Charles M. Beasley, Jr., and Roy Tamura: What We Know and Do Not Know by Conventional Statistical Standards About Whether a Drug Does or Does Not Cause a Specific Side Effect (Adverse Drug Reaction)

Overview

Charles M. Beasley, Jr.: Reply to Edward Shorter's comments
Olanzapine and Diabetes Mellitus, Evolution of Data – Illustrating the Difficulties in Identification of Adverse Drug Reactions

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First, we want to thank Prof. Shorter for his interest in our series of postings and his willingness to provide comment. We have not addressed the matter of understanding the relationship of **second-generation antipsychotics (or specifically olanzapine) with glucose homeostasis dysregulation and diabetes mellitus in our writing. However, Prof. Shorter's specific interest in** diabetes mellitus afforded us a reason to review the evolution of studies of olanzapine and dysregulation of glucose homeostasis subsequent to the early 2000s when our responsibilities within Eli Lilly and Company were shifted away from olanzapine. Other than the studies described below sponsored by Lilly and the manuscript of Ader (2005) for which an abstract appeared several years earlier, we were not familiar with any of the work that we summarize and compare.

Also, Prof. Shorter's interest allows us to illustrate two of the difficulties in detecting (with reasonable medical certainty if not 'proving' by the standard required for efficacy) an adverse drug reaction that we briefly discussed in our writing, and another difficulty that we did not discuss. We did not discuss this third difficulty because our focus was on adverse events that might or might not be adverse drug reactions that can be assessed based exclusively on events only described with text. Such events are binary entities. An investigator writes that a subject has Stevens-Johnson Syndrome (not present before entering a study). That subject has shifted from the state of absence of Stevens-Johnson Syndrome to the state of presence of the disorder, at least per the investigator.

With diabetes mellitus as an example, there are objective numerical data collected systematically throughout a study (e.g., fasting glucose, random glucose, HbA1c, urine glucose) available to determine the accuracy of text attribution of an event to a subject or attribute an adverse event to a subject even if an investigator has not described an adverse event in text. Additionally, the incidences of abnormalities of such objective numerical data can be compared between treatments to contribute to the determination of whether the test drug is causing an adverse drug reaction as identified by values considered to be indicative of pathology rather than simply relying on the comparison of incidences of text attributed adverse events that are treatment emergent..

As said above, our earlier work, we addressed adverse events where the only data sources for analysis are the text-based reports of occurrences of adverse events. Continuing with our example of Stevens-Johnson Syndrome, there are no laboratory data collected systematically throughout a study that would allow confirmation of presence or absence of Stevens-Johnson syndrome for an individual subject for whom an investigator recorded the adverse event of Stevens-Johnson syndrome or to identify the presence of the disorder even if the investigator did not record it as an adverse event. Although objective data (such as tissue biopsy with microscopic examination) might be obtained to confirm or refute the attribution of an event to a subject by an investigator, for most adverse events, there are no systematically collected data that allow identification of an event based exclusively on those objective, usually numerical, data. While having objective numerical data to aid in the identification of true adverse events is usually helpful, this data source can introduce certain difficulties as we will note below.

The first difficulty in determining adverse drug reactions that we discussed in our series of postings is when an adverse event that is an adverse drug reaction has its onset well after the initiation of the drug. The negative effect of this difficulty on the identification of adverse drug reactions increases as the central tendency estimate of time to onset of the adverse drug reaction increases and if the rate of onset increases over time. A study might require an impractical number of subjects on both drug and placebo, followed for an

impractical length of time (especially for placebo treatment) to detect even slim evidence of a difference between groups in either the incidence of text described adverse events or subjects with numerical data meeting objective criteria for an adverse event. It is likely that special analytical time-based methods would be required to be most sensitive to differences in incidence if there is a time delay in onset as also briefly discussed. Such methods are not routinely applied to all adverse events recorded nor to treatment-emergent instances of values of objective safety parameters reflective of pathology. Such analyses are often applied on a for-cause basis when more routine incidence-based analyses (difference in incidences or the ratio of incidences) suggest differences between treatment groups. Such a time delay is expected for most cases of diabetes mellitus that might be adverse drug reactions to second-generation antipsychotics, and this will be underscored when we discuss olanzapine analyses specifically.

The second difficulty impacting the detection of adverse drug reactions that we discussed in more detail is the difficulty in detecting differences between treatment groups when there is a relatively high incidence of new-onset cases of an event that are not adverse drug reactions but are completely independent of the drug treatment (background incidence) compared to the incidence of new-onset cases that are adverse drug reactions. This difficulty when a relatively high background incidence is present was illustrated in Section 4 of our work. We believe this difficulty is also relevant to finding an of excess diabetes mellitus in the development databases for second-generation antipsychotics, or at least for the agent with which we are most familiar, olanzapine.

The third difficulty, not addressed previously in our writing, is that of a ‘noisy parameter’ or a low ‘signal-to-noise ratio’ when dealing with numerical data relevant to the identification of an adverse event, a difficulty quite familiar to engineers. Here, ‘noisy’ refers to a large magnitude of unexpected and unexplained within-subject and between-subject variability across time. In the domain of drug safety, nothing illustrates this problem more clearly than the assessment of group changes in QTc. In Thorough QT Studies, the signal of interest is the maximal mean difference from placebo of 5-10 ms. However, normal within-subject beat-to-beat variability can be 25 ms even with optimal recording and measurement techniques with the collection under optimal conditions. Venous glucose

concentrations that are most useful for the assessment of glucose homeostasis and the diagnosis of diabetes mellitus are intended to be collected in a between 10-12 hour fasting state. The difficulties in obtaining such fasting values in subjects with schizophrenia when most of the values collected are collected on an outpatient basis should be easily appreciated by most clinicians who treat patients with schizophrenia. As a result of the collection procedures in the studies described below and other factors as well, the observed glucose values, especially in analyses on long-term data with olanzapine were very 'noisy'.

In order to understand the evolution of the understanding of the relationship between olanzapine as a specific example of second-generation antipsychotics and diabetes mellitus, it is important to review the evolution of the numerical diagnostic criteria[i] for that disease (Kumar, 2016) because there were substantial changes in these criteria during the period in which the early second-generation antipsychotics (e.g., risperidone, olanzapine, quetiapine, ziprasidone) were initially developed and evaluated (the mid-1980s through mid-1990s):

- World Health Organization (WHO) 1980 criteria
    - Diabetes mellitus (DM)
        - Fasting glucose: ≥7.8 mmol/L (140 mg/dL), or
        - Post-glucose [glucose tolerance test load of 75 g of glucose]: ≥11.1 mmol/L (200 mg/dL)
    - Impaired glucose tolerance (IGT)
        - Fasting glucose: <7.8 mmol/L (140 mg/dL) and post-glucose [glucose tolerance test load of 75 g of glucose]: ≥7.8 mmol/L but <11.1 mmol/L
    - Impaired fasting glucose (IFG)
        - Not defined
- American Diabetes Association (ADA) 1997 criteria and WHO 1999 criteria
    - DM

        - Fasting glucose: ≥7.0 mmol/L (126 mg/dL), or
        - Post-glucose [glucose tolerance test load] (or post-prandial / non-fasting): ≥11.1 mmol/L
    - IGT
        - Fasting glucose: <7.0 mmol/L (if measured) and post-glucose [glucose tolerance test load of 75 g of glucose]: ≥7.8 mmol/L but <11.1 mmol/L
    - IFG
        - Fasting glucose ≥6.11 mmol/L (110 mg/dL) but <7.0 mmol/L and post-glucose [glucose tolerance test load of 75 g of glucose]: <11.1 mmol/L (if measured)
- ADA 2003
    - Modified criteria for IFG
        - Fasting glucose ≥5.5 mmol/L (100 mg/dL) but <7.0 mmol/L and post-glucose [glucose tolerance test load of 75 g of glucose]: <11.1 mmol/L (if measured)
- ADA 2010 and WHO 2011
    - Incorporated HbA1c into diagnostic criteria
        - DM – HbA1c: ≥6.5%
        - IGT and IFG – HbA1c: 5.7%-6.4%

While the diagnostic criteria for diabetes mellitus based on fasting values decreased, the criteria based on a random value, unlikely to be fasting has not changed. To be precise, the random value should only be applied to a value obtained at least 2 hours after ingesting an oral 75 g glucose load in a glucose tolerance test but by inference any value observed ≥11.1 mmol/L (200 mg/dL) 2 hours or more after a meal or heavy carbohydrate load would be strongly suggestive of diabetes mellitus and by current diagnostic criteria, values

between 7.8-11.1 mmol/L (140-200 mg/dL) at any time at least 2 hours following such ingestion might suggest some degree of impaired glucose homeostasis.

1. Olanzapine Clinical Trial Data: An Illustration of the Impact of 'Noise' Combined with the Impact of Delayed Onset and Relatively High Background Incidence

Olanzapine clinical development Phase III studies were conducted between 1991-1995 with data analyses and preparation of regulatory submission documents occurring in 1995, a time at which diabetes mellitus was diagnosed based on a fasting plasma glucose ≥7.8 mmol/L (140 mg/dL) and HbA1c was not considered useful for diagnosis (and was not collected as a routine laboratory analyte during development studies). The initial development program for risperidone had occurred several years earlier, and the development programs for quetiapine and ziprasidone had occurred in the same period as that for olanzapine.

The initial development program for olanzapine for the treatment of psychosis[ii] included five studies with extensions:

1. Placebo-controlled and haloperidol-controlled, 3 variable doses of olanzapine (5±2.5 mg/d, 10±2.5 mg/d, 15±2.5 mg/d), 6-weeks, inpatient with the transition to outpatient. For subjects showing an adequate response, a 1-year continued double-blind extension was available. The extension was further extended to indefinite until approval. (Beasley, 1996a)
2. 1 mg/d (pseudo-placebo) controlled and haloperidol-controlled, 3 variable doses of olanzapine (5±2.5 mg/d, 10±2.5 mg/d, 15±2.5 mg/d), 6-weeks, inpatient with the transition to outpatient. For subjects showing an adequate response, a 1-year continued double-blind extension was available. The extension was further extended to indefinite until approval. (Beasley, 1997)
3. Placebo-controlled, 2 fixed doses of olanzapine (1 mg/d and 10 mg/d), 6-weeks, inpatient with the transition to outpatient. All subjects who completed at least three weeks and were still substantially symptomatic could switch to open-label olanzapine with an indefinite extension until approval. The open-label extension was also available to subjects completing the study. (Beasley, 1996b)

4. Haloperidol-controlled, one variable dose olanzapine (5-20 mg/d), 6-weeks, outpatient or inpatient. An indefinite open-label extension until approval was available to subjects (Tollefson, 1997)
5. Placebo-controlled, one variable dose of olanzapine (2-8 mg/d), 6-weeks, for subjects with psychotic symptoms and a diagnosis of Alzheimer's Disease. This study was not intended to support registration for an indication of psychosis with Alzheimer's Disease but was intended to allow for the study of at least 100 subjects ≥65 years of age (few subjects in this age group with schizophrenia enter randomized clinical trials). (Unpublished)

In 1995, at the time of data analyses for initial submission, the following numbers of subjects[iii] were available for analyses:

- Olanzapine (not including 1 mg/d dose) vs. placebo: 248 – 118
- Olanzapine (not including 1 mg/d dose) vs. haloperidol (up to 6 weeks): 1,796 – 810
- All olanzapine (including 1 mg/d dose): 2,500 with some subjects treated for more than four years

The following summaries of analyses performed are based on our recall in December-2018 as neither the sources documents with the results of the analyses nor the original US Prescribing Information for olanzapine were available to review.

The potential effects on glucose homeostasis must be considered in the context of any changes in body weight, especially where those changes are probably increases in adipose tissue (especially visceral adipose tissue). Weight increases with olanzapine were well characterized in the initial development studies and described in the original US Prescribing Information. Our recollection, in which we are quite confident of its accuracy, is that this Prescribing Information noted that approximately 50% of subjects in long-term treatment with olanzapine with a median exposure of approximately six months gained ≥10% body weight. Based on the manuscripts specifically reporting weight changes with olanzapine and haloperidol, in Study 2 above, haloperidol-treated subjects experienced a mean decrease in weight and in Study 4 above experienced a mean increase in weight of only 0.02 kg with 4.6% losing ≥7% weight

compared to only 2.5% of olanzapine-treated subjects experiencing such weight loss. The differences between olanzapine and haloperidol for both mean change (an increase of 1.88 kg with olanzapine) and proportions gaining ≥7% were both statistically significant in Study 4.

The weight gain data that were available at the time of approval and included in the US Prescribing Information are important in the interpretation of the glucose data and their analyses for the initial development studies and a subsequent set of analyses conducted using a larger set of data performed in the late 1990s. Additionally, these weight gain changes would allow for a conclusion that olanzapine would be temporally associated with some emergent diabetes mellitus (differs from definitive proof), especially with long-term use, on the part of a reasonably informed clinician.

