amp; Yan, 2009 (47)</td>
<td>M</td>
<td>27</td>
<td>N/S</td>
<td>S</td>
<td>R</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>2 m</td>
<td>N/S</td>
<td>Death</td>
<td>Death</td>
<td>Death</td>
</tr>
<tr>
<td>Chellamuth &amp; Gregory, 2010 (226)</td>
<td>M</td>
<td>42</td>
<td>South East Asian</td>
<td>S</td>
<td>R</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>N/S</td>
<td>N/Y</td>
<td>INS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Croarkin et al., 2000 (227)</td>
<td>M</td>
<td>42</td>
<td>C</td>
<td>O</td>
<td>R</td>
<td>4</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>N/S</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>INS</td>
</tr>
<tr>
<td>Ananth et al., 2004 (228)</td>
<td>M</td>
<td>46</td>
<td>N/S</td>
<td>BD</td>
<td>R</td>
<td>3</td>
<td>N/S</td>
<td>N/S</td>
<td>N/S</td>
<td>N</td>
<td>N/S</td>
<td>2 y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>None</td>
</tr>
</tbody>
</table>

**LEGEND FOR TABLE 2**

Y: Yes, N: No, N/S: Not stated

**Sex:** M: Male, F: Female

**Ethnicity:** C: Caucasian, AA: African-American, AC: Afro-Caribbean

**Dx** (diagnosis): BD: Bipolar Disorder, S: Schizophrenia, SD: Schizoaffective Disorder, O: Other

**AAP** (atypical antipsychotic): A: Aripiprazole, C: Clozapine, OL: Olanzapine, Q: Quetiapine, R: Risperidone

**OV/OB:** was the patient overweight or obese prior to initiating drug treatment?

**Wt change:** did the patient experience change in weight while on drug? INC: increase, DEC: decrease

**Hx Hgy:** did the patient have a history of hyperglycemia. FhX DM: did the patient have a family history of diabetes mellitus?

**Tx time:** what was the length of treatment before onset of diabetic ketoacidosis (d=days, m=months, w=weeks and y=years)

**PolyTx:** was there multiple treatment for diabetes required? INS: insulin, OHA: oral hypoglycemic agents
2.3.1 Incidence

This represents an important statistic for clinicians, although not one that is readily calculated by the present figure, as reports are not confined to a single region or country where estimates can be established based on data capturing antipsychotic use. Clearly, DKA is rare given the widespread use of antipsychotics; calculations specific to schizophrenia over a 7-year interval indicate the following risk: 0.2% (risperidone), 0.8% (olanzapine), and 2.2% (clozapine) (229). That said, the incidence of diabetes presenting as DKA in schizophrenia has been calculated as 14.93 per 10,000 patient years, 10-fold higher than the calculated risk of 1.4 per 10,000 years in the general population (229). In a 1-year follow-up of 56,849 patients with schizophrenia receiving antipsychotic monotherapy and without a history of diabetes, 0.2% were hospitalized with DKA (165). In individuals on atypical antipsychotics where diabetes is identified, the development of DKA is not uncommon; one study reported DKA developing in 5 of 11 such individuals (230).

Diagnosis was schizophrenia or schizoaffective disorder in approximately 70% of the reported cases here.

2.3.2 Antipsychotic, Dose, and Duration

Notably, the greatest number of DKA cases has been reported with the two antipsychotics also associated with the highest liability for weight gain (i.e., clozapine, olanzapine) (see Table 2). It must be taken into consideration that we do not know the actual proportion of use of each antipsychotic; that said, it warrants comment that the number reported with clozapine is so high, despite evidence that it is used in a relatively small per cent of patients (231-233). Only one atypical agent, ziprasidone, had no
associated reports of DKA, although there is one confirmed case involving severe hyperglycemia with this compound (234). Ziprasidone is an atypical antipsychotic that is considered more ‘weight neutral’; however, this is also true for aripiprazole (235), although it has been linked to six cases of DKA. The mean dose for each of the antipsychotics did not exceed recommended therapeutic ranges, with the exception of aripiprazole in one case. The mean duration of antipsychotic treatment prior to DKA, where clearly established (N=65), was just over nine months (range 4 days-4 years).

Table 3. Demographic summary of diabetic ketoacidosis cases with atypical antipsychotic treatment.

<table>
<thead>
<tr>
<th>AAP</th>
<th>N(DKA)</th>
<th>N(SCZ)</th>
<th>M:F</th>
<th>Age (avg, yrs)</th>
<th>Time to DKA (avg, mos)</th>
<th>Dose Range (mg)</th>
<th>Age Range</th>
<th>N (all AAPs)</th>
<th>% (all AAPs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aripiprazole</td>
<td>6</td>
<td>3</td>
<td>4:2</td>
<td>27.8</td>
<td>6.8</td>
<td>15-40</td>
<td>under 20</td>
<td>3</td>
<td>4.3</td>
</tr>
<tr>
<td>Clozapine</td>
<td>18</td>
<td>13</td>
<td>13:5</td>
<td>36.5</td>
<td>6.1</td>
<td>100-550</td>
<td>20-29</td>
<td>11</td>
<td>15.9</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>29</td>
<td>15</td>
<td>19:10</td>
<td>37.8</td>
<td>11.8</td>
<td>5-40</td>
<td>30-39</td>
<td>25</td>
<td>36.2</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>7</td>
<td>5</td>
<td>4:3</td>
<td>48.0</td>
<td>5.1</td>
<td>50-800</td>
<td>40-49</td>
<td>22</td>
<td>31.9</td>
</tr>
<tr>
<td>Risperidone</td>
<td>9</td>
<td>5</td>
<td>7:2</td>
<td>36.8</td>
<td>11.8</td>
<td>2-4</td>
<td>over 50</td>
<td>8</td>
<td>11.6</td>
</tr>
</tbody>
</table>

AAP: atypical antipsychotic; N(DKA): number of diabetic ketoacidosis cases; N(SCZ): number of schizophrenia cases; M:F: male to female ratio

2.3.3 Age, Gender and Ethno-cultural Background

The calculated average age was 37.5 years (range 12-80), and the largest percentage of cases occurred in people aged 30-39 (Table 2). Approximately 70% of cases were in the age range 30-49, while a further 20% of cases occurred in individuals under the age of 29. The preponderance of cases were male (47/69, 68.1%) (Table 2), and where
reported (N=56) 41% of individuals were of African-American or African-Caribbean descent, with a further 30% occurring in Caucasians.

2.3.4 Other Factors
In 39.0% of cases where information was provided (16/41), antipsychotic use was associated with either no weight gain or weight loss. Despite infection being the most common precipitating cause for DKA, occurring in 30-50% of cases (236), it was identified in only two of the cases reported here.

2.3.5 Outcome
Effective treatment of acute DKA does not equate with resolution of the underlying metabolic disturbance. A total of 50 cases provided follow-up in individuals where no previous personal history of glucose dysregulation was recorded prior to the occurrence of DKA. Eighteen (36%) did not require any further intervention, while seven (14%) were controlled with diet alone; only nine of this subgroup (18%) were continued on the antipsychotic administered in association with the DKA. Of these individuals, six (66.7%) required ongoing pharmacological treatment, two (22.2%) with an oral hyperglycemic agent and four (44.4%) with insulin therapy. Five deaths (7.25% of all reported cases) underscore the life-threatening potential of DKA.
Table 4. Post-Diabetic Ketoacidosis Antipsychotic Treatment

<table>
<thead>
<tr>
<th>Outcome/Drug</th>
<th>Aripiprazole</th>
<th>Clozapine</th>
<th>Olanzapine</th>
<th>Quetiapine</th>
<th>Risperidone</th>
</tr>
</thead>
<tbody>
<tr>
<td>DKA associated drug cont’d.</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>OHA/Insulin req’d long term.</td>
<td>1/1</td>
<td>2/3</td>
<td>4/5</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Drug discont’d w/o new compound.</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>OHA/Insulin req’d long term.</td>
<td>2/3</td>
<td>1/5</td>
<td>1/8</td>
<td>0/2</td>
<td>0/4</td>
</tr>
<tr>
<td>Switch to new antipsychotic.</td>
<td>2</td>
<td>7</td>
<td>11</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>OHA/Insulin req’d long term.</td>
<td>2/2</td>
<td>1/6</td>
<td>5/11</td>
<td>0/2</td>
<td>3/3</td>
</tr>
<tr>
<td>Fatalities.</td>
<td>None</td>
<td>None</td>
<td>4</td>
<td>None</td>
<td>1</td>
</tr>
</tbody>
</table>

2.4 Discussion

The relationship between atypical antipsychotics, weight gain, and metabolic disturbances has received a great deal of focus in the last decade (48,49,166,235,237-239), understandable given that weight gain with a drug like olanzapine is as high as 30kg over one year (68), while the prevalence of type 2 diabetes in schizophrenia is twofold greater when compared to the general population (68,96). Although altering actual practice patterns (i.e., regular metabolic monitoring) has proven a challenge (240,241), it remains that numerous guidelines are in place to assist clinicians in monitoring patients on these medications (101,102,164).

