

Metformin attenuates olanzapine-induced hepatic, but not peripheral insulin resistance

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Abstract

Antipsychotics (APs) are linked to diabetes, even without weight gain. Whether anti-diabetic drugs are efficacious in reversing the direct effects of APs on glucose pathways is largely undetermined. We tested two metformin (Met) doses to prevent impairments seen following a dose of olanzapine (Ola) (3 mg/kg); glucokinetics were measured using the hyperinsulinemic-euglycemic clamp (HIEC). Met (150 mg/kg; $n = 13$, or 400 mg/kg; $n = 11$) or vehicle (Veh) ($n = 11$) was administered through gavage preceding an overnight fast, followed by a second dose prior to the HIEC. Eleven additional animals were gavaged with Veh and received a Veh injection during the HIEC (Veh/Veh); all others received Ola. Basal glucose was similar across treatment groups. The Met 400 group had significantly greater glucose appearance (R_a) in the basal period (i.e., before Ola, or hyperinsulinemia) vs other groups. During hyperinsulinemia, glucose infusion rate (GINF) to maintain euglycemia (reflective of whole-body insulin sensitivity) was higher in Veh/Veh vs other groups. Met 150/Ola animals demonstrated increased GINF relative to Veh/Ola during early time points of the HIEC. Glucose utilization during hyperinsulinemia, relative to basal conditions, was significantly higher in Veh/Veh vs other groups. The change in hepatic glucose production (HGP) from basal to hyperinsulinemia demonstrated significantly greater decreases in Veh/Veh and Met 150/Ola groups vs Veh/Ola. Given the increase in basal R_a with Met 400, we measured serum lactate (substrate for HGP), finding increased levels in Met 400 vs Veh and Met 150. In conclusion, Met attenuates hepatic insulin resistance observed with acute Ola administration, but fails to improve peripheral insulin resistance. Use of supra-therapeutic doses of Met may mask metabolic benefits by increasing lactate.

Key Words

- ▶ atypical antipsychotics
- ▶ insulin resistance
- ▶ metformin
- ▶ olanzapine
- ▶ diabetes

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Introduction

Antipsychotics (APs) remain the cornerstone of treatment for schizophrenia (Canadian Psychiatric Association 2005). However, their use is also associated with significant concerns in terms of metabolic side effects; high

rates of metabolic syndrome, dyslipidemias, weight gain and glucose dysmetabolism have been reported (Goff *et al.* 2005, Newcomer 2005). While weight gain associated with APs remains a major risk factor for type 2 diabetes (DM2)

(Pi-Sunyer 1993), there is growing evidence, both clinical and preclinical, indicating increased liability for glucose dysregulation independent of illness-related factors or weight increases (Ader *et al.* 2005, Houseknecht *et al.* 2007, Chintoh *et al.* 2009, Vidarsdottir *et al.* 2010a, Teff *et al.* 2013, Hahn *et al.* 2014). In this regard, our own work from preclinical rodent models consistently supports pronounced, direct effects on insulin sensitivity (peripheral and hepatic) following acute dosing of specific AP agents (e.g., risperidone, olanzapine (Ola) or clozapine) (Chintoh *et al.* 2008, 2009). From a clinical perspective, acute or 'direct' effects are supported by reports of diabetic ketoacidosis occurring shortly after initiation of AP drugs in absence of notable weight changes (Guenette *et al.* 2013), as well as studies that suggest a risk of glucose dysregulation independently of weight gain (Newcomer *et al.* 2002, Henderson *et al.* 2005, Ebdrup *et al.* 2014). These observations support the notion that acute mechanisms can lead to glucose dysregulation in the absence of weight gain, but that over time, these can contribute to notable changes in adiposity.