The analyses of glucose values at the time of submission (conducted between January – August 1995) were interpreted as not suggesting an alteration in glucose homeostasis associated with olanzapine treatment. The analyses included consideration of both mean changes from baseline to endpoint and the emergence of both high and low outlier values. These analyses considered both placebo and haloperidol comparisons with simple pooling of all direct comparative data, excluding the 1 mg/d dose of olanzapine. Additionally, all data (non-comparative, open-label) data for olanzapine (including the 1 mg/d dose) were similarly analyzed and considered. While some analytes (e.g., CPK) were subjected to more complex analyses based on the results of the initial set of analyses, the initial set of analyses of glucose values were not interpreted as suggesting the need for such additional analyses. What some might consider a potentially more objective review of the data and analyses results (i.e., by the FDA) can be considered concordant with the Lilly interpretation as no additional analyses were requested of Lilly by FDA. We recall that there were a low incidences (but not zero incidences) of treatment-emergent adverse events described with the terms of “hyperglycemia” and “diabetes mellitus” during olanzapine treatment and if this is correct, they would likely have been included in lengthy lists of adverse events that might or might

not be adverse drug reactions in the Prescribing Information. However, no specific Prescribing Information text required by regulatory authorities did not discuss glucose, glucose homeostasis, or diabetes mellitus in any dedicated section or any detail.

Based on spontaneous adverse event reports received by Lilly and published case reports and case series, Lilly undertook an extensive set of analyses of all available clinical trial data in the late 1990s. Additional data were available with even longer periods of treatment relative to the data available in 1995. The results of this work were presented in several public, scientific forums with the first presentation by Beasley in 2000 (Beasley, 2000). The interpretation of these analyses was simply that the analyses failed to support the hypothesis of an association between olanzapine treatment and the development of hyperglycemia or diabetes mellitus. This interpretation is highly limited; these analyses could not be interpreted as supporting the hypothesis of a lack of association between olanzapine and the development of hyperglycemia or diabetes mellitus. The data were complex, particularly considering the 1997 ADA numerical criteria (see above, changed since the 1995 initial data analyses) relevant to diabetes mellitus and impaired glucose homeostasis. Many subjects' baseline values were elevated. For the group, as well as individual subjects, variability over time was much greater than expected. The magnitude of variability was such that some diabetologists that reviewed the results of the analyses questioned the veracity of the values. One source probably contributing to the variability was the potential for sample collection intended to be in the fasting state often being in a non-fasting state and potential within the 2-hour post consumption window during which even values ≥200 mg/dL are difficult to interpret.

Subsequent analyses of the Lilly clinical trial database (after the late 1990s analyses) are best summarized in the US Zyprexa® Prescribing Information updated in 2010 copied verbatim below.

"Olanzapine Monotherapy in Adults — In an analysis of 5 placebo-controlled adult olanzapine monotherapy studies with a median treatment duration of approximately three weeks, olanzapine was associated with a greater mean change in fasting glucose levels

compared to placebo (2.76 mg/dL versus 0.17 mg/dL). The difference in mean changes between olanzapine and placebo was greater in patients with evidence of glucose dysregulation at baseline (patients diagnosed with diabetes mellitus or related adverse reactions, patients treated with anti-diabetic agents, patients with a baseline random glucose level ≥200 mg/dL, and/or a baseline fasting glucose level ≥126 mg/dL). Olanzapine-treated patients had a greater mean HbA1c increase from baseline of 0.04% (median exposure 21 days), compared to a mean HbA1c decrease of 0.06% in placebo-treated subjects (median exposure 17 days). In an analysis of 8 placebo-controlled studies (median treatment exposure 4-5 weeks), 6.1% of olanzapine-treated subjects (N=855) had treatment-emergent glycosuria compared to 2.8% of placebo-treated subjects (N=599). Table 2 shows short-term and long-term changes in fasting glucose levels from adult olanzapine monotherapy studies."

"Table 2: Changes in Fasting Glucose Levels from Adult Olanzapine Monotherapy Studies"

			Up to 12 Weeks Exposure		At Least 48 Weeks Exposure	
Laboratory Analyte	Category Change (at least once) from Baseline	Treatment Arm	N	Patients	N	Patients
Fasting Glucose	Normal to High (<100 to ≥126 mg/dL)	Olanzapine	543	2.2%	345	12.8%
		Placebo	293	3.4%	NA[1]	NA[1]
	Borderline to High (≥100&<126 to >126 mg/dL)	Olanzapine	178	17.4%	127	26.0%
		Placebo	96	11.5%	NA[1]	NA[1]

[1] Not applicable

"Olanzapine Monotherapy in Adolescents — The safety and efficacy of olanzapine have not been established in patients under the age of 18 years. In an analysis of 3 placebo-controlled olanzapine monotherapy studies of adolescent patients, including those with Schizophrenia (6 weeks) or Bipolar I Disorder (manic or mixed episodes) (3 weeks), olanzapine was associated with a greater mean change from baseline in fasting glucose levels compared to placebo (2.68 mg/dL versus -2.59 mg/dL). The mean change in fasting glucose for adolescents exposed at least 24 weeks was 3.1 mg/dL (N=121). Table 3 shows short-term and long-term changes in fasting blood glucose from adolescent olanzapine monotherapy studies."

"Table 3: Changes in Fasting Glucose Levels from Adolescent Olanzapine Monotherapy Studies"

			Up to 12 Weeks Exposure		At Least 24 Weeks Exposure	
Laboratory Analyte	Category Change (at least once) from Baseline	Treatment Arm	N	Patients	N	Patients
Fasting Glucose	Normal to High (<100 to ≥126 mg/dL)	Olanzapine	124	0%	108	0.9%
		Placebo	53	1.9%	NA[1]	NA[1]
	Borderline to High (≥100&<126 to >126 mg/dL)	Olanzapine	14	14.3%	13	23.1%
		Placebo	13	0%	NA[1]	NA[1]

[1] Not applicable

We could not conduct inferential analyses on the mean change data in the two paragraphs above because the standard deviations are not provided. But the categorical (outlier) shifts in Tables 2 and 3 could be analyzed simply pooling the multiple studies and not appropriately adjusting for differences among the studies.

2-Sided Fisher's Exact Test p-Values – Categorical Change in Fasting Glucose, Olanzapine vs. Placebo

Age Group	**Change Category**	**p-Value**
Adults	Normal to High	0.3653
	Borderline to High	0.2213
Adolescents	Normal to High	<0.0001, less with Olanzapine
	Borderline to High	0.2222

These aggregated placebo-controlled data from 2010 Prescribing Information included larger sample sizes than the initial submission data, but the length of treatment where a comparison to placebo could be made was still extremely short. Median exposure times were of a length such that HbA1c changes are not relevant. The mean change data for glucose might have demonstrated statistically significant differences with a greater mean increase with olanzapine. Statistical significance would almost certainly be the case with the adolescent mean change results. In the general case, mean change differences (more with the drug than placebo) support the potential for the existence of a mechanistic process. Application of inferential statistical methods to mean changes in numerical safety data is prone to type 1 error (false positive identification of a difference) without adjustment for the multiplicity of comparisons that can be made. In most development programs for potential new drugs, there are more than 30 laboratory analytes that are measured, and vital signs and anthropomorphic characteristics add to this number. However, it is a difference in the incidence of treatment-emergent outliers that are more informative regarding a clinically significant process and less prone to type 1 error when subjected to inferential analyses. In the

four treatment-emergent outlier comparisons (change from normal to high [diabetic], change from borderline [prediabetic] to high [diabetic]) in both adults and adolescents, the only olanzapine-placebo comparison difference to reach statistical significance was a shift from normal values to high values in adolescents with the greater incidence associated with placebo. Our overall impression is that these 2010 data are more clearly suggestive of an adverse change in glucose homeostasis in temporal association with olanzapine but with these clinical trial data in isolation it would be difficult to conclude that olanzapine causes diabetes mellitus either through a direct or indirect process. These results best illustrate the combined problems of insufficient length of comparative treatment data for adverse drug reactions, with an infrequent rate of occurrence, with a high background incidence of the adverse event (same event as the adverse drug reaction), and further combined with 'noisy' data. Note that in the adolescent group 1 out of 53 subjects, in a period of up to only 12 weeks shifted from a normal baseline glucose value to a glucose value in the diabetic range for at least one measurement. We do not know about the variability in any of the subjects' glucose values, and with these data representing up to 12 weeks of treatment and glucose measurements being obtained potentially weekly, the value ≥126 mg/dL could be a single value out of 12. These data might, therefore, underscore the potential problem of variability in data used to identify an adverse event, the 'noise-to-signal' ratio problem.

Lilly extended its research efforts regarding glucose homeostasis and olanzapine in several ways following the late 1990s analyses of available clinical trial data. Although HbA1c had not yet become a standard for diagnosing diabetes mellitus and was considered only useful for assessing average glycemic control in patients with diagnosed diabetes mellitus, HbA1c was added as a standard laboratory analyte collected in studies and fructosamine was also added. Fructosamine is an analyte comparable to HbA1c, but while HbA1c assesses changes in average glucose throughout several months, fructosamine assesses these changes throughout several weeks. Measurement of both analytes served as an attempt to address the 'noise-to-signal' ratio problem with venous glucose measurements, where many of these values were likely for samples collected in a non-fasting state.

Additionally, Lilly undertook the conduct of three glucose clamp studies, one to assess the release of insulin from the pancreas and two to assess the body's sensitivity to insulin.

2. Clamp Studies and Mixed Meal Tolerance Tests

We will discuss three types of studies below (two types of clamp studies and the Mixed Meal Tolerance Test). The first type of clamp study is the hyperglycemic clamp study, the gold standard for assessing the capacity of the pancreas (β-cells) to produce and release insulin appropriately in the face of exposure to glucose. The second type of clamp study is the hyperinsulinemic-euglycemic clamp study, the gold standard for assessing the body's tissues ability to respond appropriately to insulin – take the glucose into tissues (liver, muscle, and fat) and for those tissues that can produce and release glucose (liver through gluconeogenesis and glycogenolysis), decrease that production and release of glucose. The third type of study is a Mixed Meal Tolerance Test (MMTT) that can assess both changes in glucose and changes in insulin in response to a standardized meal. This test allows for the estimation of both pancreatic insulin production and release as well as insulin sensitivity, although without the precision of the two clamp studies. The MMTT is being discussed because it was included in one research effort sponsored by Lilly and it was also included along with a hyperinsulinemic-euglycemic clamp study in work by another research group and results from both study types were combined in one assessment of changes in glucose homeostasis.

Some additional background information on the methods, the analytes measured, and parameters measured and computed in these studies will help understand the descriptions of research results that follow And the substantial inconsistencies in results. In the discussions below, we will focus on the analytes insulin and glucose, but other analytes of potential interest such as C-peptide and free fatty acids could be analyzed and were analyzed in some studies.

We noted above that the hyperinsulinemic-euglycemic clamp study is the gold standard for assessing the body's tissues' ability to respond to insulin. This ability to appropriately respond is referred to as appropriate insulin sensitivity. Peripheral insulin sensitivity is a measure of the body's tissues to take up glucose in response to a given amount of insulin. Hepatic insulin sensitivity is a measure of the liver's decrease (or complete cessation) of the production of glucose in response to a given amount of insulin. Whole-body insulin sensitivity is a combination of hepatic insulin sensitivity and peripheral insulin sensitivity (the greater the whole-body insulin sensitivity, the less glucose is produced, and the more that is taken up in response to a given amount of insulin).

Whole-body insulin sensitivity is best determined in the hyperinsulinemic-euglycemic clamp study, and if radio-tracer labeled glucose is used in that study, hepatic and peripheral insulin sensitivity can be determined separately.

In the hyperglycemic clamp study, the adequacy of insulin production and release can be assessed based simply on the absolute magnitude of insulin produced and released or that insulin production and release can be adjusted for whole-body insulin sensitivity (requiring a hyperinsulinemic-euglycemic clamp study or some other measure of whole-body insulin sensitivity). This adjustment might be particularly important if assessing insulin production and release before and after a treatment that might change whole-body insulin sensitivity. If this whole-body insulin sensitivity has been changed, then it would be important to know if a corresponding compensatory change in insulin production and release had occurred. This matter of adjusting a parameter assessing the adequacy of pancreatic insulin output for whole-body insulin sensitivity is relevant when a decrease in whole-body insulin sensitivity has occurred. Whole-body insulin sensitivity can also be assessed in the hyperglycemic clamp study but with potentially less accuracy than in the hyperinsulinemic-euglycemic clamp study because insulin is not being clamped (should, however, be stable and constant late in the study) while in the hyperinsulinemic-euglycemic clamp study, both insulin (rate of infusion, not necessarily concentration) and glucose (concentration) are clamped.

Both types of clamp studies are begun in a fasting (basal) state where stable plasma insulin and glucose concentrations are expected.