In contrast, considerably less attention has been given to DKA, although this is not surprising; even assuming underreporting, the risk of DKA is quite rare (165). However, its acuity and potential lethality argue for clinician awareness, as well as vigilance regarding its possible occurrence.
2.4.1 Clinical Implications

For psychiatrists, what is paramount is early identification of DKA to ensure appropriate treatment is initiated as quickly as possible. First and foremost in this process is recognizing that DKA can occur at any time following the onset of antipsychotic treatment, and independent of weight gain. To this point, in a review of 45 cases of new-onset diabetes and DKA following initiation of atypical antipsychotics, 42% presented as DKA (169). Of the reported cases here with the necessary information, almost one-third recorded no weight gain or even weight loss prior to the occurrence of DKA. At the same time, weight gain over the course of treatment and the resulting metabolic consequences cannot be ignored as significant risk factors, reflected in the increased liability of DKA with an agent like olanzapine versus risperidone. For example, the adjusted risk of DKA for olanzapine, compared to risperidone, has been calculated to increase from 1.7 following >30 days of treatment to 3.5 after >180 days of treatment (242). Based on current case reports, it appears that all atypical antipsychotics are at risk of causing DKA, with the caveat that to date there have been no published cases involving ziprasidone, although there is a report of severe hyperglycemia (234). Albeit rare, clinicians should also be aware that DKA has also been reported with conventional antipsychotics and other psychotropic compounds such as lithium (243-245).

There has been one report assessing the risk factors of DKA versus type 2 diabetes in the context of atypical antipsychotic administration (169). A total of seven demographic variables were examined: gender, race, adjunctive medications, overweight at baseline, weight gain, family history of diabetes, age, and weeks on atypical antipsychotic. The DKA group was significantly different on three of these measures: higher proportion of
females (26.3% vs. 3.8%); lower proportion of overweight at baseline (58.3% vs. 100%); and, younger (37 vs. 43 years of age).

Although DKA is generally associated with type 1 diabetes, it can also occur in the type 2 form of the disease (64,167,168), which is more prevalent in African Americans (246). In line with this, our data, and that of a previously published report (169), indicate 40-50% of reported DKA cases occur in this ethno-cultural group. A further study identified 4 of 5 individuals (80%) with DKA related to atypical antipsychotics as African American (247).

While DKA has been reported to occur twice as frequently in females in the general population (248), evidence related to atypical antipsychotics indicates that males constitute over two thirds of the sample; this too was noted in an earlier report (169). Along similar lines, infection is known as the commonest cause of DKA in the general population (167), but this has not been observed with DKA linked to atypical antipsychotics, both in the present sample and elsewhere (169).

As noted, evidence indicates a number of individuals will initially present with DKA, and in the cases gathered here 5 (7.25%) were fatal, higher than the overall mortality rate of <5% that has been reported in the general population (236). For those who survive, DKA does not represent a temporary and time-limited medical emergency; in those cases gathered here with the information available, over 60% continued treatment with an oral hyperglycemic agent and/or insulin.

Table 5 details the signs and symptoms of DKA. Psychiatrists are unlikely to be directly involved in treatment as DKA represents a medical emergency that often entails
emergency hospitalization (236); however, the reader is referred to several reviews regarding current treatment recommendations (64,167,168).

*Table 5. Signs and Symptoms of Diabetic Ketoacidosis (Source: Trachtenberg 2007)*

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>&gt;250</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.25-7.30</td>
<td>7.00-7.24</td>
<td>&lt;7.00</td>
</tr>
<tr>
<td>Serum bicarbonate (mEq/L)</td>
<td>15-18</td>
<td>10-&lt;15</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Urine ketones</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Serum ketones</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Beta-hydroxybutyrate</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Effective serum osmolality (mOsm/kg)</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Anion gap</td>
<td>&gt;10</td>
<td>&gt;12</td>
<td>&gt;12</td>
</tr>
</tbody>
</table>

**Physical**

- Polyuria
- Polydipsia
- Polyphagia
- Weakness
- Kussamul’s respirations/fruity breath
- Nausea/vomiting+coffee-ground emesis
- Body temperature normal/low
- Dehydration (e.g. dry mucous membranes, tachycardia, hypotension)
- Altered consciousness (e.g. alert to coma)
2.4.2 Mechanisms of Action

There are several facets to this topic. Much of the work to date has focused on the weight gain issue, premised on the notion that the metabolic side effects of atypical antipsychotics are secondary to this. From the standpoint of weight gain liability, there are notable differences between the atypical antipsychotics, with clozapine and olanzapine carrying the highest risk \((48,49)\). While the precise underlying mechanisms are not well understood, various factors including genetics, appetite, food choice, activity level, metabolism and environmental factors (e.g., socioeconomic status) have each been implicated \((235,249,250)\). Over and above weight gain per se, evidence has also highlighted changes in body composition and the adverse effects of increased visceral adiposity \((251-254)\) with atypical antipsychotic treatment. What aspects of these compounds account for these changes also remains poorly understood; the atypical antipsychotic are characterized by heterogeneous receptor binding profiles, and considerable attention has been given to the role of specific receptors in the associated weight gain \((228,235,255,256)\).

The risk of atypical antipsychotic-related DKA, which frequently occurs in the absence of weight gain, suggests that these drugs also carry a risk that is independent of changes in adiposity. That a drug like aripiprazole, one of several antipsychotics thought to be more ‘weight neutral’ \((235)\), has also been linked to DKA, further fuels this argument. A number of animal studies have been able to confirm acute effects on glucose and/or insulin metabolism following a single injection of atypical antipsychotics \((146,147,149,150,257-263)\); although not entirely consistent, there is evidence from investigations examining multiple antipsychotics that the atypicals with the highest
liability for weight gain (i.e., olanzapine, clozapine) also appear to demonstrate the greatest acute effect (146,149,257,261). One human study, involving 3 days of olanzapine administration, reported elevated plasma glucose levels consistent with alterations in insulin sensitivity and/or pancreatic beta-cell secretion (264). Other human studies have addressed this same issue but employed dosing intervals in the range of 8-21 days (83,84,265-268). The results, again not entirely consistent (83,84), also suggest an acute effect; however, interpretation of these findings is compromised by weight gain (83,84,266,267). Furthermore, none of these studies examined changes in body composition. Of note, this work is being carried out in control subjects since schizophrenia itself has been linked to an increased risk of diabetes (76), thereby providing another possible confound in studies of this nature.

Work has also extended to the etiological factors underlying this acute effect. It is intuitively appealing that the same mechanisms responsible for antipsychotic-related weight gain account for the acute effects on glucose dysregulation, and evidence in animal studies looking at multiple antipsychotics offer at least some support for this position (146,147,257,261,262). Again, the fact that a drug like aripiprazole is associated with DKA suggests the story may not be so straightforward. In addition, there is evidence investigating the role of specific receptors in the acute model that would suggest the same. For example, selective H₁ blockade has not been found to affect insulin secretion acutely (258), although it is thought to play a role in the weight gain associated with compounds like olanzapine and clozapine (235,255,256,269).
Finally, more recent work involving an acute animal model implicates a role for central mechanisms, with evidence that intra-cerebroventricular (ICV) olanzapine administration activates hypothalamic AMPK and peripheral hepatic insulin resistance (270).

2.4.3 Limitations

In carrying out this review, we included only published reports and those in which at least an abstract was available in English. Any calculations regarding incidence and prevalence are likely to be compromised by underreporting; furthermore, the details provided by the authors varied considerably between reports, and the retrospective nature of the information leaves certain questions unanswered (e.g., prevalence of diabetes or pre-diabetes before DKA crisis). More than half of the reports involved individuals where there was polypharmacy, complicating the interpretation of a specific agent's contribution to the occurrence of DKA. The significant level of heterogeneity that exists among individuals who develop DKA with antipsychotic treatment cannot be denied, however, more detailed and longitudinal data may permit the characterization of the greatest risk factors involved. Prospective trials are therefore needed that capture first episode populations being started on antipsychotics. Extensive baseline testing and systematic monitoring throughout treatment will no doubt enable physicians to diagnose more cases of latent or new onset glucose dysregulation, possibly circumventing the development of potentially fatal metabolic crises such as DKA.