The medical consequences of these side effects are troubling. Patients with serious mental illness, including schizophrenia, have increased rates of cardiovascular disease, which contribute to the twofold increase in the standardized mortality ratio observed in this population (Hennekens *et al.* 2005, De Hert *et al.* 2009). Furthermore, the risk of metabolic syndrome is estimated to be twofold higher, and the prevalence of DM2 three- to fivefold higher than the general population (McEvoy *et al.* 2005, De Hert *et al.* 2006). Despite these implications, those with serious mental illness and DM2 are insufficiently monitored and undertreated (Frayne *et al.* 2005, Kreyenbuhl *et al.* 2006, Goldberg *et al.* 2007). Moreover, mortality among those with serious mental illness and DM2 is higher than in those diagnosed with DM2 or serious mental illness alone (Jackson *et al.* 2007, Vinogradova *et al.* 2010). Clinical studies examining anti-diabetic agents in the context of frank glucose dysregulation in schizophrenia are lacking. As a case in point, to our knowledge, only two clinical studies exist specifically evaluating insulin resistance or impaired glucose tolerance respectively in the context of antipsychotic treatment (Henderson *et al.* 2009, Smith *et al.* 2013). Both examined thiazolidinediones and failed to establish significant improvements in indices related to glucose metabolism. Given strong preclinical evidence of direct antipsychotic effects on glucose pathways, independent of and in addition to illness and adiposity, the question remains as

to whether anti-diabetic drugs are efficacious in reversing these direct molecular effects.

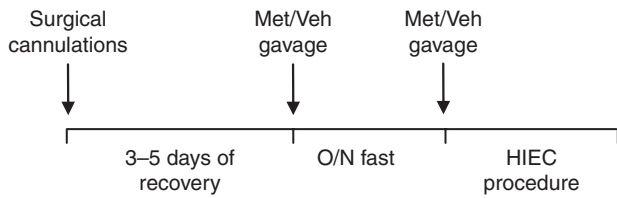
Metformin (Met), a first-line treatment in the general population for pre-diabetes and DM2 (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee *et al.* 2013, American Diabetes Association 2014), has been studied as an adjunctive pharmacological intervention for AP-related weight gain. Eleven double blind, randomized, controlled trials have examined Met in this role (Baptista *et al.* 2006, 2007, Klein *et al.* 2006, Arman *et al.* 2008, Wu *et al.* 2008a,b, 2012, Carrizo *et al.* 2009, Wang *et al.* 2012, Chen *et al.* 2013, Jarskog *et al.* 2013). Of these, nine examined a surrogate measure of insulin sensitivity, the homeostasis model assessment of insulin resistance (HOMA-IR), with six demonstrating beneficial effects favoring Met (Wu *et al.* 2008a,b, Carrizo *et al.* 2009, Wang *et al.* 2012, Chen *et al.* 2013, Jarskog *et al.* 2013). To our knowledge, no studies have specifically examined the effect of Met on glucose metabolism in patients on APs with DM2. In the preclinical literature, a recent rodent study examining effects of three classes of anti-diabetic drugs, including Met, suggested only partial reversal of glucose intolerance induced by Ola during a glucose tolerance test (GTT) (Boyda *et al.* 2014). In the present investigation, we tested two doses of Met to assess the drug's ability to reverse Ola-induced whole-body insulin resistance; this was done using the hyperinsulinemic-euglycemic clamp (HIEC) technique, the current 'gold standard' allowing the separate determination of drug effects on peripheral and hepatic insulin sensitivity.

Materials and methods

The Centre for Addiction and Mental Health and University of Toronto Animal Care Committee approved the following protocol. Healthy, male Sprague-Dawley rats (*Rattus norvegicus*) (12 weeks old, 300–325 g, Harlan, IN, USA) were kept on a 12 h light:12 h darkness cycle and allowed to feed *ad libitum*. Testing was conducted during the light cycle, with all protocols initiated between 0800 and 0900 h, in free-running rats. Figure 1 provides an overview of the experimental timeline.