In the hyperglycemic clamp study, glucose is initially infused as a bolus to result in a rapid rise to a specified glucose concentration (a concentration believed high enough to stimulate maximum insulin production and release). Then glucose is slowly infused at a sufficient rate (glucose frequently monitored and the infusion rate adjusted if necessary) to maintain a constant concentration at the target concentration. The glucose is maintained at this target concentration for some specified period after the expected achievement of maximal, steady-state insulin release. Insulin concentrations and concentrations of other analytes of interest (e.g., C-peptide) are measured frequently, especially early during the study (e.g., at 2 min intervals from 0-10 min, at 15-30 min intervals from 10-120 min, and 20 min intervals from 120-240 min) in a clamp study lasting 240 min with one target glucose concentration. An alternative approach to the hyperglycemic clamp study is to clamp glucose at multiple concentrations (each concentration referred to as a 'step'; reaching a final concentration expected to elicit a maximum insulin response) and assess the insulin response to those various concentrations of glucose and the change in the insulin response across the concentrations of glucose

In the hyperinsulinemic-euglycemic clamp study, insulin is infused at one or more different rates (steps) and glucose infused to maintain a glucose concentration approximating a fasting concentration. A maximal rate of insulin infusion would be expected to completely suppress hepatic glucose production and maximize glucose uptake into peripheral tissues. At the one or more insulin steps a steady rate of glucose infusion is achieved and held for some period. If hepatic (and therefore peripheral), as well as whole-body insulin sensitivity, is being accessed, the test is begun with an infusion of radio-tracer labeled glucose for a period before beginning the insulin infusion, and radiotracer labeled glucose is also added to the cold (unlabeled) glucose being infused to maintain euglycemia. At steady-state, before beginning the actual clamp study, circulating glucose is comprised of hepatically produced cold (non-labeled) glucose and radio-tracer labeled glucose being infused. During the clamp study, at steady-state, any cold glucose is a hepatic product plus the cold glucose infused to maintain euglycemia (a known quantity of glucose). The ratio of cold glucose to total glucose represents the ratio of

hepatically produced glucose plus infused cold glucose to total glucose. These ratios (that involve total, radiotracer labeled, and cold glucose), knowledge of the radiotracer labeled and cold infused glucose amounts/concentrations/rates, and knowledge of the glucose concentration maintained allow computation of hepatic glucose output and peripheral glucose uptake. Any lack of decrease in hepatic glucose output during hyperinsulinemia between two clam studies, one before some treatment and the other at the end of the treatment, would indicate a decrease in hepatic insulin sensitivity, possibly due to the treatment (unless the same was observed with placebo treatment). Likewise, any decrease in peripheral glucose uptake would indicate a decrease in peripheral insulin sensitivity. Assuming complete suppression of hepatic glucose production, at steady-state, the rate of glucose infusion is the rate of peripheral glucose uptake. If hepatic glucose production has not been completely suppressed, then peripheral glucose uptake is the sum of infused glucose plus the hepatically produced glucose. Whole-body insulin sensitivity is equivalent to the glucose infusion rate as this rate will increase as peripheral glucose uptake increases and hepatic glucose production decreases. In some hyperinsulinemic-euglycemic clamp study protocols, somatostatin is infused suppress any residual endogenous insulin production. Endogenous insulin production would be expected to be suppressed by an insulin concentration that completely suppresses hepatic glucose production and maximizes peripheral glucose uptake. However, somatostatin guarantees that only infused insulin (a known concentration and known constant rate) is responsible for peripheral uptake of glucose and suppression of hepatic glucose production.

The computation of separate hepatic glucose production and peripheral glucose uptake is conceptually simple in that it is based on the ratio of radio-tracer labeled (infused) to cold (hepatically produced insulin plus infused) and known rates of infusion as described above. In practice, computation is non-trivial, requiring knowledge of the complex pharmacokinetics of glucose and alternative methods have been suggested as optimal that have been used across laboratories conducting such clamp studies (Finegood,1987; Molina, 1990 among others). Furthermore, there are several alternative forms of radio-tracer labeled glucose (2-^{3}H, 3-^{3}H, 6-^{3}H, 6,6-$^{2}H_2$, 6-^{14}C) that can be used, and the use of alternative forms between studies might complicate the comparison of study results. Whole-body insulin sensitivity

is more straightforward as it is measured by the rate of glucose infusion required to maintain euglycemia at steady state during steady-state insulin infusion/concentration. Steady-state insulin might be best developed when somatostatin infusion is used.

The MMTT assesses area under the curve (AUC) for both glucose and insulin changes from a fasted state with frequent sampling before and then after a standardized breakfast through a post-standardized lunch time point. Calories are fixed for each subject the proportions of those total calories from carbohydrates, fats, and protein are standardized across subjects (e.g., carbohydrates – 55%, fats – 30%, proteins – 15%). AUCs are computed for total values and values above the baseline AUC for both glucose and insulin (and other analytes of interest). Additionally, the peak values of these analytes can be determined. Some MMTTs are be performed with a single meal.

The paragraphs above describe the basic methods of these two types of clamp studies and the MMTT. However, there are differences across laboratories in specific details of study conduct and perhaps more importantly how parameters that are measured (infusion rates of glucose and/or insulin, glucose concentrations, insulin concentrations, concentrations of other analytes of interest) are then used to compute the parameters of interest (insulin production, insulin production relative to insulin sensitivity, hepatic insulin sensitivity, peripheral insulin sensitivity, whole-body insulin sensitivity). What follows is a description of the measured and computed parameters and a high-level overview of some of the different alternatives for the computed parameters. Some of the computed parameters are computed in both types of clamp studies, but the methods of computation differ between the two types of study as well as across laboratories. Also, see Krentz (2015), Muniyappa (2008), and Bergman (1985) for discussions of measured and computed parameters in the hyperinsulinemic-euglycemic clamp study and the conduct of this study type.

Also, different abbreviations for the parameters have been used. We will use a single abbreviation throughout this summary, even if the authors of a manuscript being summarised used a different abbreviation. In some cases, these abbreviations have not been used by any authors, but they are intended to be intuitive to assist in following the text.

The following are insulin-related parameters:

- Insulin (I): measured at a specific time or during a time interval of interest
    - Multiple insulin measurements contribute to the computation of the AUC of insulin concentrations during an interval of interest
- Insulin Response (IR): the weighted average AUC of insulin concentrations during a period of interest after beginning the glucose infusion in a step of a hyperglycemic clamp study from which is subtracted the AUC of insulin at the previous step (or basal in the first or only step) [AUC-$I_{stepX+1}$ – AUC-I_{stepX}] in a hyperglycemic clamp study
    - This parameter does not take into account the adaptation of insulin response to any change in whole-body insulin sensitivity
    - Calculated but based on direct measurements of insulin concentrations
- β-cell Slope: an intermediate variable in the computation of a parameter that adjusts insulin response for any change in whole-body insulin sensitivity
    - Computed using a 3 step hyperinsulinemic-euglycemic clamp study protocol
        - β-cell slope: the slope of the linear regression line for the values of insulin response for the multiple steps
    - Used by Richard Bergman's laboratory
- Disposition Index (DI): the parameter that assesses β-cell function (insulin production and output) between two clamp studies for any changes in whole-body insulin sensitivity
    - DI = (β-cell slope) * (whole-body insulin sensitivity)

        - The above is the computation used by Richard Bergman's laboratory
        - In one Lilly manuscript, DI = IR * (whole-body insulin sensitivity) with IR being obtained from the first 10 minutes of a 1-step hyperglycemic clamp study
        - See below for various alternatives for computation of whole-body insulin sensitivity
    - Important to not confuse this with the rate of glucose disappearance that is the rate at which glucose is being taken up by tissues, abbreviated as R_d and will be discussed below as a glucose-related parameter
- Insulin infusion rate (IIR): the rate at which insulin is infused in a hyperinsulinemic-euglycemic clamp study
    - Generally adjusted for body mass or body surface area
- Change in Mean Insulin Concentration (ΔI): the change in the mean insulin concentration (or AUC) from the basal state to a step in a hyperinsulinemic-euglycemic clamp study
    - Calculated but based on direct measurements of insulin concentrations

The following are glucose-related parameters:

- Glucose Concentration at Steady State (GLU_{SS}): the weighted average AUC of glucose concentrations during a steady-state period of interest in a clamp study
- Glucose Infusion Rate (GIR): the rate at which glucose is being infused to maintain either hyperglycemia or euglycemia
    - At steady state, represents whole body (muscle, adipose tissue, hepatic tissue) glucose uptake and suppression of hepatic insulin suppression
        - Is the sum of any infused glucose plus any hepatically produced glucose
    - Often adjusted for body mass or fat-free body mass
    - Directly measured
- Change in Glucose Infusion Rate (ΔGIR); the change in the GIR from a basal state or step to a step up in a clamp study

- Rate of Glucose Appearance (R_a):
    - Two alternative definitions
        - The rate at which total glucose is added to the body and is the sum of hepatic glucose production plus infused glucose
            - With this definition, it's used in computing an insulin sensitivity index result in an index for whole-body sensitivity
        - The rate of only hepatically produced glucose (Finegood,1987 definition)
            - With this definition, it's used in computing an insulin sensitivity index result in an index for hepatic sensitivity
            - If R_a is the total glucose added, then hepatically produced glucose is R_a – infused glucose `

Rate of Glucose Disappearance (R_d):

- The rate at which glucose is taken up by tissues
- Used to compute peripheral insulin sensitivity
- Will be equivalent to GIR at steady-state if no hepatic glucose production and equivalent to GIR plus hepatically produced glucose at steady-state if there continues to be hepatically produced glucose

- Endogenous Glucose Production (EGP – will use EGP throughout this writing), also referred to as Hepatic Glucose Output (HGO)
- Change in Endogenous Glucose Production or Hepatic Glucose Output (ΔEGP): change in glucose production: this is the change across steps in a clamp study

- EGP (and R_a and R_d) and therefore the relative contributions of hepatic insulin sensitivity (suppression of hepatic glucose production by a given amount of insulin) and peripheral insulin sensitivity (facilitation of glucose uptake by tissues by a given amount of insulin) are computed in a hyperinsulinemic-euglycemic clamp study by infusing radio-tracer labeled glucose before and during a study that allows computation of hepatic glucose production that contributes to total glucose. These computations are based on the known quantity of infused glucose, the concentration of glucose, and changes in the ratio of hot to cold glucose. Multiple methods of computation exist:
    - Finegood (1987)
    - Molina (1990)

Insulin Sensitivity parameters:

- Whole-body insulin sensitivity index (ISIw):
    - Several alternative calculations that are very similar
        - ISIw = ΔGIR / (ΔI * GLUss)
            - Most common computation
        - ISIw = ΔGIR /Δ I
            - Because whole-body insulin sensitivity is being compared between pre-treatment and post-treatment states and glucose concentrations that are used in both types of clamp studies would be equivalent in the pre-treatment and post-treatment clamp studies, the GLU_{SS} term would effectively cancel out in the comparison as the two GLU_{SS} values would be equivalent under the assumption that the two clamps were successful in maintaining a constant glucose at the target value; this applies to ISIh, and ISIp discussed below as well
        - ISIw = GIR / I

        - This formula computes an ISI value that is an absolute value at a step rather than being a change from basal state to a step or from one step to another
        - Lilly studies have computed in this way
    - When determined in a hyperinsulinemic-euglycemic clamp, the ΔI (or I) is influenced by the insulin infusions (especially if a somatostatin infusion is used or at a very high rate of insulin infusion is used) but when determined in a hyperglycemic clamp, the ΔI (or I) is influenced by the insulin release elicited by the hyperglycemia; also in a single-step hyperglycemic clamp study ΔGIR is the absolute of glucose infusion during one clamp study and whole-body insulin sensitivity is relevant when comparing values before and after some treatment
- Peripheral insulin sensitivity (ISIp): ISIp = ΔR_d / (ΔI * GLUss)
    - If EGP is not 0, it adds to GIR to give R_d and at steady-state $R_d = R_a$ if R_a is being defined as total glucose being added to the system rather than just hepatic glucose
- Hepatic insulin sensitivity (ISIh): ISIh = ΔEGP / (ΔI * GLUss)
    - Lilly: ISIh = ΔEGP / $EGP_{(basal)}$ * 100 (the ΔEGP as a percentage of the basal EGP)
        - Lilly reverses the order of subtraction of basal/clamp EGP from some other authors
        - As insulin is being infused, even at the higher rate, we would believe that insulin concentrations might be changing within a clamp study and the absolute basal and step 1 values might be different before and after some treatment; therefore we believe that these two computational formulas might be different
- ISIw, ISIp, and ISIh might be calculated as absolute values at a step but are more customarily calculated as changes from basal to step 1 in a 1-step study or changes between steps in a multi-step study

In discussing the Lilly conducted and non-Lilly studies that follow, even when the summaries are detailed, not all findings will be summarized. We focus on food intake, activity level, weight, fat tissue, insulin sensitivity, and pancreatic β-cell insulin

production/release, and other factors that potentially have a direct impact on glucose homeostasis. In some instances, we summarize findings for other analytes/parameters.