2.5 Final Comments

The prescription of antipsychotics has expanded dramatically in the last decade, both in terms of indicated and off-label use (271-275). With this much broader utilization there is
risk of complacency regarding adverse side effects that can occur with this class of drugs. Weight gain represents the driving force behind metabolic monitoring for individuals on atypical antipsychotics, but the risk of DKA reminds clinicians that monitoring should be carried out at baseline and routinely throughout treatment. From a research perspective, it remains important to distinguish the acute effects of atypical antipsychotics on glucose metabolism from those on weight gain, at least until we better understand the underlying mechanisms that characterize each.
Chapter 3

Atypical Antipsychotics and Effects of Adrenergic and Serotonergic Receptor Binding on Insulin Secretion In-Vivo: An Animal Model
3 Atypical Antipsychotics and Effects of Adrenergic and Serotonergic Receptor Binding on Insulin Secretion In-Vivo: An Animal Model

3.1 Introduction

The US Food and Drug Administration and Health Canada’s Therapeutic Product Division have each issued monograph warnings outlining weight gain and metabolic abnormalities as established side effects of all atypical antipsychotic (AAPs) compounds. The propensity of each AAP to induce weight gain, lipid abnormalities and type 2 diabetes (DM2) has, however, been shown to vary considerably, with clozapine and olanzapine conferring the greatest risk (163).

Adiposity has been inextricably linked to glucose dysregulation; specifically, the increased plasma concentration of free fatty acids (FFAs) characteristic of obesity (113,114) has been shown to cause insulin resistance (115-118) and pancreatic beta cell apoptosis (132-134). Because weight gain is an acknowledged risk factor for glucose dysregulation (276), it is reasonable to attribute the association between metabolic sequelae and AAPs to significant increases in body mass.

While weight gain with AAP treatment usually starts early in treatment and continues over time (66-68), findings suggest that AAP-induced metabolic abnormalities, at least in part, may be acute in nature and independent of changes in weight. There is a small but growing body of evidence using pre-clinical models demonstrating that AAPs cause significant changes in glucose metabolism after a single dose (146,147,149-151,257). Further, within the clinical population, a spate of reports exist describing diabetic ketoacidosis (DKA) in the context of AAP treatment (277). Cases occurring very early in
treatment, and in patients without a history of impaired glucose metabolism or weight gain, further support the possibility of an acute effect of these agents (174,208,209,218,221).

A clear distinction between the acute (i.e. weight gain independent) and chronic (i.e. attributed to weight gain) effects of AAPs on glucose metabolism has yet to be defined. Interestingly, DKA has been reported in patients taking AAPs for as little as six days (185) and as long as five years (201). It has been stipulated that the rapid onset of DKA in these patients results from an acute effect on insulin secretory mechanisms; these may share a common etiology with the progressive decline in secretory capacity observed in DM2 (258). However, recent work from our group indicates a similar stratification of AAPs based on their effect on insulin sensitivity and weight gain, suggesting a common physiological mechanism may underlie both processes (150). It is likely that several mechanisms are at play, some having immediate effects, and others accounting for a longer-term effect mediated primarily through weight gain.

The mechanisms underlying AAP-induced acute glucose dysregulation remain elusive. AAPs are generally characterized by heterogeneous binding profiles, thought to account for the variable metabolic liability observed across this class of agents; the presence and influence of dopaminergic, histaminergic, serotonergic, adrenergic and muscarinic receptors on tissues involved in glucose metabolism has been reviewed elsewhere (278,279). Work from our group has focused on ‘deconstructing’ the pharmacological binding profile of high liability AAPs (e.g. clozapine and olanzapine) in an effort to parse out those receptors that may play a role (258). Results to date indicate that antagonism at the muscarinic (M3) and serotonergic (5HT2A) receptors attenuates insulin response
to glucose challenge, whereas blockade of the dopaminergic (D2/D3) receptor increased insulin secretion.

The aim of the present study was to continue this line of investigation and further deconstruct the pharmacological binding profile of AAPs within the context of an acute versus chronic model. To achieve this, we used a single dose of selective receptor antagonists, assessing their in vivo effects on the secretory capacity of pancreatic beta cells using the hyperglycemic clamp, which allows the estimation of peripheral tissue insulin sensitivity (145).

3.2 Materials and Methods

3.2.1 Animals

Healthy male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300-325g were housed at the University of Toronto’s Division of Comparative Medicine and maintained on a 12h light/dark cycle. Prior to surgery, animals were housed in pairs and post-cannulation, were kept in individual cages to avoid injury. 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.

3.2.2 Surgery

All surgeries were performed in the University of Toronto’s Division of Comparative Medicine. Rats were anaesthetized with isoflurane and polyethylene catheters (PE-50, Cay Adams, Boston, MA) capped with 2.5 cm of silastic tubing (Dow Corning Corp., Midland, MI) were introduced and advanced to the right atrium and aortic arch of the jugular vein and carotid artery, respectively. The catheter lines were externalized
dorsally and blocked with a pin. All animals received a single dose of analgesic (buprenorphine 0.3mg/kg) during cannulation to manage post-operative discomfort. All animals were given 3-4 days to recover before the application of a hyperglycemic clamp.

3.2.3 Drug and Dose Selection

Rats were randomly assigned to one of six treatment groups: vehicle (0.9% NaCl saline, DMSO or cyclodextrin), prazosin (0.25mg/kg), idazoxan (0.5mg/kg), SB242084 (0.5mg/kg), WAY100635 (0.1mg/kg), and MDL100907 (0.5mg/kg). All drugs were delivered via sub-cutaneous injection and in a volume of 1mL/kg. Prazosin was dissolved in 100% DMSO, SB242084 was dissolved in cyclodextrin, and idazoxan, MDL100907 and WAY100635 were dissolved in 0.9% NaCl saline.

To determine dosing, an exhaustive literature review was conducted for each compound to establish doses that produced significant behavioural or physiological responses. Prazosin dose was chosen based on previous work done on metabolic disturbances with antipsychotic treatment in the male rat (260). MDL100907 and SB242084 doses were chosen on the basis of altered response time in the five-choice serial reaction time test (280). The dose of WAY100635 was chosen based on its ability to modulate the fear-potentiated startle response in the rat (281). An initial dose of 2mg/kg was chosen for prazosin, however acute and extreme hyperglycemia developed, causing increased attrition. A reduction of dose to 0.25mg/kg was chosen, as it had been shown to induce a less pronounced rise in blood glucose (260).
MDL100907 and prazosin were obtained from Toronto Research Chemicals (Toronto, Ontario, Canada). Idazoxan, SB242084 and WAY100635 were obtained from TOCRIS (Minneapolis, Minnesota, USA).

3.3 Procedures

3.3.1 Hyperglycemic Clamp

To assess for glucose control, we employed the hyperglycemic clamp procedure. This method enables the quantification of the secretory capacity of insulin-producing beta cells, and can assess the sensitivity of peripheral tissues (145). It is considered the gold standard for evaluating the secretory function of beta cells in-vivo (144).

Following an overnight fast (14 to 16 hours), the catheter lines were extended to allow connection of the jugular line to the infusion pump and enable blood sampling through the carotid line (see Figure 11 for a schematic of the experimental protocol). Rats were then injected subcutaneously with vehicle (0.9% NaCl saline, n=8; DMSO, n=8; cyclodextrin, n=5), prazosin (n=16), idazoxan (n=10), SB242084 (n=10), WAY100635 (n=10) or MDL100907 (n=8). The hyperglycemic clamp began 90 minutes post-injection, with the injection of a glucose bolus (50% Dextrose 1mL/kg, Abbott Lab, 500mg/kg) into the jugular catheter to raise plasma glucose above 300mg/dL. After bolus administration, the jugular catheter line was connected to a pump for glucose infusion. Variable infusion of exogenous glucose maintained plasma glucose levels at approximately 300mg/dL (or 17mM). Plasma samples were taken at pre-determined and regular intervals throughout the clamp for insulin and C-peptide assay.
3.3.2 Laboratory Methods

Plasma glucose was measured with an Analox GM9 Glucose Analyzer (Analox Instruments USA Inc.). Plasma insulin and C-peptide levels were determined by radioimmunoassay using kits (Linco Research, St. Charles, MO) specific to rat insulin (with 100% cross reactivity with porcine insulin used for infusion) and C-peptide. The coefficients of variation were less than 9% and 10.5% for insulin and C-peptide, respectively.

3.3.3 Statistical Analysis

Glucose infusion (GINF) rate was calculated based on each animal's weight and glucose infusion pump rate. An index of insulin sensitivity (SI) was calculated by dividing GINF by the insulin levels during the final 30 minutes of the clamp. The relationship between insulin sensitivity and secretion is hyperbolic in healthy subjects. The product of insulin sensitivity and secretion is a constant defined as disposition index (DI) and represents a measure of adequate beta cell compensation in response to insulin resistance, also considered a predictor of early impairments in glucose metabolism.
The DI was calculated as the product of insulin sensitivity and 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 a) whether glucose, insulin, C-peptide, GINF, SI, and DI values change over the course of the experiment, b) whether these values differ across the five study groups, and c) whether the magnitude of any group differences present vary over the course of the experiment.

Full maximum likelihood (FML) estimation was selected as the method of estimation for each of these analyses, and a variety of appropriate potential covariance structures were investigated (variance components, spatial Gaussian, spatial power, spatial spherical, and unstructured). The covariance structure resulting in the lowest Bayesian information criterion (BIC) value was ultimately selected for use in each of the final models.