Vessel cannulations

After a 7-day acclimatization period, animals underwent vascular catheterization surgery. Rats were anaesthetized with isoflurane and polyethylene catheters (PE-50, Cay Adams, Boston, MA, USA), with 2.5 cm of silastic tubing advanced into the right atrium and aortic arch through

**Figure 1**

Experimental timeline. Outline of experimental procedures: metformin (Met); vehicle (Veh); overnight (O/N); hyperinsulinemic-euglycemic clamp (HIEC).

the jugular vein and carotid artery respectively. The catheter lines were externalized dorsally. Buprenorphine (0.3 mg/kg) (Reckitt Benkiser Healthcare Ltd, Berkshire, UK) was administered for post-operative analgesia and animals were allowed 3–5 days of recovery before the clamping experiments, which were performed in conscious, fasted rats.

Drug and dose selection

Ola (Toronto Research Chemicals, Toronto, Canada) was dissolved in 1% acetic acid buffered with 1 M NaOH to a pH > 5.5. S.c. dosing of Ola (3 mg/kg) was based on > 70% D₂ brain occupancy, reflecting clinically relevant thresholds for therapeutic efficacy (Kapur *et al.* 2003), as well as established effects on peripheral and hepatic insulin sensitivity (Chintoh *et al.* 2008, 2009). Animals were randomly assigned and treated with one of two Met doses (400 mg/kg, *n* = 11; 150 mg/kg, *n* = 13) or vehicle (Veh) (*n* = 11), administered through gavage preceding an overnight fast, followed thereafter by a second dose the morning prior to the HIEC. Eleven rats were additionally gavaged with Veh, and injected with Veh directly preceding hyperinsulinemia (*t* = 90 min) (Veh/Veh; *n* = 11) to serve as controls. All other animals received Ola at the 90 min time point (Met 400/Ola, *n* = 11; Met 150/Ola, *n* = 13; Veh/Ola; *n* = 11). The lower dose of Met (150 mg/kg × two doses, administered 15–16 h apart) was based on evidence that similar doses improve measures of glucose metabolism in rat models of diabetes (Matthaei *et al.* 1993, Borst *et al.* 2000, Cleasby *et al.* 2004, Yasuda *et al.* 2004). We also examined a higher dose (400 mg/kg × two doses) based on the possibility of dose-dependent effects on metabolic indices (Suzuki *et al.* 2002, Hauton 2011).

Hyperinsulinemic-euglycemic clamp

The goal of the HIEC is a new steady state, of higher-than-fasting (i.e., hyperinsulinemic) insulin levels across

groups and without a change in the basal glucose level, which would initiate a counterregulatory response and influence glucose kinetics. Hyperinsulinemia is induced via an i.v. insulin infusion, while the 'euglycemic' clamp is achieved via a variable glucose infusion. The amount of glucose infusion (GINF) rate necessary to 'clamp' the glucose level is a measure of whole body insulin sensitivity. Radioactive glucose tracer infused before and during the clamp can also allow the separate determination of glucose production (or rate of glucose appearance; R_a) and utilization (or rate of disappearance; R_d) by a tracer dilution principle. The suppression of glucose production by insulin during the clamp is a measure of hepatic insulin sensitivity, and the stimulation of glucose utilization during the clamp is a measure of peripheral insulin sensitivity.

Following the overnight fast and gavage with the second dose of Met or Veh, catheter lines were immediately extended to the infusion pumps. Radioactive tracer ([3-H³]-glucose, 20 μCi/ml) was then infused into the jugular vein for 90 min prior to the clamp, and throughout the clamp (0.4 ml bolus plus 7.5 μl/min infusion) (*t* = 90–180 min). The insulin infusion (5 mU/kg × min) began at *t* = 90 min, and euglycemia was maintained via exogenous glucose given at a variable rate according to plasma glucose levels determined every 5 min. The exogenous glucose was labeled (specific activity = 48 μCi/g) to maintain plasma glucose-specific activity constant, which minimizes errors of estimation of glucose production (Finegood *et al.* 1987). At onset of the 'clamp' (*t* = 90 min), animals received a single s.c. dose of Ola (3 mg/kg) or a corresponding volume of Veh (Veh/Veh). Plasma samples were collected every 10 min during the 30-min tracer equilibration period before the clamp (*t* = 60–90 min), and during the clamp following Ola injection (*t* = 150–180 min). Samples were stored at –80 °C for subsequent tracer and insulin analysis. Figure 2 provides an overview of the HIEC procedure.