Lilly Studies and Analyses

These studies and analyses were initiated in the late 1990s. All Lilly work is presented first although a supplemental analysis of one study (Hardy, 2007) and the last Lilly study (Hardy, 2011) was conducted after an important study conducted in dogs (Ader, 2005).

The hyperglycemic clamp study (Beasley, 2000; Sowell 2002) involved placebo (n=18), olanzapine (10 mg/d, n=17) or risperidone (4 mg/d, n=13) administered to healthy volunteers for 15-17 days. A single concentration (1-step) of glucose was used (200 mg/dL). The time intervals of interest for Lilly were: 1) first phase (0-10 min); second phase (10-240 min); and total (0-240 min). Steady-state for purposes of computing ISIw as considered to be between hours 3 and 4 (last of 4 hours). Important results are as follows.

Change from Pre-treatment to Post-treatment in the Change within Study in Insulin Response (IR [pmol/L]) During Time Interval of Interest:

	Placebo	**Olanzapine**	**Risperidone**
First Phase	-4.8 (3.45%)	+69.0 (38.7%)[2]	+35.4 (30.2%)[3]
Second Phase	-82.2 (17.9%)	+117.0 (22.4%)[2]	+90.0 (22.5%)[3]
TIR[1]	-~80(~13%)	+~200 (~29%)[4]	+~70 (~13%)[5]
At Steady State	-112.8	+111.0	+81.6

[1] Estimated from figure

[2] $p<0.01$, within group

[3] Inferential test results not reported, described as comparable to olanzapine

[4] p<0.01 within group; p<0.001 vs. placebo

[5] p=0.054 within group; p=0.014 vs. placebo

Change from Pre-treatment to Post-treatment in the Glucose Infusion Rate (GIR [(mmol/kg)/min] [x10^{-3}]) During Time Interval of Interest [1]:

	Placebo	**Olanzapine**	**Risperidone**
At Steady State	+0.3	-2.4	-7.8

[1] Inferential test results (if any performed), not reported

Change from Pre-treatment to Post-treatment in the Whole-Body Insulin Sensitivity Index (ISI) [x10^{-5}] During Time Interval of Interest:

	Placebo	**Olanzapine**	**Risperidone**
At Steady State	+0.92	-4.63[1,2]	-3.7[2]

[1] p<0.05, within group

[2] p≥0.05 vs. placebo

Mean weight gain with the three treatments was: placebo-0.5 kg; olanzapine-2.8 kg; risperidone-3.1 kg. Changes with both olanzapine and risperidone were significant (p<0.01) within treatment and significant (p<0.001) vs. placebo. Multivariate regression analyses with

therapy and BMI as covariates were performed for parameters of interest, including TIR and M/I to assess the potential influence of weight gain on these parameters.

Change from Pre-treatment to Post-treatment in the Change within Study in the Insulin Response (IR [pmol/L]) and Change from Pre-treatment to Post-treatment in the Whole-body Insulin Sensitivity Index (ISIw) [x10^{-5}] During Time Interval of Interest; Multivariate Regression Analyses Including BMI:

	Placebo	**Olanzapine**	**Risperidone**
IR-TIR	-111.6[1]	+42.6	-24.0
ISIw - SS	+2.8	+1.9	+2.8

[1] $p<0.05$, within group

This study's results for olanzapine can be interpreted as follows:

1. Significantly increased insulin output
2. Significantly decreased whole-body insulin sensitivity
3. Weight gain might explain the decreased whole-body insulin sensitivity, and when accounted for, olanzapine did not decrease whole-body insulin sensitivity

The hyperinsulinemic-euglycemic clamp study (Beasley, 2001; Sowell 2003) included placebo (n=19), olanzapine (10 mg/d, n=22) and risperidone (4 mg/d, n=14) administered to healthy volunteers for ~21 days. As a secondary method of assessment, an MMTT was included in the study.

The clamp study was a 2-step study (insulin infused at 20 µU/m^2/min for 3 hr and 120 µU/m^2/min for 2 hr). Somatostatin was not infused, and radiotracer labeled glucose was not infused. Steady-state for the two steps were defined as 140-160 min and 240-260 min (20 minutes excluding the last 20 minutes of each step). Glucose was clamped at 90 mg/dL.

Weight gains with olanzapine (+1.95 kg) and risperidone (+1.6 kg) were significant within treatment, and both were significantly different from the weight loss with placebo (-0.22 kg).

Change from Pre-treatment to Post-treatment in Change within Study

Treatment	**Low Dose Insulin**		**High Dose Insulin**	
	ΔGIR ((mg/kg)/min)	**ISIw[1,2]**	**ΔGIR ((mg/kg)/min)**	**ISIw[2,3]**
Placebo	↑[4]	↑	NC[5]	↓(-4.7%)
Olanzapine	NR[6,7]	↑	NC[5]	↑(+6.9%)
Risperidone	↓[8]	NC[5]	NC[5]	↓(-0.7%)

[1] Shown in a figure

[2] No significant within treatment changes or between treatment differences based on either absolute values or percentages

[3] Absolute values are shown in a figure; actual percent changes cited in the text

[4] p=0.019, within group

[5] No change

[6] Direction of any change not reported (likely a decrease as with risperidone but smaller in magnitude because not significantly different from change with placebo whereas risperidone-placebo difference was significant

[7] p=0.332 vs. placebo; within treatment p-value not reported

[8] p=0.045 vs. placebo, p=0.215 vs. olanzapine; with treatment change p-value not reported

Olanzapine was associated with slight, non-significant numerical increases in whole-body insulin sensitivity under both low and high insulin steady-state conditions.
Results of the MMTT are as follows.

Change from Pre-treatment to Post-treatment:

Treatment	**Glucose AUC ((mg/dL)/min) * 10^3)**		**Insulin AUC (µU/min * 10^3)**	
	Total	**Above Fasting**	**Total**	**Above Fasting**
Placebo	-0.18	0.32	1.0	1.0
Olanzapine	1.85[1]	0.80	1.4	1.1
Risperidone	0.47	0.50	0.7	1.0

[1.] p=0.033 vs. placebo; p=0.018 within treatment

Weight gain was better controlled with olanzapine and risperidone in this study but still not completely controlled, and placebo-treated subjects lost weight, as described above. These weight changes introduce a potential confound in the interpretation of the results of the MMTT.

This study's results for olanzapine can be interpreted as follows:

1. Based on the hyperinsulinemic-euglycemic study, whole-body insulin sensitivity was slightly numerically increased (not decreased)

2. The MMTT suggests the possibility of a slight decrease in insulin sensitivity. The results of these two study types might be viewed as contradictory. The authors (Sowell, 2003) concluded: "Nevertheless, results from the euglycemic clamps strongly suggest that the small changes in postprandial glucose and insulin observed during the MMTT in subjects treated with olanzapine or risperidone were not clinically significant and were unlikely to be due to a change in insulin sensitivity."

Some might wonder about the sensitivity of the hyperinsulinemic-euglycemic clamp study with a brief treatment period of 21 days and a small number of subjects. As the authors (Sowell, 2003) pointed out, comparable studies with β blockers demonstrate an ~25% decrement in insulin sensitivity with 4-8 weeks treatment and in 10 healthy volunteers. Prednisone, 30 mg/d for 7 days in 10 subjects was shown to be associated with a 2-fold (50%) reduction. The protease inhibitor, indinavir, was shown to be associated with a 34% decrease after a single dose. The hyperinsulinemic-euglycemic clamp method is extremely sensitive in the detection of changes in insulin sensitivity.

In response to the Ader (2005) study with its finding of a decreased pancreatic insulin response to a non-significant decrease in whole-body insulin sensitivity that will be discussed below, Lilly (Hardy, 2007) conducted additional analyses of the results of its initial hyperglycemic clamp study (Beasley 20000, Sowell 2002).

Hardy and colleagues (Hardy, 2007) analyzed insulin release during the first 10 min of the hyperglycemic clamp. The presumption was that the insulin response during this first 10 min (when bolus glucose was being administered) is the most sensitive indicator of the adequacy of pancreatic β-cell function. The following parameters were computed:

- Incremental (change from baseline) in insulin AUC from 0-10 min ($AUC_{0\text{-}10}$). Insulin, C-peptide, and glucose were measured every 2 min during this period.
- Steady-state whole-body insulin sensitivity (ISIw):

- Homeostasis model assessment-1 of insulin resistance (HOMA1-IR); calculated from mean baseline glucose and insulin values. HOMA1-IR= ((fasting plasma insulin (µU/ml) * fasting plasma glucose (mmol/L))/22.5)
    - An alternative to ISIw that estimates whole-body insulin sensitivity
- Glucose disposal index (DI):
    - Computation method 1: DI = I $AUC_{0\text{-}10}$ * ISIw
    - Computational method 2: DI = I $AUC_{0\text{-}10}$ * HOMA1-IR
    - This DI value is the product of insulin sensitivity multiplied by the change in insulin production from the pre-glucose infusion. This DI is not equivalent to Richard Bergman's laboratory's computation of DI that is the product of multiplying ISIw by the slope of the insulin production line across a 3-step hyperglycemic clamp test. Richard Bergman's laboratories method of DI computation was described above, and a result for olanzapine that found a decrement in DI computed by Bergman's method will be described below (Arder, 2005). However, this DI as computed by Hardy (2007) is a parameter that does adjust insulin output for insulin sensitivity.

Changes from Pre-treatment to Post-treatment

Treatment	**Δ $AUC_{0\text{-}10}$ Insulin (pmol/L/min)**	**Δ $AUC_{0\text{-}10}$ C-peptide (pmol/L/min)**	**ISIw (((mg/kg)/min)/(pmol/L))**	**DI - Method 1**
Placebo	-3.3	8.9	0.002	0.10
Olanzapine	44.0[1]	39.4	-0.007[3]	-0.18
Risperidone	22.9	75.8[2]	-0.005	0.07

[1] $p<0.05$ vs. placebo; $p<0.05$ within treatment

[2] $p<0.05$ within treatment

[3] $p<0.05$ within treatment

When DI was computed with method 2, the results did not change.

These analyses clearly show a robust pancreatic β-cell response (significant for insulin and directionally consistent for C-peptide) to an initial bolus of glucose and this served to support Hardy and colleagues conclusion that olanzapine did not negatively influence pancreatic function with adjustment for any change in whole-body insulin sensitivity. As additional evidence of lack of any impairment of pancreatic function, Hardy and colleagues pointed out that DI was not significantly changed, and pancreatic function is one component of DI (the other component being a measure of insulin sensitivity). Hardy and colleagues acknowledged that the two components of DI might be interdependent and that with method 1 of computing DI, both components are derived from the same clamp study. However, method 2 of computing DI used an independent measure of insulin sensitivity (based on fasting glucose and insulin) and found no significant decrement in DI.

To further argue against Ader and colleagues' (Ader, 2005) conclusions regarding the impact of olanzapine on pancreatic function, Hardy and colleagues (2007) pointed out that in the Ader and colleagues study, DI did not show an actual within-treatment statistically significant decrease and that the decrease in pancreatic function inferred by Ader and colleagues was based on olanzapine-treated animal not showing the same increase in DI as animals induced to gain adipose tissue through dietary manipulation (not exposed to olanzapine), based on a statistically significant difference between these two groups. Finally, Hardy and colleagues pointed out that another group of animals who had fat-induced obesity and had not been treated with a drug showed a 62% decrement in DI as reported by the Bergman laboratory that conducted the Ader and colleagues (2005) study and that the olanzapine-treated and fat-treated dogs received different insulin infusion rates.

Hardy and colleagues acknowledged that a 2004 Technical Review published by the American Diabetes Association concluded that olanzapine and risperidone were associated with an increased risk of diabetes. However, Hardy and colleagues stated that the results of this supplemental analysis of the Lilly hyperglycemic clamp study argued: "against a substantial and generalized impairment of insulin secretion with these agents after short-term treatment." Hardy and colleagues (2007) also stated that the results of Lilly the hyperinsulinemic-euglycemic clamp study did not show "differential effects" on insulin sensitivity "in normal individuals". Hardy and colleagues acknowledged that limitations of these two Lilly studies included small numbers of subjects (probably not a limitation given observation with other drug classes), short duration of the studies, and the lack of inclusion of subjects with risk factors for the development of diabetes (including excess adipose tissue and schizophrenia itself [literature supports the possibility of this association]).

We believe that it is important to note that in Hardy and colleagues (2007) supplemental analysis of the hyperglycemic clamp results, olanzapine is the only treatment associated with a slight (non-significant vs. placebo and within treatment) decrement in DI. This DI decrement might hint at some overall decrement in glucose homeostasis where the parameter is dependent on both pancreatic function and insulin sensitivity.