Significant findings were further investigated through a series of Bonferroni-adjusted pairwise comparisons (SI, DI, and GINF) and linear contrasts (glucose, insulin, and C-peptide). The linear contrasts were constructed to determine whether glucose, insulin, and C-peptide values differ between the five study groups and their respective control groups during the baseline phase (80 and 90 minutes), during the secretion phase (92, 97, and 102 minutes), during the post-secretion phase (120, 150, 170, and 180 minutes), and during the post-baseline study phase (92, 97, 102, 120, 150, 170, and 180 minutes).
Due to a heavily right-skewed distribution of the model residuals, a natural-logarithm transformation was applied to SI and DI prior to testing. Because of this log-transformation, the interpretation of the parameter estimates and confidence intervals is slightly different for SI and DI than it is for the other metabolic measures. Instead of obtaining an estimate (and confidence interval (CI)) for the difference between the arithmetic means of subjects in each treatment group relative to the control group, we now obtain an estimate (and confidence interval) for the ratio between the geometric means of subjects in the active treatment and control groups. As a result, the interpretation of our confidence intervals is different. When assessing CIs based on the raw data, we conclude that a difference between groups exists if the CI does not contain 1. For log-transformed data, we establish whether the value 1 is included in the interval. If there is no difference between study groups, we expect the ratio of their geometric means to be 1, so we determine whether 1 lies within the confidence interval to assess significance.

Significance was accepted at p<0.05 and all p-values provided were adjusted for multiple comparisons.

3.4 Results
The MMRM 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 course of the clamping procedure (data not shown). Glucose values in each of the experimental conditions do not differ from control values during any of the pre-specified phases of the study (Fig. 12A-C).
Accordingly, no differences in LS means were seen for baseline insulin and C-peptide between treatment groups compared to their respective controls.

During the entire clamp, prazosin significantly decreased insulin (p=0.0155) (Fig. 13B) and C-peptide (p=0.0070) values compared to DMSO (Fig. 14B). These effects were mirrored by a significant decrease in GINF required to maintain hyperglycemia (p<0.0001) (Fig. 15B), as well as a significantly lower DI (p<0.0001) (Fig. 17B).

Similar to prazosin, during the post-secretory phase of the clamp MDL 100 097 significantly decreased insulin levels (p=0.0205); these were marginally lower during the entire post-baseline phase (p=0.0760) (Fig. 13A). C-peptide levels were significantly lower throughout the entire clamp for this group (p=0.0035) compared to saline controls (Fig. 14A). While MDL 100 907 affected both insulin and C-peptide levels, this was not replicated with GINF levels required to maintain hyperglycemia (p=0.3985) (Fig. 15A) or SI and DI values (p=0.1665 and p=0.3555, respectively) (Fig. 16A, 17A).

Treatment with idazoxan was not associated with any significant change in insulin, C-peptide or GINF values; however, there was a significant increase in both SI (p=0.0015) and DI (p=0.0125) measures. Treatment with SB242084 and WAY100635 did not result in significant changes in any of the examined parameters.
Figure 12. Effect of a single subcutaneous dose of representative antagonist on plasma glucose levels during the hyperglycemic clamp in male Sprague-Dawley rats.

Figure 13. Effect of a single subcutaneous dose of representative antagonist on plasma insulin levels during the hyperglycemic clamp in male Sprague-Dawley rats. †p<0.05 versus control.

Figure 14. Effect of a single subcutaneous dose of representative antagonist on plasma C-peptide levels during the hyperglycemic clamp in male Sprague-Dawley rats. †p<0.05 versus control.
Figure 15. Effect of a single subcutaneous dose of representative antagonist on plasma glucose infusion rates (GINF) during the hyperglycemic clamp in male Sprague-Dawley rats. ††p<0.001 versus control.

Figure 16. Effect of a single subcutaneous dose of representative antagonist on the sensitivity index (SI), calculated during the last 30 minutes of hyperglycemic clamp in male Sprague-Dawley rats. †p<0.05 versus control.

Figure 17. Effect of a single subcutaneous dose of representative antagonist on the disposition index (DI), calculated during the last 30 minutes of hyperglycemic clamp in male Sprague-Dawley rats. †p<0.05 and ††p<0.001 versus control.
3.5 Discussion

While poorly understood, the mechanisms underlying metabolic sequelae of AAP treatment are likely numerous and complex. The use of pre-clinical models has shown that these compounds can cause acute and significant changes in glucose homeostasis (146,147,150,151,257) that are in contrast to the significant weight gain and development of diabetes observed with chronic exposure. Insulin secretion and signaling involves many tissues (e.g. brain, liver, pancreas, fat, muscle) and receptors (279,283), all of which are susceptible to modulation with AAP treatment. It is within this context that we sought to examine the acute changes in glucose metabolism induced by AAPs through the deconstruction of their heterogeneous pharmacology. Using representative antagonists and the hyperglycemic clamp technique, we examined the potential impact of the $\alpha_1$, $\alpha_2$, 5HT$_{1A}$, 5HT$_{2A}$ and 5HT$_{2C}$ receptors on pancreatic beta cell function in-vivo.

The sympathoadrenal system is capable of providing rapid increases in blood glucose levels. Noradrenaline is released from post-ganglionic axon terminals, while adrenaline and noradrenaline are secreted from the chromaffin cells of the adrenal medulla; these hormones elevate plasma glucose by inhibiting insulin, and increasing glucagon secretion from the endocrine pancreas, while also increasing glucose producing mechanisms (i.e. gluconeogenesis and glycogenolysis) in the liver (284).

Our results suggest that $\alpha_1$ antagonism with prazosin confers a robust attenuation of insulin and C-peptide secretion in response to glucose challenge. The role of prazosin on plasma glucose regulation, however, remains unclear. Several studies report an acute hyperglycemic effect with prazosin administration (260,285), as was our
experience with an initial dosing of 2mg/kg; furthermore, prazosin has been shown to potentiate post-prandial increases in plasma glucose levels (286,287) in the rat. Indeed, the use of prazosin (a thiazide diuretic) in humans has itself been associated with hyperglycemia (288). In line with these findings, agonism of the $\alpha_1$ receptor has been shown to lower plasma glucose in rats lacking insulin (due to treatment with streptozotocin) through increased glucose utilization of peripheral tissues, and increased glycogen synthesis; this effect was blocked using an $\alpha_1$ receptor antagonist (289,290). Support for the presence and involvement of the $\alpha_1$ receptor in glucose metabolism comes from data indicating that prazosin completely inhibits phenylephrine (an $\alpha_1$ agonist) stimulated glucose uptake in rat white adipocytes (290,291) L6 muscle cells (292).

With respect to insulin secretion, our data do not support that of the existing literature. The use of a pig pancreas-duodenum model revealed that prazosin abolishes both noradrenaline and phenylephrine inhibition of insulin secretion (285). Another study using a rat model showed that acute infusion of prazosin resulted in a significant increase in plasma insulin concentration; this effect was steeply attenuated by the addition of somatostatin (which suppresses insulin secretion), and re-established once somatostatin was removed (293). Still other studies report effects of prazosin on insulin levels that are dependent on the nutritional status of the animal (286). By way of example, animals fed chow supplemented with prazosin over several weeks did not experience a change in insulin sensitivity; furthermore, potentiation of the post-prandial increase in plasma glucose and insulin levels was not affected by chronic treatment, but required the acute administration of prazosin to occur (287).
While the focus of the present study was on the acute (single dose) effects of receptor antagonism, it is important to note that an alteration in sympathetic nervous system function has also been suggested to play a role in the development of obesity and diabetes (294, 295). The administration of a norepinephrine re-uptake inhibitor such as reboxetine, for example, has been shown to mitigate the weight gain associated with olanzapine treatment (296) in the clinical population. Sympathetic activation, norepinephrine secretion and the $\alpha_1$ receptor are also known to influence adipocyte metabolism by the way of lipolytic activity (297) in the rodent.

Some atypical antipsychotics possess a high affinity for the $\alpha_1$ receptor (298), although in contrast to certain histaminergic, muscarinic and dopaminergic subtypes (298-300), there has been no work indicating a strong association between $\alpha_1$ binding and metabolic risk. Of note is the high affinity of clozapine, risperidone and aripiprazole for this receptor; these agents represent high, moderate and low metabolic risk compounds, respectively (163). While the $\alpha_1$ receptor clearly played a role in acute glucose metabolism in the work presented here, its alignment with existing data is varied and more work is needed to fully elucidate its role.