Lilly performed a third clamp study (hyperinsulinemic-euglycemic) (Hardy, 2011) along with DEXA measurements of whole-body fat mass and fat-free whole-body mass as well as CT scans to distinguish subcutaneous fat from visceral fat changes in a 12-week (sufficient time to observe initial change in HbA1c) comparison of olanzapine (n=41 completing both clamp studies, dose- 5-20 mg/d) and risperidone (n=33 completing both clamp studies, dose-2-6 mg/d). Placebo-control was not included as subjects were patients with schizophrenia and the intended treatment period was 12 weeks. While we are not including other human or animal studies in this review that did not include placebo, we include this Lilly study because this is, in part, a response regarding work that Lilly performed. Whole-body insulin sensitivity (ISIw) was adjusted to fat-free mass by adjusting the ΔGIR for fat-free mass ($ISIw_{ff}$ = (ΔGIR/(fat-free mass)) / ΔI). Also, weight was included as a covariate in the analytical model. Importantly, this study included (as a supplemental study with

separate informed consent) the administration of radio-tracer labeled glucose (3-^{3}H) to assess suppression of hepatic glucose production (specific hepatic insulin sensitivity) due to the results (Ader, 2005 summarized below). Methods for estimating the hepatic-specific insulin sensitivity can be reviewed in the manuscript. Results for changes in insulin sensitivity and selected other metabolic parameters were as follows:

Fasting Metabolic Parameters and Insulin Sensitivity Indices – Change from Pre-Treatment to Post-treatment (Clamp Study Completers – at least 1 step)

	Olanzapine (Low insulin N=41 High insulin N=40)	**Risperidone (Low insulin N=33 High insulin N=30)**
HbA1c (completers)	+0.07%[1] (N=36)	-0.04%[2] (N=28)
Fasting glucose (completers) (mg/dL)	+5.41[3]	+1.62[2]
Basal (fasting) insulin (completers) (µ/mL)	+2.60[4]	+1.25[2]
ISIh (low insulin step)	-5.28%[1] (N=5)	-4.33%[2] (N=4)
$ISIw_{ff}$ (low insulin step)	-9.0%[1]	-13.2%[5]
ISIh (high insulin step)	+9.13%[1] (N=5)	No change[2] (N=4)
$ISIw_{ff}$ (high insulin step)	-10.4%[6]	-2.1%[2]
Total fat body mass (kg)	+1.73[7]	+1.08%[2]
Total lean body mass (kg)	+1.53[7]	+0.64[2]
Total weight (kg)	+3.90[7]	+2.16[8]

[1] p-value non-significant within treatment and vs. risperidone

[2] p-value non-significant within treatment

[3] p=0.007 within treatment; p non-significant vs. risperidone

[4] p=0.002 within treatment; p non-significant vs. placebo

[5] p=0.047 within treatment

[6] p=0.036 within treatment; p non-significant vs. risperidone

[7] p<0.01 within treatment; p non-significant vs. risperidone

[8] p<0.05 within treatment

In this study without placebo-control, at the low insulin dose step, olanzapine was only associated with a numerical decrement in whole-body insulin sensitivity after adjusting the parameter to fat-free mass and including weight as a covariate in the analytical model. The lack of a statistically significant decrease in ISIw at the low insulin step was maintained even if weight was not a covariate in the analytical model and separately if ISIw was not adjusted for fat-free mass. Olanzapine was associated with a significant decrement in whole-body insulin sensitivity at the high insulin step, but the authors maintain that the low insulin step is most appropriate for assessing whole-body insulin sensitivity. For olanzapine, these results were directionally consistent with the results of the hyperinsulinemic-euglycemic clamp study conducted in healthy volunteers described above, but in that study ISIw (no adjustment to free-fat mass) neither the decrease at the low insulin nor high insulin step reached significance within a treatment or compared to placebo. The small numbers of subjects participating in the assessment of hepatic insulin sensitivity might limit the ability to draw conclusions regarding hepatic insulin sensitivity, but Hardy and colleagues argued that the sample size was adequate to detect the decrement noted by Ader and colleagues (2005) with only 10 dogs treated with olanzapine.

The Hardy (2011) work concludes the discussion of Lilly work published in this area. However, subsequent work with olanzapine has been performed by multiple laboratories.

3.2 Non-Lilly Clamp Studies and Mixed Meal Tolerance Tests

In 2005, Ader and colleagues (2005) published an extremely important and complex study. This work was conducted in the laboratory of Richard Bergman, a prominent researcher in diabetes who, along with colleagues, has made substantial contributions to the development of methods for assessing both pancreatic function and insulin sensitivity. The study is not without potential caveats concerning extrapolation of results to patients treated clinically with risperidone, olanzapine, or other second-generation antipsychotics that are associated with significant weight gain as with olanzapine.

Mongrel dogs were studied with a mean weight at baseline of 28.6 kg. The doses were: olanzapine – 15 mg/d (n=10); risperidone – 5 mg/d (n=10); placebo (n=6) with all animals allowed ad libitum access to a standardized food during the study. A separate group of 6 dogs received no treatment but were fed with a food isocaloric but higher in fat content than the dogs receiving comparative treatments. The purpose of this group was to produce a group showing a marked increase in adiposity, as was expected with olanzapine and possibly risperidone. This group was thought to be useful in determining if any alterations in glucose homeostasis that was observed with an antipsychotic that produced weight gain, were due to weight gain (in which case comparable alterations would be expected to be observed in the fat-fed, obese animals) or were more likely explained by an additional action of the drug. The dogs were treated for 4-6 weeks with a series of pre-treatment and post-treatment examinations. The dogs were treated at about a 2-fold greater dose on a body-mass basis than what is likely a maximum dose for a majority of patients treated clinically with the two test drugs. Olanzapine-treated dogs received a dose of 0.52 mg/kg/d, based on mean overall weight. For patients weighing a mean of 80 kg (accounting for greater body mass in the majority of patients compared to a population without schizophrenia), they would generally receive a maximum dose of olanzapine of 20 mg/d [0.25 mg/kg/d].

The parameters measured and computed for assessing pancreatic function and insulin sensitivity in the hyperglycemic and hyperinsulinemic-euglycemic clamp studies of Ader and colleagues (2005) described below differ in some ways from those described above for the Lilly studies and other researchers.

Three different examinations were performed on the dogs on separate days in random order at baseline and after the 4-6 weeks of treatment:

- An abdominal MRI to measure trunk fat (subcutaneous and visceral). Normalized to the volume of non-fat tissue as cm^3 / cm^3 of non-fat tissue. Expressed as cm^3.
- A hyperinsulinemic-euglycemic clamp study but using radio-tracer labeled glucose
- Labeled glucose (3-^{3}N) and somatostatin. Radio-tracer labeled glucose and somatostatin were begun 3 hr before beginning the clamp. With the use of somatostatin, insulin was infused at 0.15 mU/kg/min to standardize a basal insulin exposure. The hyperinsulinemic-euglycemic clamp was a single step with 1 mU/kg/min insulin infusion for 3 hours. The glucose concentration target was not specified in the manuscript other than as "euglycemic".
- A hyperglycemic clamp with 3 steps. Glucose was clamped at three concentrations of 100, 150, 200 mg/dL over a total of 4 hours (60-, 90-, and 90-minutes for the 3 steps).

The important findings were as follows:

Changes from Pre-treatment to Post-Treatment

	Placebo (N=6)					Fat-Fed (N=6)						Olanzapine (N=10)						Risperidone (N=10)					
	BL[1]	**EP[2]**	**Δ[3]**	**% Δ**	**P within[4]**	**BL**	**EP**	**Δ**	**% Δ**	**P within**	**P OZ[5]**	**BL**	**EP**	**Δ**	**% Δ**	**P within**	**P PLC[6]**	**BL**	**EP**	**Δ**	**% Δ**	**P within**	**P PLC**
Food intake (calories)			inc[8]					inc						inc		.031	0.82			Slight dec[9]		.17	
Anthropomorphic Parameters																							
Body weight (kg)			1.5	4.8	.006			inc						1.7	6	.001				inc		.09	
Total abdominal fat (cm3)				27-30[7]	.042	21.0	35.8	14.8	70		.60	24.9	43.4	18.5	74	<.000001	.0088	21.9	31.8	9.9	45	.005	
Visceral fat (cm3)				27-30	.046	13.5	20.5	7.0	52		.65	13.1	21.8	8.7	66	<.000001	.025	11.4	17.3	5.9	52	.001	
Subcutaneous abdominal fat (cm3)				27-30	.044	7.6	15.4	7.8	103		.60	11.8	21.6	9.8	83	.0001	.0078	10.5	14.4	3.9	37	.053	
Insulin Sensitivity Parameters																							
ISIw ((dL/min)/kg))/(μU/mL)	25.6	28.9	3.3	13	.6			-8.9			.63			-6.2		>.1				-6.9		>.1	
ISIp ((dL/min)/kg))/(μU/mL)	20.1	25.3	5.2	26	>.3							24.3	23.3	-1.0	-4	>.3		24.7	19.9	-4.8	-19	>.3	
ISIh ((dL/min)/kg))/(μU/mL)	5.5	3.3	-2.2	-40	.12							6.1	1.5	-4.6	-75	.009		4.3	3.0	-1.3	-30	.35	
β-cell function (insulin release)																							
IR-step1 (μU/mL)			inc		NS[11]									inc		.005				inc		.015	
IR-step2 (μU/mL)			inc		NS									inc		NS				inc		.007	
IIR-step3 (μU/mL)			dec		NS									NC		NS				inc		NS	
β-cell function (insulin release) – Relative to Insulin Sensitive Changes Parameters																							
β-cell response (slope) (((μU/mL)/(mg/dL)))			NC[10]		NS	0.74	2.18	1.44	195	.01		1.24	1.07	-0.17	-14	.58		0.64	0.97	0.33	52	.038	
DI (SIclamp * β-cell slope)	33.3	37.9	4.6	14	.80	14.4	32.7	18.3	127	.053	.02	35.7	24.8	-10.9	-31	.222		19.8	21.8	2.0	10	.74	

[1] Baseline (pre-treatment) mean

[2] Endpoint (post-treatment) mean

[3] Difference between endpoint mean and baseline mean (NOT mean of the individual, within dog changes)

[4] p-value for test of within treatment change

[5] p-value for test of change compared to change with olanzapine

[6] p-value for test of change compared to change with placebo

[7] Single range for percentage fat increase was reported that applied to total, visceral, and subcutaneous fat stores

[8] Increase

[9] Decrease

[10] No to minimal change

[11] Not significant

The Ader (2005) manuscript did not describe important results in an easily readable table as above but included most in lengthy text sections. Also, the authors switched between describing results as pre- and post-values, as absolute change values, as percent change values, and sometimes only by providing a p-value for a within treatment change. Additionally, there were apparent errors in the manuscript. For example, in the Abstract, the increase in subcutaneous fat with olanzapine was described as +106% while in the text this increase was described as 83% (the 83% value is correct). There are several additional examples of such inaccuracies. All of this makes the manuscript difficult to read with regard to key details. However, we doubt that any of the important findings or conclusions are inconsistent with the data collected.

This study's results for olanzapine treatment can be summarized as follows:

1. While olanzapine did not result in significantly decreased whole-body insulin sensitivity, it did result in a significant decline in hepatic insulin sensitivity
2. Insulin production did increase (significantly in step 1 and numerically in step 2 of the hyperglycemic clamp) but the increase was not sufficient for the numerical decrease in whole-body insulin sensitivity observed with olanzapine leading to a conclusion that olanzapine did negatively affect β-cell function
3. The lack of β-cell adaptation to a decrement in whole-body insulin sensitivity was not observed with risperidone or with fat-fed dogs

It should be noted that this conclusion regarding insulin production (pancreatic function) was based on parameters computed in Bergman's laboratory and that whole-body insulin sensitivity with olanzapine did not decrease significantly relative to placebo. Finally, insulin production increased, significantly in step 1 and numerically in step 2 of the hyperglycemic clamp study.

Note that the only olanzapine-placebo differences in change from baseline that were described as statistically significant were greater increases in abdominal total- and subcutaneous-fat and visceral fat with olanzapine. Lack of significant differences from placebo should be carefully considered when assessing Ader and colleagues' conclusions regarding the effects of olanzapine on factors influencing glucose homeostasis. These conclusions were based on within olanzapine-treatment statistically significant changes and absence of within placebo-treatment significant changes. The parameters measured and computed are not subjective experiences described by the dogs to the investigators during measurements and, therefore, any within treatment changes that are significant suggest a drug-related effect. However, the absence of a significant difference from placebo, or at least a strong trend toward statistical significance, raises the strong possibility that observed within treatment changes with olanzapine could be due to random variability or to systematic factors in study methods other than drug influence.

Google Scholar and Pub Med searches on the text string ("olanzapine" and ("diabetes" or "glucose")) performed on December 22, 2018, returned 19,500 and 893 citations, respectively. The text that follows is not an exhaustive review of subsequent studies not sponsored by Lilly but brief summaries of studies we view as important in understanding the phenomenon under discussion or suggesting a hypothesis about the phenomenon. These are presented first for human studies and then for animal studies, both sets in chronological order of publication. For both human and animal studies, we are only considering those that employed the hyperinsulinemic-euglycemic clamp method (specifically not considering any that employed an oral glucose tolerance test or the frequently sampled intravenous glucose test) and that included placebo (vehicle in the case of animal studies) control treatment. We are also not reviewing comparisons of olanzapine with another second-generation antipsychotic that did employ a hyperinsulinemic-euglycemic clamp assessment but did not include a placebo-treatment arm. A Pub Med search on the text string (("olanzapine" AND ("placebo" OR "vehicle")) AND ("hyperinsulinemic" or "euglycemic")) performed on December 23, 2018, returned 11 unique citations (one citation was included twice for a total of 12 citations), one of these being the Sowell (2003) publication, one human and one animal study did not include placebo controls. This search text string within Pub Med was not sufficient to return all manuscripts describing a hyperinsulinemic-euglycemic clamp study of olanzapine that included a placebo control as evidenced by the search not returning the Ader (2005) manuscript for example. This search did, however, add to my list of manuscripts of which we were previously aware through personal knowledge and other searches. A similar search but using (("olanzapine" AND ("placebo" OR "vehicle")) AND "hyperglycemic") performed on February 6, 2019, to search for hyperglycemic clamp studies yielded no additional manuscripts. This hyperglycemic search also failed to find the Ader (2005) manuscript. There are many additional studies conducted in both humans and animals that employed both types of clamp studies but included only active drugs and no placebo/vehicle control.

Kopf and colleagues (2012) performed a one-stage hyperinsulinemic-euglycemic clamp assessment followed immediately by a hyperglycemic clamp assessment in 10 subjects. The treatment design was a 3-way crossover, single oral dose of placebo, olanzapine 10 mg, and amisulpride 200 mg administered 1 hour before initiation of the clamp studies. In the hyperinsulinemic-euglycemic clamp

component, ISIw with olanzapine was only slightly numerically less than with placebo. Based on C-peptide, the pancreatic β-cell response was virtually identical between olanzapine and placebo. The insulin response (C-peptide response) relative to the slight decrease in ISIw (DI) was not computed. This single, oral dose study without a positive finding is unlikely to be particularly informative regarding changes in glucose homeostasis associated with second-generation antipsychotics.

The final human study was reported by Teff and colleagues (2013). Healthy, non-overweight volunteers not engaged in active exercise other than walking (10 per treatment) were treated for 9 days with olanzapine (10 mg/d) associated with weight gain, aripiprazole (10 mg/d) associated with less weight gain, or placebo. Importantly, subject activity levels (daily caloric expenditure) were actively encouraged to be maintained at pre-study levels throughout the treatment period. Subject underwent both an MMTT and a hyperinsulinemic-euglycemic clamp pre- and post-treatment. Radiotracer labeled glucose was used in the clamp study. Insulin was initially infused at 1.6 μU/kg for 10 minutes followed by 0.8 μU/kg for 240 minutes. Glucose was clamped at 90 mg/dL. The MMT was with a single breakfast meal. The meal consisted of 10 kcal/kg with 45% carbohydrate, 15% protein, and 40% fat calories. Radio-tracer labeled glucose (6,6-$^{2}H_{2}$) was included in the meal. Parameters of interest were assayed for 330 minutes from initiation of the meal.

In this hyperinsulinemic-euglycemic clam study, the following parameters that were measured or computed were as follows:

- From the hyperinsulinemic-euglycemic clamp study
    - GIR
    - R_d
    - EGP both at basal state and during the 1-step hyperinsulinemic-euglycemic clamp study
- From the MMTT
    - ΔIR (an increase from baseline in insulin AUC during the first 10 minutes after meal initiation)

- From data from both the hyperinsulinemic-euglycemic clamp study and the MMTT
  - $ISIp = \Delta R_d / (\Delta IR * GLU_{SS})$
  - $DI = ISIp * \Delta IR$
    - This DI computation is more analogous to that used by Hardy (2007), except that it is based on peripheral insulin sensitivity rather than whole-body insulin sensitivity, than it is analogous to DI as computed in Bergman's laboratory (Ader, 2005)

The results of the study are summarized in the table below:

Change from Pre-treatment to Post-treatment

Parameter	Placebo (N=10)					Olanzapine (N=10)						Aripiprazole (N=10)					
	BL[1]	EP[2]	Δ[3]	% Δ	P within	BL	EP	Δ	% Δ	P within[4]	P PLC[5]	BL	EP	Δ	% Δ	P within	P PLC
Energy intake and Output																	
Food intake (calories/day)[4]	3300	3500	200		NS[5]	3750	4200	450		NS		3500	3200	-300		NS	
Activity (steps/day)[4]	6200	6000	-200		NS	10000	9000	-1000		NS		7000	6600	-400			
Body weight (kg)	68.1	68.5	0.4		NS	65.9	66.7	0.8			NS	67.8	67.3	-0.5			.08
Insulin Sensitivity Related																	
EGP_{clamp} (mg/kg)/min		slight dec[6]			NS			slight dec		NS				slight dec		NS	
R_d (mg/kg)/min		slight dec			NS				-26	<.05					-28	<.05	
R_d/I ($Rd/(\mu U/mL)_{SS}$)		-.007			NS			-.025		<.01				-.028		<.01	
GIR		slight dec			NS				-21	<.05					-23	<.05	

ISIp (mg/kg)/min		slight inc[7]			NS	11.7	9.1	-2.6	-22	<.05		9.2	7.0	-2.2	-24	<.05	
Pancreatic Function Related																	
IR (µU/mL)/10min (from MMTT)		slight dec			NS	26.0	38.2	12.2	47	<.05		23.8	27.4	3.6	15	NS	
DI (SI * IR)		slight inc			NS			inc		NS				inc		NS	

Change from Pre-treatment to Post-treatment

Parameter	**Placebo (N=10)**					**Olanzapine (N=10)**						**Aripiprazole (N=10)**					
	BL[1]	**EP[2]**	**Δ[3]**	**% Δ**	**P within**	**BL**	**EP**	**Δ**	**% Δ**	**P within[4]**	**P PLC**	**BL**	**EP**	**Δ**	**% Δ**	**P within**	**P PLC[5]**
Glucose AUC			1600					850			NS[5]			1600			NS
Insulin AUC			600	5	NS			4650	73		<.05			3000	24		NS
ΔIR_{1-10}	See Previous Table					See Previous Table						See Previous Table					
C-peptide AUC			16		NS			170			NS			173			NS
C-peptide/insulin AUC ratio	.12	.10	.02		NS	.18	.10	-.08		<.05		.13	.10	-.03		<.05	
GLP-1 AUC			30					220			<.05			-85			NS
Glucagon AUC			-2200					1900			<.05			-2200			NS

Change from Pre-treatment to Post-treatment

[1] Pre-treatment

[2] Post-treatment

[3] Some values estimated from figures

[4] Within treatment

[5] Versus placebo

[6] Decrease

[7] Increase

Pre-treatment to Post-treatment Changes in Change from within study from basal/fasting - MMTT

[1] Pre-treatment baseline

[2] Post-treatment endpoint

[3] Some values estimated from figures

[4] Within treatment

[5] Versus placebo

Recall that C-peptide is a cleavage by-product product of insulin production (links insulin A-chain and B-chain and is cleaved). In general, C-peptide is a marker of insulin production and release as is insulin itself. Insulin is cleared by hepatic metabolism. Therefore, as the C-peptide/insulin ratio decreases, it is likely that there is a decrease in the hepatic metabolism of insulin that can be a response to decreased insulin sensitivity.

This study's results for olanzapine treatment can be summarized as follows:

1. Did not increase post-prandial glucose
2. Reduced peripheral insulin sensitivity
3. Did not reduce (significantly) hepatic insulin sensitivity
4. Did not decrease (actually increased) the pancreatic β-cell insulin response, with a reduction in whole-body insulin sensitivity (DI increased)
5. Prandial glucagon increase might increase prandial/post-prandial glucose concentration
6. Increase in GLP-1 and a decrease in hepatic clearance of insulin, along with the increased insulin release might serve to mitigate decrees in whole-body insulin sensitivity
7. The findings above are not explained by weight gain or food intake in the olanzapine-treated subjects

The methods employed in many animal studies could not be employed in humans, and while their relevance to human oral ingestion of olanzapine or other second-generation antipsychotics, even in large doses, could represent questions, these studies suggest important mechanistic hypotheses.

Houseknecht (2007) and colleagues performed a hyperinsulinemic-euglycemic clamp study, sponsored by Pfizer, in female rats. Radio-tracer labeled (3-^{3}H) glucose was used to assess EGP and in a separate study, (U-^{14}C) 2-deoxyglucose was used to assess glucose uptake into muscle, fat, and liver. At steady state infusion (insulin, somatostatin [to suppress endogenous insulin release], glucose), animal were given single subcutaneous (s.c.) injections of either vehicle (placebo control) or olanzapine or clozapine or ziprasidone (multiple doses of each were tested) in doses that would result in D_2 occupancy in brain tissue comparable to what would be expected in humans receiving the medications clinically. Risperidone was studied but without radio-tracer labeled glucose. These single, acute doses of olanzapine and clozapine significantly reduced whole-body insulin sensitivity. Peripheral glucose uptake was not adversely impacted. These findings suggest that the drugs exert a direct adverse effect on whole-body insulin sensitivity, primarily through a negative effect

on hepatic insulin sensitivity as it could be detected after a single dose without any opportunity for a clinically relevant change in fat mass.

Martins and colleagues (2010) administered vehicle and olanzapine both by intravenous (IV) infusion and by intracerebroventricular injection (single dose) into separate groups of male rats. The rats were assessed after administration (basal period) and then during a hyperinsulinemic-euglycemic clamp study. IV olanzapine increased EGP during the basal period. During the clamp study, GIR (suggesting a decrease in whole-body insulin sensitivity) and peripheral glucose uptake were reduced (reduced peripheral insulin sensitivity), and EGP were further increased relative to vehicle (reduced hepatic insulin sensitivity). These findings are consistent with both acute impairment of hepatic and peripheral insulin sensitivity (as well as whole-body sensitivity), but the results of computations of ISIh, ISIp, and ISIw were not presented. There was also an increase in mRNA levels for hepatic enzymes required for hepatic glucose production. Intracerebroventricular olanzapine resulted in similar changes to IV olanzapine except that there was no decrease in peripheral glucose uptake. The study suggests that both peripherally circulating olanzapine and olanzapine in the CNS (presumably without substantial peripheral exposure) decreases insulin sensitivity, especially hepatic insulin sensitivity. IV olanzapine was also found to increase phosphorylation of hypothalamic AMPK, and intracerebroventricular olanzapine increased levels of hypothalamic neuropeptide-Y underscoring the possibility that olanzapine has a direct effect on glucose homeostasis through effects in the hypothalamus.

Park and colleagues (2010) experimented with groups of ovariectomized (OVX) and non-ovariectomized (nOVX) female rats, all rats being diabetic through 90% pancreatectomy. The OVX rats were treated with estrogen replacement or placebo-estrogen. All three groups (OVX with estrogen replacement, OVX without estrogen replacement, nOVX were treated for eight weeks with placebo, risperidone 0.5 mg/kg/d, or olanzapine 2 mg/kg/d. All rats were fed high-fat diets. Olanzapine-induced more food intake, body weight gain, and fat gain in both OVX and nOVX rats. Changes from pre- to post-treatment hyperinsulinemic-euglycemic clamp study results

demonstrated a decrease in hepatic insulin sensitivity with increased glucose output (and increase in hepatic enzymes involved in gluconeogenesis) with olanzapine in both OVX and nOVX rats but to a lesser extent in nOVX rats than in OVX rats. Estrogen replacement in the OVX rats attenuated the decrement in hepatic insulin sensitivity. These changes with olanzapine were not observed with risperidone.

Albaugh and colleagues (2011) conducted perhaps the most complex study reviewed in male rats. Assessments were performed after two days of acute administration of vehicle, olanzapine 4 mg/kg/d or olanzapine 10 mg/kg/d. Assessments were also performed after chronic administration of vehicle or olanzapine titrated up to 12 mg/kg/d over 14 days. Chronic treatment was continued up to week five, and the multiple assessments were performed at various times during chronic treatment. The overall study included assessments of: 1) locomotor activity; 2) actual energy expenditure using indirect calorimetry; 3) weight; 4) body composition using NMR; 5) an oral glucose tolerance test (OGTT); 6) an acute intraperitoneal (IP) administered dose insulin tolerance test (ITT); 7) a hyperinsulinemic-euglycemic clamp study (with methods to allow measurement of tissue-specific glucose uptake) and tissue-specific (e.g., hepatic) insulin sensitivity; 8) adipose tissue fatty acid uptake; 9) tissue lipogenesis; and 10) an isoproterenol challenge test to assess hepatic glucose response and adipose tissue response (glycerol and free fatty acids release Multiple metabolic-related analytes (e.g., glucose, insulin, C-peptide, free fatty acids) were measured during the assessments. A number of these assessments compared the vehicle-treated group to the drug-treated group at the time of assessment rather than a change from pretreatment to the time of assessment between the two treatments.