As mentioned above, disruption of sympathetic tone has been associated with the development of diabetes (294, 295). Activation of sympathetic tone leads to increased gluconeogenesis, glycogenolysis and lipolysis, all processes that result in higher plasma glucose concentration (279). Peripherally, the $\alpha_2$ receptor is expressed on pancreatic $\beta$-cells, where it inhibits the release of insulin (279). Several antipsychotics antagonize this receptor, albeit to different degrees (299), potentially increasing insulin secretion as was noted in one rat study (301). Here, we demonstrated a significant increase in both
sensitivity and disposition indices upon administration of a selective α₂ receptor antagonist, idazoxan. This was not corroborated, however, by changes in glucose infusion rate, insulin or C-peptide levels. While we failed to detect an acute effect of α₂ antagonism on glucose metabolism, it is possible that more prolonged exposure could produce an effect. As well, a central effect cannot be ruled out; for example, the injection of norepinephrine into the paraventricular nucleus of rats has been shown to result in increased food intake and weight gain (302). This is postulated to occur because of a self-feedback mechanism by which the release of norepinephrine is regulated by an α₂ auto-receptor on the pre-synaptic axon; when activated, neurotransmitter release is inhibited. Antagonism of this auto-receptor by antipsychotics would impede this regulatory process, leading to excessive norepinephrine release in the paraventricular nucleus and abnormal feeding behaviour. That being said, clozapine, risperidone and aripiprazole have the highest affinity for the α₂ receptor (300); their marked disparity in metabolic liability argues against a role for the α₂ receptor in AAP’s risk of metabolic side effects. In addition, the relative affinities of atypical antipsychotics for the α₂ receptor have not been clearly correlated with weight gain or associated diabetes, making the role of this receptor in antipsychotic-induced metabolic sequelae uncertain.

The atypicality of second-generation antipsychotics is often attributed to their increased 5HT₂A receptor binding (303). Recent work from our laboratory (258) has shown that pre-treatment with ketanserin (a non-selective 5HT antagonist) induces a significant attenuation of insulin secretion during the application of a hyperglycemic clamp. Similarly, using a highly selective 5HT₂A antagonist, MDL 100 907, we have shown here
a significant decrease in both insulin and C-peptide secretion. Ketanserin displays a robust affinity for not only the 5HT, but also the α1 and histaminergic (H1) receptors (304). Serotonin is synthesized within the pancreatic beta cell (305) and co-localized to the insulin secretory granules (306). Serotonin is thus co-secreted upon stimulation of pancreatic islets with glucose (307, 308). Paulmann and colleagues (309) demonstrated that lack of intracellular serotonin in transgenic mice leads to diabetes while replenishment of serotonin rescues insulin secretion. Further characterization of serotonin involvement comes from work demonstrating an increase in 5HT2 receptor mRNA in beta cells following glucose stimulation (310). Overall, the 5HT2 receptor has been implicated in insulin sensitivity rather than secretion; however, the results have been inconsistent (311-315). 5HT2 receptors have been shown to have different effects; central antagonism with ketanserin reduces DOI (non-specific serotonin agonist) mediated increases in sympathetic tone (311), while peripheral blockade may attenuate glucose uptake by skeletal muscle cells and reducing peripheral sensitivity (313, 316). The ability of ketanserin to cross the blood-brain barrier may be responsible for such inconsistencies. The use of MDL 100 907, however, implicates the 5HT2A receptor specifically, bolstering findings of serotonin involvement in insulin secretion and glucose homeostasis. Chronic administration of both clozapine (317) and olanzapine (318) downregulate the expression of 5HT2A receptors and decrease neuropeptide Y (NPY) mRNA levels (319). This downregulation of NPY receptors has been postulated as a mechanism whereby chronic 5HT2A antagonism leads to NPY over-secretion and orexigenic signal disinhibition (320). However, weight-neutral drugs such as aripiprazole and ziprasidone (163) display a high affinity for the 5HT2A receptor, implying that
although there may be some involvement in energy metabolism, it is likely not significant.

While the 5HT\textsubscript{2C} receptor has been most closely linked to increased risk of diabetes and weight gain (321), the lack of effect of SB242084, a selective 5HT\textsubscript{2C} antagonist, on insulin, C-peptide or glucose infusion rate, does not support an acute effect of this receptor on beta-cell function. Recent studies provide a role for the 5HT\textsubscript{2C} receptor in satiety, food intake and body weight control. Agonists of this receptor reduce food intake and increase energy expenditure (322). Inhibition of serotonin and norepinephrine re-uptake by sibutramine, venlafaxine, duloxetine, or by a combination of fluoxetine and nisoxetine, has been shown to produce a marked reduction of food intake in freely feeding rats (323). Transgenic mice for the 5HT\textsubscript{2C} receptor are hyperphagic and experience greater white adipose tissue (WAT) depots and later-onset obesity (324). Knockout mice also develop hepatic insulin resistance, impaired glucose tolerance, and hyperleptinemia (325-327). The combined blockade of the H\textsubscript{1} and 5HT\textsubscript{2C} receptors has been associated with weight gain (328); both clozapine and olanzapine have high affinities at both receptors and have greater weight gain liability than other antipsychotics such as chlorpromazine, which has appreciable H\textsubscript{1} but little 5HT\textsubscript{2C} affinity (255, 329). The role of 5HT\textsubscript{2C} in weight gain is uncertain however, as some studies show a correlation between drugs with high affinity at this receptor and increased propensity to cause weight gain (tricyclic antidepressants, atypical antipsychotics) (330), while others do not (331). Further complicating the issue is the relative binding affinity of atypical antipsychotics at this receptor; ziprasidone, olanzapine and clozapine are among the most potent, whereas risperidone, quetiapine and aripiprazole have almost
no affinity whatsoever (300).

The 5HT$_{1A}$ receptor is expressed on pancreatic β-cells and is known to influence their responsiveness to plasma glucose (279). It has been suggested that antagonism of this receptor by antipsychotics may decrease responsiveness, resulting in hyperglycemia (331). However, using a selective 5HT$_{1A}$ antagonist, WAY100635, we found no significant effect on plasma glucose, insulin or C-peptide values during a glucose challenge. Studies investigating the role of 5HT$_{1A}$ in glucose metabolism however, remain inconsistent. For example, administration of 8-OH-DPAT, a selective 5HT$_{1A}$ agonist, triggers a dose-dependent hyperglycemia in the conscious rat that is associated with inhibition of insulin release (332, 333). Administration of an α$_2$ antagonist (idazoxan), and the mixed 5HT$_{1A}$/5HT$_{1B}$ antagonist (-)propranolol, prevent 8-OH-DPAT hyperglycemia and hypoinsulinemia (332). An increase in adrenaline secretion has been implicated in this 8-OH-DPAT-induced hyperglycemia (332) and is thought to be mediated through the action of central 5HT$_{1A}$ receptors (334). The occurrence of hyperglycemia through both antagonism (at β-cells) and agonism (at brain) of the 5HT$_{1A}$ receptor may be due to differing actions at central versus peripheral sites; however, the lack of effect here with WAY100635 is not in agreement with either mechanism. With regards to feeding behaviour, the use of 5HT$_{1A}$ agonists has also been shown to cause opposing effects depending on the nutritional status of the animal (335, 336)

While little work has been done on the use of 5HT$_{1A}$ antagonists on glucose metabolism, it is appropriate to comment on potential implications of agonistic activity at this receptor. Both aripiprazole and risperidone (337, 338) are partial agonists at the 5HT$_{1A}$ receptor and are known as dopamine-serotonin system stabilizers (339).
Olanzapine, on the other hand, has low affinity at this receptor, similar to that of haloperidol, and clozapine, risperidone and quetiapine lie somewhere in between (338, 340). With respect to its function on the pancreatic β-cell, agonism at the 5HT₁₅ receptor has been suggested to act as a protective mechanism against the development of diabetes (279). Given data suggesting acute hyperglycemia with the use of agents like 8-OH-DPAT, further investigation is required before a specific role for this receptor in glucose homeostasis can be defined.

Several limitations must be considered in evaluating the results of the current work. While every effort was made to choose the most selective antagonists for each studied receptor, the compounds used display some degree of pleiotropy. WAY100635, in particular, acts as a full agonist at the dopamine (D₄) receptor (341). The hyperglycemic clamp provides an index of insulin secretory capacity, but leaves many questions unanswered regarding changes in glucose metabolism at specific sites (i.e. muscle and liver). Hyperinsulinemic-euglycemic clamps are considered the gold standard in the assessment of insulin sensitivity, and when done using radioactive tracers, provide information on the site of insulin resistance (i.e. muscle or liver) (342). Further work using this technique would be beneficial in evaluating the effects of acute antagonism at the α₁, α₂ and 5HT₂₅ receptors. While compensatory mechanisms likely exist, these are difficult to control, as are additive or synergistic receptor interactions, which would not be captured with this approach. Although a number of receptors involved in atypical antipsychotic pharmacology have now been examined (α₁, α₂, 5HT₁₅, 5HT₂₅, 5HT₂₇, D₂/₃, M₃, H₁) in the context of glucose metabolism, there remain others merit ing investigation (e.g. β₃, found on adipocytes and pancreatic β-cells and D₄, for which
clozapine, in particular, displays a high affinity). Lastly, because both central and peripheral mechanisms are involved in glucose homeostasis, these complex and interacting systems are also likely involved in the metabolic sequelae of atypical antipsychotic treatment. Central administration of selective antagonists, through the use of intracerebroventricular (ICV) cannulas, followed by a hyperglycemic or hyperinsulinemic-euglycemic clamp, may provide us with more information regarding the central influence of the receptors studied. This is especially important given that certain receptors have opposing effects at the central and peripheral level.