In the hyperinsulinemic-euglycemic clamp studies, the basal/fasting period was 120 minutes. During this basal period, radio-tracer labeled glucose (3-^{3}H) was infused to measure hepatic glucose production. The insulin infusion was a single step of 1 µU/kg/min infusion (following an initial bolus of 75 µU/kg) that was continued for 180 min. Glucose was infused, and glucose was clamped at 100

mg/dL. As with other clamp studies described here, the parameters computed to assess insulin sensitivity were conceptually similar to those used in other laboratories but unique in actual details of computation.

This study's results for olanzapine treatment can be summarized as follows:

1. Acute treatment
    a. Decreased locomotor activity
    b. Increased energy expenditure during daylight hours without decreasing it during dark hours
    c. Increased glucose in the OGTT
    d. Did not increase insulin in the OGTT
    e. Decreased the response to insulin in the ITT
    f. Did not decrease hepatic insulin sensitivity
    g. Decreased whole-body insulin sensitivity
2. Chronic treatment
    a. Did not change food consumption
    b. Did not change bodyweight
    c. Increased body fat (and decreased lean body mass)
    d. Increased glucose in the OGTT
    e. Increased insulin in the OGTT
    f. Decreased the response to insulin in the ITT
    g. Did not decrease hepatic insulin sensitivity
    h. Decreased whole-body insulin sensitivity by decreasing muscle tissue glucose uptake and therefore a selective decrease in peripheral insulin sensitivity (decreased glucose uptake in cardiac as well as slow and fast twitch muscle)

i. Markedly increased insulin sensitivity in adipose tissue (increased glucose uptake/storage)
j. Increased lipogenesis
k. Decreased lipolysis

This study suggests that olanzapine has a direct adverse effect on insulin sensitivity (based on acute results in the OGTT) without a compensatory acute increased pancreatic insulin response. An acute decrement in activity occurs, but there is an inexplicable lack of decrease in energy expenditure. Without a change in body weight (probably due to loss of muscle mass), there is an increase in fat mass (decreased activity, no decrease in food consumption, increased lipogenesis, decreased lipolysis) with chronic exposure. Additionally, there is a sustained decrease in whole-body insulin sensitivity due to a decrease in uptake of glucose by muscle but no decrease in hepatic insulin sensitivity. While in the OGTT after both acute and chronic treatment glucose increased in both, insulin did not increase after acute dosing but did after chronic dosing. The OGTT cannot be considered a robust test of pancreatic insulin response. Increasing adiposity could augment a direct adverse effect suggested by the acute results.

Girault and colleagues (2012) reported the results of three experiments conducted in separate groups of male rats. One group of rats was treated with intragastric infusion of a total of 3.66 mg/rat of olanzapine over a 165 min period. A second group was treated with intracerebroventricular infusion of 36.6 µg/rat of olanzapine over a 165 min period. Doses were intended to result in equivalent CNS dopamine receptor occupancy of approximately 70%. Comparable groups received vehicle by intragastric and intracerebroventricular infusion. Experiment 1 assessed total endogenous glucose production, using a radiotracer labeled glucose ($6,6\text{-}^{2}H_2$) infusion, before and during the administration of vehicle or olanzapine by both intragastric and intracerebroventricular routes of administration. Experiments 2 and 3 were hyperinsulinemic-euglycemic clamp studies using radio-tracer labeled glucose ($6,6\text{-}^{2}H_2$) infusion using different insulin doses (Experiment 2 [low insulin dose]: initial bolus of 7.2 µU/kg/min for 5 minutes followed by 3 µU/kg/min for the rest of the experiment. Experiment 3 [high insulin dose]: initial bolus of 21.6 µU/kg/min for 5 minutes followed by 9 µU/kg/min for

the rest of the experiment) during the administration of vehicle or olanzapine by both intragastric and intracerebroventricular routes of administration.

This study's results for olanzapine treatment can be summarized as follows:

1. Experiment 1 – endogenous glucose production
    a. Intragastric olanzapine (vehicle: n=5; olanzapine: n=6)
        i. Increased glucose concentration from baseline substantially
        ii. Slightly numerically increased endogenous glucose production
            1. The increased concentration without a substantial increase in endogenous production might suggest less peripheral uptake
        iii. Increased corticosterone concentration from baseline
        iv. Did not increase insulin concentration
2. Experiment 2 – peripheral insulin sensitivity (tissue glucose uptake) and hepatic insulin sensitivity (endogenous glucose production) with lower dose insulin
    a. Intragastric olanzapine (vehicle: n=8; olanzapine: n=7)
        i. Lower-dose insulin did increase glucose uptake from the basal state with olanzapine statistically comparable to, but numerically less than that increase with vehicle
            1. Olanzapine, therefore, only marginally negatively affected peripheral insulin sensitivity with low dose insulin
        ii. Resulted in only slight numerical suppression of endogenous glucose production by lower-dose insulin relative to the significant suppression observed with vehicle
            1. Olanzapine, therefore, significantly negatively affected hepatic insulin sensitivity with low dose insulin

iii. Increased corticosterone concentration from baseline

3. Experiment 3 – peripheral insulin sensitivity (glucose uptake) and hepatic insulin sensitivity (endogenous glucose production) with higher dose insulin
    a. Intragastric olanzapine (vehicle: n=8; olanzapine: n=7)
        i. Higher-dose insulin did not increase the absolute amount of glucose uptake with olanzapine compared to vehicle
            1. Olanzapine, therefore, significantly negatively peripheral glucose sensitivity with high dose insulin
        ii. Resulted in only numerically less suppression of endogenous glucose production by higher-dose insulin
        iii. Increased corticosterone concentration from baseline

With low dose insulin, olanzapine resulted in a larger negative effect on hepatic insulin sensitivity while with higher dose insulin there was a large negative effect on peripheral insulin sensitivity.

None of these effects were observed with intracerebroventricular olanzapine administration. The overall interpretation of these results could be that olanzapine can have an immediate (therefore independent of weight gain) adverse effect on glucose homeostasis, due to peripheral effects and not mediated through central nervous system activity.

Boyda and colleagues (2013) performed single-dose studies of multiple doses of intraperitoneal (IP) asenapine, iloperidone, and olanzapine compared to vehicle in female rats. Separate experiments were performed with an intravenous glucose tolerance test (IGTT) and a hyperinsulinemic-euglycemic clamp study. For olanzapine, the IGGT was performed with 5 doses, the 3 highest being 1.5, 5, and 15 mg/kg. These 3 doses resulted in statistically significantly higher glucose levels in the IGTT. In the hyperinsulinemic-euglycemic clamp study, two doses of olanzapine were used, 1.5 and 15 mg/kg. The lower dose resulted in a slightly lower glucose infusion rate

required to maintain euglycemia than with vehicle and the higher dose resulted in statistically significantly lower glucose infusion rate to be required. Both findings would suggest impaired glucose homeostasis, likely due to impaired whole-body insulin sensitivity.

Wu and colleagues (2014), conducted a study identical in design to that described above by Boyda (2013) and colleagues (same laboratory affiliation). In this set of experiments, the rats received vehicle, lurasidone or olanzapine (1.5 mg or 15 mg/kg by IP injection in the hyperinsulinemic-euglycemic experiment). Results were similar to those observed in the Boyd (2013) work. The lower dose resulted in a slightly lower glucose infusion rate required to maintain euglycemia than with vehicle and the higher dose resulted in statistically significantly lower glucose infusion rate to be required. The finding would suggest impaired glucose homeostasis, likely due to impaired whole-body insulin sensitivity. An IGTT with a single olanzapine dose resulted in similar findings to those in Boyda (2013) with olanzapine as well.

The authors of the following three manuscripts were affiliated with the same laboratory.

Hahn and colleagues (2014) studied male rats with both a hyperinsulinemic-euglycemic clamp and a hyperglycemic clamp in 3 separate experiments. In experiment 1 (hyperglycemic clamp), rats received vehicle (N=8) or 75 µg olanzapine (N=9) by intracerebroventricular injection. The clamp study was begun immediately after treatment. Glucose was clamped at 300 mg/dL.

In experiment 2 (hyperinsulinemic-isoglycemic [isoglycemic – when the glucose clamp target is a subject's basal glucose concentration, the study is an isoglycemic study]), the clamp was begun 90 min before experimental treatment. After the bolus insulin, 5 mU/kg/min insulin was infused. Target glucose was the basal glucose concentration for each animal. Rats received vehicle (N=6) or 75 µg olanzapine (N=6) by intracerebroventricular injection. The steady-state period was between 190- and 220-minutes following initiation of the insulin.

In experiment 3 (hyperinsulinemic-euglycemic clamp [somatostatin administered with insulin]) the clamps were begun immediately after experimental treatment). The rats received vehicle (N=10) or 75 µg olanzapine (N=10) by intracerebroventricular injection. Insulin was infused at 3 mU/kg/min along with the somatostatin. The glucose clamp target was 120 mg/dL. In both hyperinsulinemic-isoglycemic/euglycemic clamp studies, radio-tracer labeled glucose was used (3-^{3}H) that was begun 90 min before clamp study initiation.

Hyperglycemic clamp study parameters:

- GLU
- GIR (adjusted for weight)
- I
- C-peptide
- ISIw = GIR / $(I * GLU)_{SS}$ (although authors describe as a measure of peripheral insulin sensitivity)
- DI = ISIw * (C-peptide Concentration)^{iv}

Hyperinsulinemic-euglycemic (isoglycemic) clamp study parameters

- GLU
- GIR (adjusted for weight)
- I
- R_a basal and during the clamp (basal R_a = EGP; during clam if R_a is referring to total glucose and not just hepatic glucose, then R_a = EGP + infused glucose and EGP = R_a – infused glucose)
- EGP

- R_d

This study's results for olanzapine treatment can be summarized as follows:

1. Hyperglycemic clamp study
    a. Decrease insulin concentrations
    b. Decreased C-peptide concentrations
    c. No decrease ISIw
    d. Decreased DI
2. Hyperinsulinemic-euglycemic clamp study (experiments 2 and 3 same results)
    a. No increase in EGP (ISIh)
    b. No decrease in R_d (ISIp)

This study found that an acute, CNS dose of olanzapine reduced pancreatic insulin output but was without other effects on parameters relevant to glucose homeostasis.

Remington and colleagues (2015) reported the results of a study conducted in male rats intended to determine if metformin would attenuate the negative effects of acute, oral olanzapine administration. Rats administered olanzapine alone received a single 3 mg/kg s.c. dose, immediately before the hyperinsulinemic-isoglycemic clamp study that included radio-tracer labeled glucose (3-^{3}H). The protocol parameters were similar to those for the Hahn (2014) study above, except for the steady-state period relative to beginning the clamp studies. The steady-state period for this study was between 150- and 180-minutes following initiation of the insulin.

This study's results for olanzapine treatment can be summarized as follows:

1. Reduced hepatic insulin sensitivity based on higher R_a
2. Reduced peripheral insulin sensitivity based on lower R_d

Kowalchuk and colleagues (2017), performed a study in the laboratory's male rat model, performed a study intended to assess a potential mechanistic contribution to dysregulation of glucose homeostasis by olanzapine. The researchers hypothesized, based on their earlier work (Hahn, 2014) and the work of others that olanzapine disrupts the CNS actions of insulin leading to disruption of peripheral glucose homeostasis. To evaluate this hypothesis, the researchers employed what they term a pancreatic-euglycemic clamp study. In this clamp study, insulin (or vehicle) infused into the 3rd ventricle (intracerebroventricular [IVC]) and radio-tracer labeled glucose is infused peripherally (P). At 90 minutes, the clamp is initiated. A peripheral infusion of somatostatin and insulin, 1 mU/kg/min (somatostatin to suppress endogenous insulin and sufficient insulin to bring peripheral insulin exposure to basal levels and a level such that the peripheral insulin will not enter the CNS). Glucose is infused to maintain euglycemia. A single, s.c. injection of olanzapine (2 mg/kg) or vehicle was administered immediately before beginning the clamp study. Therefore, four combinations of treatments were studied: 1) vehicle+IVC-vehicle; 2) olanzapine+IVC-vehicle; 3) vehicle+IVC-insulin; 4) olanzapine+IVC-insulin.

This study's results for olanzapine treatment can be summarized as follows:

1. Compared to vehicle, olanzapine did not affect R_d: No decrease in ISIp
2. Compared to vehicle, olanzapine did not affect R_a: No decrease in ISIh
3. Olanzapine+insulin compared to Vehicle+insulin: no effect on CNS insulin's capacity to increase R_d - olanzapine had no effect on CNS insulin's ability to increase ISIp
4. Olanzapine+insulin compared to Vehicle+insulin: decreased CNS insulin's capacity to decrease R_a - olanzapine reduces CNS insulin's ability to increase ISIh

We have reviewed 17 studies above. There were many differences in methods, both protocols, and computations, across these studies for both hyperglycemic clamp studies and hyperinsulinemic-euglycemic (or isoglycemic) clamp studies. We will not specifically summarize these differences.

However, it is important to summarize the inconsistencies among study results that could be due to methodological differences or other unknown factors. The results, focusing on pancreatic insulin production and insulin sensitivity (ISIh, ISIp, ISIw) and inconsistencies among studies with respect to these results are summarized in a table in the next Section.