These limitations aside, the current work provides support for an acute, weight-gain independent effect of antipsychotics on pancreatic β-cell function, implicating adrenergic and serotonergic mechanisms. While we now have more information regarding the potential receptors involved in antipsychotic-induced glucose dysregulation, much remains unknown about the specific interactions and mechanisms involved. It is also unclear why certain individuals go on to develop metabolic sequelae while others remain unaffected. Lastly, it remains unknown whether the same mechanisms that are responsible for the acute changes in glucose metabolism are distinct from those incurred with chronic treatment and the associated weight gain/diabetes often observed.
Chapter 4
General Discussion
4 General Discussion

4.1 Summary of Work

The aim of this work was to 1) review case reports of diabetic ketoacidosis in association with atypical antipsychotic use in order to understand the scope of the problem, its relationship to different compounds, risk factors, outcome and putative mechanisms of action; 2) deconstruct the receptor binding profiles of these agents by investigating the acute changes in glucose regulation after a single injection of the each of following five selective receptor antagonists: prazosin (α₁ antagonist, 0.25mg/kg), idazoxan (α₂ antagonist, 0.5mg/kg), MDL100907 (5HT₂A antagonist, 0.5mg/kg), WAY100635 (5HT₁A antagonist, 0.1mg/kg), and SB242084 (5HT₂C antagonist, 0.5mg/kg).

4.2 Discussion

Atypical antipsychotics readily supplanted their first generation predecessors because of their decreased propensity to cause extrapyramidal side effects (343). While atypicals now represent the first line of treatment in psychotic disorders (34), and are increasingly used in a number of other conditions (51,52), these agents are known to cause metabolic side effects such as weight gain and glucose dysregulation (40). The mechanisms responsible for these side effects remain elusive and a better understanding is needed in order to distinguish the pharmacology involved in therapeutic response versus that of undesired side effects, knowledge which could translate into future drug development.
The objective of this work was to deconstruct the receptor binding profiles of atypical antipsychotic drugs in relation to glucose homeostasis. This was done in the context of a pre-clinical murine model using the hyperglycemic clamp, considered the gold standard for assessing the insulin secretory capacity of pancreatic beta cells \textit{in-vivo} (144). We anticipated varying degrees of change in measures of glucose homeostasis following the administration of different selective receptor antagonists; we confirmed this, however, not all antagonists studied displayed an effect. Recent work using a similar paradigm to ours confirmed the acute effects of several atypical antipsychotics (150) on glucose metabolism, lending support to the idea that these compounds cause acute changes in energetic homeostasis. The acute model is important given evidence that patients being treated with atypical antipsychotics can experience metabolic side effects soon after treatment initiation and independent of weight gain, an effect that is seen under more chronic administration (163). For example, sudden onset diabetic ketoacidosis points to a direct, weight gain-independent effect on beta cell function.

In examining candidate receptors for their role in insulin secretion, it may be possible to shed light on the molecular events taking place in the context of the aforementioned acute metabolic effects. The mechanisms responsible for acute changes in glucose metabolism have yet to be characterized; doing so could permit their comparison to the postulated mechanisms responsible for weight gain and associated development of insulin resistance and type 2 diabetes with atypical antipsychotic treatment.

We shall now turn to a brief review of the results obtained with each of the examined selective receptor antagonists.
4.2.1 Prazosin, a selective $\alpha_1$ antagonist
Administration of 0.25mg/kg of prazosin resulted in a significant attenuation of insulin secretion; this was confirmed by a concomitant and significant reduction in C-peptide secretion. In addition, the glucose infusion (GINF) rate required to maintain hyperglycemia was significantly lower compared to control (DMSO). There was also a significant decrease in the disposition index (DI) compared to control, while the sensitivity index (SI) remain unaffected.

4.2.2 Idazoxan, a selective $\alpha_2$ antagonist
Administration of 0.5mg/kg of idazoxan resulted in a significant increase in both sensitivity and disposition indices compared to control (saline); however, these findings were not supported by a change in insulin, C-peptide or GINF values.

4.2.3 WAY100635, a selective 5HT$_{1A}$ antagonist
Administration of 0.1mg/kg of WAY100635 did not result in an appreciable change in any of the parameters examined (insulin, C-peptide, GINF, SI or DI) compared to control (saline).

4.2.4 SB242084, a selective 5HT$_{2C}$ antagonist
Administration of 0.5mg/kg of SB242084 did not result in an appreciable change in any of the parameters examined (insulin, C-peptide, GINF, SI or DI) compared to control (cyclodextrin).

4.2.5 MDL100907, a selective 5HT$_{2A}$ antagonist
Administration of 0.5mg/kg of MDL100907 resulted in a significant attenuation of insulin secretion; this was confirmed by a concomitant and significant reduction in C-peptide secretion;
secretion. The glucose infusion (GINF) rate required to maintain hyperglycemia, however, was not significantly lower compared to control (saline). The SI and DI were unaffected by treatment with MDL100907.

4.3 Caveats
The current work consisted of 1) generating a concise review of case reports of diabetic ketoacidosis in the context of atypical antipsychotic treatment and 2) deconstructing the receptor binding profiles of atypical antipsychotic drugs in relation to acute glucose metabolism.

4.3.1 Diabetic Ketoacidosis with Atypical Antipsychotic Treatment
With regard to the literature review of DKA case reports, figures depicting incidence and prevalence are likely conservative due to a lack of reporting by prescribing physicians. In addition, the retrospective nature of these reports is associated with an inherent lack of complete information regarding patient risk factors and long-term follow-up. The reports included here were limited to those where at least an abstract was published in the English language, necessarily making certain details unavailable for inclusion. Prospective trials are needed in first-episode, drug-naïve patients being started on antipsychotics agents; baseline and regular metabolic monitoring throughout treatment in this population will enable a more cohesive understanding of risk factors, while potentially guiding treatment algorithms.

4.3.2 Deconstruction of Atypical Antipsychotic Binding Profiles
Glucose homeostasis is a complex and multifaceted process involving many physiological systems and molecular mechanisms. With this in mind, several caveats
merit mention in the interpretation and translational applicability of the aforementioned data.

A subcutaneous route of administration was chosen for this project because this method is minimally invasive and unlikely, when carried out properly, to cause excessive stress in the rat. This is an important consideration in conducting metabolic research, as stress itself can cause changes in glucose metabolism (344). In addition, this approach avoids the reduction in serum drug concentration due to the first-pass effect of the liver (i.e. biotransformation) observed with oral administration (345). However, the molecular structure and solvent used in any injected drug affects its absorption and distribution (346). Unfortunately, data evaluating the pharmacokinetics of the compounds used in this study are sparse, leaving questions such as the extent to which they cross the blood-brain barrier unanswered. These compounds, however, are commonly used in behavioural research using a murine model. The doses chosen here have been shown to cause significant changes in rodent behaviour, and have therefore been accepted for the current metabolic work. It is possible, however, that the metabolic side effects of antipsychotics are caused through different mechanisms than those affecting behaviour; it is difficult to ascertain, therefore, which doses truly affect the metabolic system under study. By way of example, to produce the same magnitude of glucose intolerance seen in humans treated with olanzapine, the required dose in rats is much larger than that required to cause a significant change in behaviour (264).

As noted, glucose metabolism involves several different physiological systems and molecular mechanisms. Antagonizing a specific receptor, while informative, does not accurately mirror the behaviour of atypical antipsychotics in-vivo. These drugs have
widely heterogeneous binding profiles (152,153) that differ significantly from one compound to another (152,153), and both first- and second-generation antipsychotics have been associated with metabolic side effects (243-245). In addition, the relative binding affinity of each drug for a given receptor varies, further complicating data interpretation. It is possible that several different receptors being antagonized concomitantly leads to a synergistic or additive effect, and that a drug’s metabolic liability is related to its complement of receptor binding, not necessarily to a specific receptor. Furthermore, compensatory mechanisms have been shown to exist in chronic blockade of certain G-coupled receptors (347), potentially masking a true effect, while a ‘paradoxical’ downregulation has been seen in others (e.g. 5HT\textsubscript{2A} and 5HT\textsubscript{2C}) (348). Because schizophrenia per se has been linked to increased risk of diabetes (101), it is unclear as to what role this may have in the effects of antipsychotics in this regard.

While data exist on the localization of certain receptors involved in glucose homeostasis (279), it is likely that existing knowledge is incomplete. In the periphery (i.e. outside the central nervous system) it is liver, muscle and fat cells that play an important role in insulin signaling and glucose metabolism. We have shown here that prazosin, a highly selective α\textsubscript{1} antagonist, significantly impairs insulin secretion, and while it is known that this receptor is involved in glucose uptake by muscle and fat cells (290-292), it is not clear if and how it is involved in the synthesis and release of insulin. As the sympathetic nervous system has been shown to modulate glucose and insulin signaling (349,350), it is important to consider the central role of the α\textsubscript{1} receptor in this regard (see 4.6 Future Directions).
While the hyperglycemic clamp is considered the gold standard for the assessment of beta cell secretory capacity (144), this technique does have its limitations. Using insulin, C-peptide and glucose infusion rate (GINF) data, it is possible to derive indices of insulin sensitivity and disposition; the former enables an approximation of the responsiveness of cells to the insulin signal, while the latter informs on the ability of the beta cell to compensate in the instance of insulin resistance (282). These indices, while useful, do not explain where abnormalities lay, an issue central to the differentiation of hepatic versus peripheral insulin resistance. The pitfalls of the hyperglycemic clamp can be largely overcome through use of the euglycemic-hyperinsulinemic technique (see 4.6 Future Directions).

4.4 Relevance of Atypical Antipsychotic-Induced Glucose Dysregulation

4.4.1 Clinical

Atypical antipsychotics are associated with a number of metabolic side effects, weight gain being the most familiar to prescribing physicians (351,352). All atypical compounds, with the exception of ziprasidone and aripiprazole, are associated with some degree of weight gain (48), and individuals with schizophrenia are more likely to be obese, even in the absence of antipsychotic treatment (162). The Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study highlights the negative effects of increased body mass index (BMI) in patients taking atypical antipsychotics, linking it to “poor self-rating of physical health and increased somatic preoccupation,” (39,67). Using the Psychological Well-Being Index, Allison and colleagues (353) found weight gain to be significantly associated with perceptions of poorer quality of life, general health, and
vitality. Patients report “a sense of...being the target of social stigma,” (354) which can negatively affect compliance, resulting in compromised treatment management and long-term outcome (355,356).

Unfortunately, the effects of diabetes on patient outcome are also unfavourable. As with weight gain, individuals with schizophrenia are at increased risk of developing diabetes, irrespective of treatment (96). Serious mental illness, which includes schizophrenia, is often associated with persistent symptoms and the need for chronic antipsychotic treatment (357). Coping with diabetes is stressful and those who are diagnosed have lower quality of life (358,359). Excess central adiposity is a component of the metabolic syndrome and an established risk factor for the development of type 2 diabetes, hypertension and cardiovascular disease (90). Once diabetes develops, the risk of acquiring coronary heart disease (CHD) increases, more so than with obesity alone (90). The standardized mortality ratio from cardiovascular disease is doubled in schizophrenia and the lifespan of these individuals is reduced by as much as 25% (105).

What makes these facts all the more disturbing is the evidence suggesting individuals with schizophrenia have reduced access to, and use of, appropriate medical care (360-362).

In short, it is critical that the weight gain and metabolic side effects of antipsychotics be addressed, not only to improve indices of quality of life, but also to minimize the risk of potentially fatal medical complications such as heart attack and stroke.
4.4.2 Economic

The fiscal burden of caring for individuals with schizophrenia is thought to be as high as $6.85 billion per year in Canada (363). These values are likely conservative as many ‘hidden’ or ‘indirect’ costs to patients, families and the society-at-large exist. Interestingly, health care costs of treating and supporting individuals with schizophrenia remain high despite a shift in providing care outside of the hospital (364). In addition, because only approximately 20% of this population has paid work, much of the financial burden stems from lost productivity (365). With regards to diabetes, the number of individuals diagnosed in Canada is expected to increase from 1.4 million in 2000 to 2.4 million by 2016; the total cost of care will increase from $4.66 billion to $8.14 billion in that time period. (366).

Given the extreme cost of schizophrenia and diabetes to the Canadian government, much debate exists surrounding the cost-effectiveness of second-generation compounds in comparison to their first-generation counterparts. The atypicals were heralded because of their reduced propensity to cause movement disorders, but recent evidence challenging their claims of superior clinical efficacy, coupled with their increased costs (67) have called their utility into question. A meta-analysis of 140 clinical trials concluded that clozapine, amisulpride, risperidone and olanzapine were significantly more efficacious than first-generation drugs (367). Furthermore, the atypicals were seen as providing better functional recovery and ‘cost-effective’ because the reduction of other costs (e.g. hospitalization) offset the increased cost of these agents (367). Basu (368), however, found that in the treatment of chronic schizophrenia, the cost-effectiveness results do not support the use of one class of
drugs over another. In addition, the Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUTLASS) found that first-generation were no worse than second-generation agents in terms of symptom treatment and quality of life measures (369). And lastly, the CATIE study found no significant outcome differences between perphenazine (a first-generation agent) and any atypical drug, although monthly costs of the former remained 20-30% lower (370).

It is obvious that opinions remain divided on the cost-effectiveness of atypical antipsychotics, however their use continues to surge, especially in non-psychotic disorders such as anxiety, depression, panic disorder, and agitation and dementia (365). Regardless of their cost-effectiveness, the widespread use of atypicals emphasizes the need to further explore the impact of their side effects and indirect costs.

4.5 Implications

Our findings have important clinical implications, emphasizing the need for metabolic monitoring of patients prior to, and throughout all stages of antipsychotic treatment. Regular monitoring of patients is important because:

1. Screening for components of metabolic syndrome (i.e. increased fasting glucose levels; increased waist circumference; hypertension; increased triglyceride levels; or reduced HDL cholesterol) will flag patients at increased risk for progressive metabolic decompensation.
2. Identification of high-risk patients can help guide physicians in their selection of antipsychotic treatment; taking into consideration patient and drug liability will minimize risk of adverse events.

3. Identification of existing pathologies (e.g. diabetes) enables active intervention and treatment (e.g. lifestyle or pharmacological), thus reducing the risk of serious sequelae upon initiation of antipsychotic treatment.

4. Metabolic crises such as DKA have been reported with all atypical antipsychotics (with the exception of ziprasidone) and at markedly different intervals following the onset of treatment; baseline values can be used to track changes in metabolic status and enable physicians to act promptly should adverse changes become apparent.

Early diagnosis and prompt treatment of diabetes are important for favourable long-term outcome. Practice guidelines for metabolic monitoring of the general population are well established, although in psychiatry this is in its earliest stages. Part of the challenge stems from the fact that psychiatrists are not normally charged with metabolic screening. Mounting evidence of increased morbidity in schizophrenia (99) and increased off-label use of atypical compounds (271-275) add to the urgency in designing standardized guidelines. It is encouraging that several sites have established baseline monitoring recommendations for patients taking antipsychotics, the details of which are outlined in table 6 below. In addition, continuous monitoring protocols have also been put forth; those of the American Diabetes Association are outlined in table 7.
Table 6. Factors included in monitoring protocols for patients prescribed atypical antipsychotics. (Source: Adapted from Cohn et al., 2006)

<table>
<thead>
<tr>
<th>Workup</th>
<th>Mount Sinai SCZ &amp; any AP</th>
<th>Australia All pts, any AP</th>
<th>ADA-APA All pts, SGA</th>
<th>Belgium SCZ, SGA</th>
<th>United Kingdom SCZ, any AP</th>
<th>Canada SCZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPG</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Random glucose</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>If FPG not feasible</td>
<td></td>
<td>No</td>
<td></td>
<td>In addition to FPG or random glucose</td>
<td></td>
</tr>
<tr>
<td>OGTT</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>To follow up IFG</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Family History</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Tobacco</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Diet activity</td>
<td>x</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Signs &amp; Symptoms of DM</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Factors included in monitoring protocols for patients prescribed atypical antipsychotics. (Source: Adapted from Bermudes et al., 2007 with data from American Diabetes Association, 2004; Marder et al, 2004.)

<table>
<thead>
<tr>
<th>Metabolic Parameter</th>
<th>Baseline</th>
<th>Each visit for 6 months</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>4 months</th>
<th>Every 6 months</th>
<th>Annually</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Plasma Glucose</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin A1c</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Lipid Profile</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The measurement of baseline laboratory values is of paramount importance in the schizophrenia population because of increasing evidence supports the acute effects of atypical agents on glucose metabolism, both through the use of animal models and
clinical reports. While several studies show that switching from a ‘high risk’ to ‘lower risk’ agent improves metabolic indices (371-373), it is crucial to remember that all atypicals have been associated with some degree of glucose dysregulation and at highly varied times into treatment. Using compounds with decreased metabolic liability, however, does not guarantee against dangerous metabolic sequelae. In addition, clinical response to antipsychotic treatment is varied and patients are more often than not tried on several compounds before finding one that effectively manages psychotic symptoms (374). Clozapine remains distinct in its effectiveness in treating refractory schizophrenia (375), however it (along with olanzapine) is also considered the drug with greatest metabolic liability (25,163). And because significant polypharmacy is exceedingly common in schizophrenia (376), this puts patients at increased risk of metabolic syndrome (377). In addition, many patients who experience a metabolic crisis such as diabetic ketoacidosis do not fully recover and remain diabetic, requiring long-term insulin and/or oral hypoglycemic agent treatment.