4. Summary of Study Findings: Insulin production and Insulin Sensitivity

Summary of Study Findings Related to Olanzapine and Insulin Sensitivities as well as Pancreatic Insulin Response[1]

	CNS Effect					Peripheral Effect										Wt 2
	Acute Dose					Acute Dose					Repeated Dose					
	ISI h	ISI p	ISI w	Insulin Response		ISI h	ISI p	ISI w	Insulin Response		ISI h	ISI p	ISI w	Insulin Response		
				Absolute	Relative				Absolute	Relative				Absolute	Relative	
Lilly Studies / Analyses																
Sowell (2002)													Y	N		Y
Sowell (2003)													N			
Hardy (2007)													N	N	N	
Hardy (2011)											N		N			
Single Studies from Different Laboratories																
Kopf (2012)								N	N							
Teff (2013)											N	Y		N	N	
Ader (2005)											Y		N	N	Y	N
Houseknecht (2007)						Y	N	Y								N
Martins (2010)	Y	N	Y			Y	Y	Y								N
Park (2010)											Y					

Albaugh (2011)						N		Y			N		Y[3]			N[4]
Girault (2012)	N	N				Y	Y									N
Studies from University of British Columbia Laboratory																
Boyd (2013)								Y								N
Wu (2014)								Y								N
Studies from University of Toronto Laboratory																
Hahn (2014)	N	N	N	Y	Y											N
Remington (2015)											Y	Y				
Kowalchuck (2017)	N[5]	N														

[1] Y: the study found olanzapine to have a negative effect on the parameter; N: the study found olanzapine not to have a negative effect on the parameter; If a cell is empty, the study did not evaluate the parameter

[2] Yes if the study suggested that weight or fat gain could be a variable leading to the negative effects observed; no if the effects unlikely to be influenced by weight/fat gain (findings observed with acute dose, findings not observed with weight gain without olanzapine, findings observed without weight gain)

[3] Decreased ISIp in muscle tissue but glucose uptake increased in adipose tissue

[4] Although a weight change was not found, a substantial increase in adipose tissue was found that might have mediated observed effects with chronic dosing only

[5] No direct effect of acute CNS olanzapine on ISIh but CNS olanzapine reduced CNS insulin's ability to increase ISIh (decrease EGP)

It is very clear that olanzapine, as well as some other second-generation antipsychotics, can be associated with substantial weight gain and that an excess incidence of diabetes mellitus is observed in patients treated with these agents. However, we do not believe that the question has been adequately addressed as to whether these agents have a direct diabetogenic effect in the absence of weight/fat gain due to increase appetite and or decreased satiety, sedation leading to a decrease in active caloric expenditure with ensuing weight/fat gain, high risk for diabetes due to impaired fasting glucose, a decrease in basal metabolic rate due to direct influence of drug that might in turn result in acutely impaired glucose homeostases or lead to weight/fat gain, impaired glucose tolerance, or genetic factors without observable impairments in homeostatic-related parameters. Furthermore, we believe the question of whether these agents are directly diabetogenic or indirectly diabetogenic, specifically in human subjects, is critically important in optimal patient treatment and management. Weight gain or lack thereof can be easily monitored and possibly predicted early in treatment (Lipkovich, 2009). Activity level can almost as easily be monitored. Total and regional body fat (but not specifically visceral fat) can be monitored with DEXA that is a relatively quick (15 min) procedure and not excessively costly at many centers ($50-75). Risk-benefit of any individual agent could be more precisely assessed for individual patients with better knowledge regarding direct or indirect effects.

5. A Hypothetical Study to Resolve the Important Uncertainties

We believe that the labeling of these agents in 2003 with a warning regarding diabetes without some acknowledgement of the potential for differential risk among the agents and the potential for some of the risk to be due to indirect effects effectively curtailed any interest in funding the types of studies necessary to address the question of direct effects that could not be predicted versus indirect effects that could be predicted either before selecting a treatment agent or early in treatment by monitoring ongoing changes or lack thereof. Pharmaceutical companies would be the source of funding that would have a vested interest in funding such studies. However, the required labeling was a major disincentive to the funding of high-quality research in humans. It is virtually impossible to alter, remove, or not include (for a new product) class labeling once it has been required. While additional human clamp studies have been performed,

they have lacked placebo-control and been aimed at comparing a new agent or agent perceived as lacking a diabetogenic effect to an active agent thought to have the effect, generally olanzapine and sometimes clozapine.

Disruption of glucose homeostasis (a decrement in pancreatic insulin production, and/or a decrement in peripheral glucose uptake and/or excess hepatic production of glucose along with changes in a number of other endogenous substances such as glucagon, free fatty acids, GLP-1, other substances influencing glucose production and/or disposal) that if substantial and prolonged constitutes clinical diabetes mellitus is an adverse event that can be assessed in a set of clinical pharmacology studies. This spectrum of adverse events is one of the few such spectrums where early changes can be detected with great sensitivity before the onset of overt disease. The hyperglycemic and hyperinsulinemic-euglycemic/isoglycemic clamp studies, accompanied by measurements of weight, lean body mass, subcutaneous fat, visceral fat, activity, and basal (or resting) metabolic rate as well as the total caloric expenditure when olanzapine is administered orally to healthy adults should be able to address the question above. While the actual basal metabolic rate is difficult to measure, relatively inexpensive, hand-held devices can measure resting metabolic rate.

Twenty subjects per treatment group treated for 4 weeks should be sufficient to detect an effect or lack of effect (two-step sequential analysis, first for difference and then if difference not observed, non-inferiority). Given the findings in the studies reviewed, a number of treatment groups would be important:

1. Olanzapine 10 mg/day with no restriction on food consumption and no mandated activity level – a group expected to gain weight as well as fat and have less total caloric expenditure, at least early in treatment
2. Olanzapine 10 mg/day with no restriction on food consumption but mandated to continue daily activity at the same level as before beginning treatment (activity level of all subjects assessed at baseline) – a group that might not gain weight/fat if such gain is solely due to decreased active caloric expenditure

3. Olanzapine 10 mg/day mandated to continue daily activity level as before beginning treatment and if necessary, restrict food consumption to maintain same body weight and total body fat as before beginning treatment – a group that would not gain weight/fat due to any combination of increased caloric consumption, decreased activity, and decreased basal metabolic rate
4. Placebo mandated to continue daily activity level as before beginning treatment and if necessary, restrict food consumption to maintain the same body weight and total body fat as before beginning treatment – a placebo-treated group without weight gain
5. Placebo with no restriction on food consumption and no mandated activity level – a placebo-treated group that might gain weight/fat due to being in a restricted environment
6. Placebo with no mandated activity level and placed on a high-fat diet to induce weight and fat gain comparable to what will be observed in the olanzapine-treated subjects with no restriction on food consumption and no mandated activity level – a placebo-treated group that will gain weight/fat but without olanzapine exposure

None of these treatment groups remove a change in basal metabolic rate with olanzapine as a potential etiological contributor to disruptions in glucose homeostasis, and this would have to be assessed in the statistical model used for analysis.

Important inclusion criteria:

- Age: 20-40
- BMI: 20-25 – only necessary with the fat criteria below to limit muscle mass
- Total body fat and visceral fat between 35^{th}- and 65^{th}-percentiles for age and sex-adjusted norms
- No current or history of a psychiatric disorder
- No current medical illness
- Taking no medications on a chronic basis
- No family history of any form of diabetes mellitus

Expert consensus, if possible, to achieve, would need to be acquired regarding the optimal conduct of both types of clamp studies, the parameters to be measured, and how to compute those parameters requiring computation.

In the hyperglycemic clamp study major considerations would include:

- The number of steps
- The glycemic targets at each step
- What to consider the time of greatest interest for the most meaningful insulin responses
- The time interval at each step during which the system would be considered at steady-state
- How to compute DI – the parameter assessing the adequacy of insulin response adjusted for any change in insulin sensitivity (requires ISIw from the hyperinsulinemic-euglycemic clamp study)
- As this study type is not the gold standard for assessing insulin sensitivity, this study would not be used for this purpose

In the hyperinsulinemic-euglycemic (or isoglycemic) clamp study major considerations would include:

- The radiotracer labeled glucose tracer to use and when to begin infusion before beginning the clamps
- Euglycemic or isoglycemic
- The number of steps
- The insulin infusion rate at each step
- The time interval at each step during which the system would be considered at steady-state
- How to compute whole-body, hepatic, and peripheral insulin sensitivity
    - How to compute endogenous glucose production used in the computation of hepatic insulin sensitivity and peripheral insulin sensitivity (if not 0)

An additional major consideration for both types of clamp studies would be any additional metabolic analytes to collect.

Besides the clamp studies, total fat, subcutaneous fat, and visceral fat would be measured at least once weekly and preferably twice weekly. Weight, activity and resting metabolic rate would be measured daily. Best methods of measuring these fat deposits would require a consensus of experts. While DEXA is probably adequate for total fat by region, the choice between CT and MRI as optimal for quantitating subcutaneous and visceral fat might require discussion. However, given radiation exposure concerns with human subjects, MRI would likely have to be used.

Both clamp studies would be performed at baseline (before treatment), early in treatment, such as when CNS exposure would be expected to reach or be close to steady-state (because of the research suggesting the acute onset of effects) and at the end of four weeks of treatment (endpoint).

Adequacy of insulin response adjusted for any change in whole-body insulin sensitivity, hepatic insulin sensitivity, and peripheral insulin sensitivity would be the primary dependent variables of interest. The primary comparative groups of interest would be: 1) group #3 vs. group #4 (no weight/fat gain in either group); 2) group #1 vs. group #6 (weight/fat gain in both groups). The comparison of groups #1 vs. #3 adds information regarding the influence of weight gain vs. no weight gain with olanzapine. A comparison across groups #1, #2, and #3 would increase the knowledge regarding relative contributions of increased food consumption and decreased activity to weight/fat gain (another group treated with olanzapine and food restricted but not forced to maintain activity would further assist in this objective). A comparison of group #4 and #5 serves to assess whether restriction to a closed unit, by itself facilitates weight gain.

Expert statistical consultation would be required to plan sequential analyses. Maximal differences that would allow declaration of non-inferiority would need to be established *a priori* in the event that olanzapine did not differ from placebo in one or more of the appropriate

group comparisons for one or more of the dependent variables of primary or secondary interest. It would be understood that if these differences could not be established with reasonable clinical certainty, then failure to find differences indicating an adverse effect associated with olanzapine could simply not be interpreted. Any strong trend toward statistical significance for an adverse effect with the drug would require reconsideration of the sample size and potentially the need for an additional study.

The study outlined above would be very expensive. The equipment necessary to perform the clamp studies in humans exists in a limited number of research facilities, and the study would require identical equipment in all participating laboratories. The equipment is very expensive and requires frequent maintenance and requires frequent calibration. MRIs are expensive. One-hundred-twenty subjects is a large number of subjects for such a study. However, we have had experience with a complex Thorough QT study that required over 120 subjects who screened positive for being CYP-2D6 poor metabolizer and that involved 24-hour continuous, high-fidelity, 12-lead ECG monitoring. Still, the study suggested above would likely be considerably more expensive and difficult to complete than this Thorough QT study.

Finally, this suggested design has limitations. The design would not address the questions as to whether greater differential risk of dysregulation of glucose homeostasis exists between persons taking olanzapine versus those not taking olanzapine among those with diabetes mellitus or with risk factors for developing diabetes mellitus (potentially including having a severe psychotic disorder) compared to those without diabetes mellitus or risk factors for its development. After addressing the question of risk in the absence of known risk factors for the development of the disorder and knowing if any excess risk associated with the drug with the drug was through direct or indirect and controllable mediators, the question of differential risk in already at risk persons could be better addressed.

If there was an excess risk with the drug and this was through direct effects, there would likely be little practical need in assessing at-risk persons. If there was no excess risk with the drug or any excess risk was mediated through indirect effects, then at risk persons could be studied while controlling those indirect effects.

6. A Post-script Caveat and Apology

A large quantity of numerical data was transcribed directly into this work and extracted from various types of figures and then transcribed by us as the single author. These transcriptions went through multiple checks but not by an independent reviewer, except in the case of data in the Arder (2005) manuscript. It is a virtual certainty that some typographical errors exist in the transcribed data. However, conceptual summaries are faithful to the data. Finally, the tabular summary of finding from the 17 manuscripts that allows easy comparison in Section 4 is likewise consistent with the data.

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[i] Complete diagnostic criteria include symptom criteria and confirmation of numerical values.
[ii] Psychosis was the indication for which olanzapine was initially approved in the US. The indication was subsequently changed in the US to schizophrenia per FDA.
[iii] Not all subjects assigned to treatment contributed data to every analysis. Therefore, these numbers are approximations for each analysis.
[iv] In rats, C-peptide concentration used rather than insulin concentration because the pharmacokinetics of insulin are not sufficiently known to calculate the insulin secretion rate

July 4, 2019