Metabolic monitoring programs, while useful in theory, have not yet been systematically evaluated or analyzed for their cost-effectiveness; these programs also differ in their vigor and frequency of testing. The American Diabetes Association (101) for example, recommends a fasting plasma glucose test for all patients (regardless of diagnosis) taking an atypical antipsychotic; they do not, however, recommend any sort of glucose tolerance test, something that has been shown to capture more cases of diabetes than fasting plasma glucose alone (378). In Canada, an oral glucose tolerance test is recommended for patients with schizophrenia taking an atypical agent, but only after an impaired fasting plasma glucose result (379). That no systematic studies have been
done is unfortunate, as the determination of the most effective scheme is paramount to capturing the most cases of diabetes, while minimizing the cost to government. That being said, it is known that screening for type 2 diabetes on its own, particularly in high-risk groups, is cost-effective insomuch as it reduces costs due to complications, while improving quality of life and extending life expectancy (380). According to Cohn et al. (2007), the major challenge and cost associated with such programs likely stems from the resources needed to organize and coordinate clinical services to streamline the monitoring practice (381).

4.6 Future Directions
The current findings offer several possibilities for future work. As mentioned, the hyperglycemic clamp provides little information related to glucose homeostasis. However, the application of a euglycemic-hyperinsulinemic clamp would enable, through the use of radioactive tracers, the determination of the site of putative insulin resistance. This method allows the differentiation of hepatic versus peripheral (i.e. at the myocytes and adipocyte level) insulin resistance, which has already been shown to vary across atypical antipsychotic agents in a pre-clinical model (150). The same approach can therefore be applied with the representative antagonists used here. Given the aforementioned limitations (see 4.3.2 of Caveats), it may also prove useful to explore multiple doses and at different time intervals following drug administration.

As noted, both central and peripheral signals control the metabolism of glucose. In order to distinguish the effects of a drug at either level, the compound must be administered directly into the brain where its effects can be localized. Intracerebroventricular cannula implantation enables the application of a drug into the ventricles, and both the
hyperglycemic and euglycemic-hyperinsulinemic can be applied thereafter. This method would be particularly useful in further examining the effects of prazosin and MDL100907 on glucose metabolism, specifically for prazosin as it relates to sympathetic innervation of insulin release from pancreatic beta cells.

It is well known that there exists significant cross-talk between various neurotransmitter systems; in addition to the brain-gut interaction, it may be useful to combine antagonists in different computations to further elucidate the complex mechanisms responsible for insulin signaling and glucose metabolism.

The use of different strains of rats may also prove useful in this paradigm. Here we used the metabolically intact Sprague-Dawley, with the intent of elucidating the effects of the employed antagonists on normal metabolism. However, this does not accurately reflect the vast heterogeneity of the clinical population of individuals with schizophrenia taking atypical antipsychotics. We could therefore use the Zucker rat, which has been bred as a genetic model for obesity and hypertension. As the majority of individuals taking atypical agents gain a significant amount of weight, the obese strain of Zucker rat could prove useful; interestingly, these animals have high levels of lipids and cholesterol and are insulin resistant, without being hyperglycemic. This is because their weight gain is due primarily to an increase in the number and size of adipocytes, resulting from a mutation in the leptin receptor. While this neither is a perfect model, it may help to disentangle the interaction between obesity-mediated and direct, acute, drug-induced glucose dysregulation. This is important because it remains unknown if excessive adiposity, caused by antipsychotics, is a separate contributor to metabolic sequelae, or if it shares a common mechanism with direct drug effects on acute glucose regulation.
At the very least, the obese Zucker rat would be a good model to mirror a sub-population of individuals who are obese and develop metabolic complications without a pre-existing diagnosis of diabetes.

The hyperglycemic and hyperinsulinemic clamps can also be performed on human subjects. While ethical considerations limit the nature of experimentation using humans, these techniques have indeed been used safely before (83,84). In the context of antipsychotic treatment and as part of baseline data collection, patients could be subjected to a clamp in order to ascertain early changes in glucose metabolism (e.g. hepatic insulin resistance, dysfunction in peripheral glucose uptake); ones that cannot be inferred from simple laboratory tests such as glycosylated hemoglobin or fasting plasma glucose levels. In addition to standardized laboratory tests, data from clamps could be used to generate a profile that would enable for better stratification of high and low risk patients. While no one group of patients seems exempt from metabolic complications, as underscored by the analysis of diabetic ketoacidosis with atypical antipsychotic use (see Chapter 2), identifying the most vulnerable is of practical use, both clinically and financially. It is clear that all patients require some level of monitoring, however, it may be useful to formally assign a patient to one of a small number of groups based on risk. This would, from a cost-benefit point of view, allocate more resources where they are most likely to be used, i.e. on patients that are most likely to develop metabolic complications.
4.7 Conclusions

Atypical antipsychotics now represent the mainstay of treatment for psychotic disorders. With their increased use, there has also been an increased prevalence of metabolic syndrome in the schizophrenia population, presenting an important clinical challenge to psychiatrists. Our findings highlight the highly heterogeneous contexts in which diabetic ketoacidosis takes place. This information is important for physicians and emphasizes the need to engage in careful metabolic monitoring of patients prescribed such compounds, both at baseline and at regular time points throughout treatment. We have also presented work outlining the potential receptors involved in acute glucose metabolism. This information can be used to guide drug selection and future design. In conclusion, the metabolic impact of atypical antipsychotics on human physiology remains a poorly understood process and is without a doubt exceedingly complex and multifaceted. We have, however, provided some useful information and suggested several avenues for further work in this field.
References


(18) Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998 Jan;78(1):189-225.

(19) Seeman P. Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1987;1(2):133-152.


(28) Kapur S, Remington G. Dopamine D(2) receptors and their role in atypical antipsychotic action: still necessary and may even be sufficient. Biol Psychiatry 2001 Dec 1;50(11):873-883.


(52) Balf G. Olanzapine treatment and weight gain: considering the lipid side effects of antipsychotics. Am J Psychiatry 2008 Sep;165(9):1206-7; author reply 1207.


(64) Trachtenbarg DE. Diabetic ketoacidosis. Am Fam Physician 2005 May 1;71(9):1705-1714.


of Intervention Effectiveness (CATIE) schizophrenia trial and comparison with national estimates from NHANES III. Schizophr Res 2005 Dec 1;80(1):19-32.


(85) Melkersson KI, Dahl ML. Relationship between levels of insulin or triglycerides and serum concentrations of the atypical antipsychotics clozapine and olanzapine in patients on treatment with therapeutic doses. Psychopharmacology (Berl) 2003 Nov;170(2):157-166.


(120) Boden G. Free fatty acids-the link between obesity and insulin resistance. Endocr Pract 2001 Jan-Feb;7(1):44-51.


(123) Ravichandran LV, Esposito DL, Chen J, Quon MJ. Protein kinase C-zeta phosphorylates insulin receptor substrate-1 and impairs its ability to activate phosphatidylinositol 3-kinase in response to insulin. J Biol Chem 2001 Feb 2;276(5):3543-3549.


Minet-Ringuet J, Even PC, Goubem M, Tome D, de Beaurepaire R. Long term treatment with olanzapine mixed with the food in male rats induces body fat deposition with no increase in body weight and no thermogenic alteration. Appetite 2006 May;46(3):254-262.


(250) Muller DJ, Kennedy JL. Genetics of antipsychotic treatment emergent weight gain in schizophrenia. Pharmacogenomics 2006 Sep;7(6):863-887.


(280) Fletcher PJ, Tampakeras M, Sinyard J, Higgins GA. Opposing effects of 5-HT(2A) and 5-HT(2C) receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. Psychopharmacology (Berl) 2007 Dec;195(2):223-234.


(305) Richmond JE, Codignola A, Cooke IM, Sher E. Calcium- and barium-dependent exocytosis from the rat insulinoma cell line RINm5F assayed using membrane capacitance measurements and serotonin release. Pflugers Arch 1996 Jun;432(2):258-269.


Davis JM, Chen N, Glick ID. A meta-analysis of the efficacy of second-generation antipsychotics. Arch Gen Psychiatry 2003 Jun;60(6):553-564.


(377) Correll CU, Frederickson AM, Kane JM, Manu P. Does antipsychotic polypharmacy increase the risk for metabolic syndrome? Schizophr Res 2007 Jan;89(1-3):91-100.