Plasma assays

Plasma glucose was measured with the glucose oxidase method (Glucose analyzer GM9; Analox Instruments, Lunenburg, MA, USA). Plasma radioactivity from [3-³H] glucose was determined after deproteinization with Ba(OH)₂ and ZnSO₄ and subsequent evaporation to remove tritiated water. Insulin was analyzed by RIA specific for rat insulin (Linco Research, St Charles, MO, USA). All assays were run in duplicate.

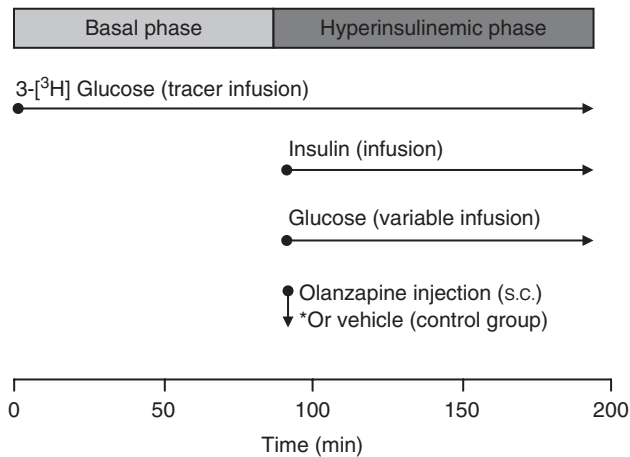


Figure 2
Hyperinsulinemic-euglycemic clamp (HIEC) procedure.

Lactate measurement

Lactate concentrations were measured in duplicate from plasma samples using an assay kit (Eton Bioscience, San Diego, CA, USA) according to manufacture instructions. The method is based on conversion of lactate and NAD⁺ by lactate dehydrogenase into pyruvate and NADH. Due to limited remaining plasma volumes, samples across basal time points were combined for each rodent, and analyzed according to treatment group for lactate measurements.

Calculations

GINF rate was derived based on each animal's weight and GINF pump rate. Glucose turnover (rate of glucose appearance, R_a , determined with [³-³H] glucose) was calculated using steady state formulae (Stetten *et al.* 1951), which also took into account the extra tracer infused with the glucose infusate (Finegood *et al.* 1987). In the basal state, the total rate of glucose appearance corresponds to the endogenous glucose production. During the clamps, endogenous glucose production was calculated by subtracting the exogenous GINF rate from the total rate of glucose appearance. At steady state, glucose disappearance, R_d , corresponds to the rate of glucose appearance, and at euglycemia, glucose disappearance corresponds to tissue glucose utilization, because renal glucose clearance is zero (Finegood *et al.* 1987).

Statistical analyses

A series of mixed models repeated-measures (MMRM) analyses were conducted in order to determine whether

glucose, insulin, GINF, R_a and R_d values changed over the course of the experiment, differed across treatment groups or changed differentially over time across the groups. If a significant time by group interaction was found, indicating that the magnitude of difference between the two treatment groups changed over time, a series of linear contrasts were constructed to further explore the nature of the interaction, with Bonferroni adjustment for multiple comparisons. For lactate measurements, ANOVAs were conducted across treatment groups, followed by *post-hoc* *t*-tests where applicable, and Bonferroni adjustments for multiple comparisons.

Results

There were no significant time by group interactions, or group effects for differences in glucose or insulin levels between the four groups (Fig. 3A and B; Table 1). GINF values, reflective of whole-body insulin sensitivity, demonstrated a significant time by group interaction ($F_{3,126} = 27.02$; $P = 0.006$), leading to further exploration of group effects (Fig. 3C). *Post-hoc* examination of differences between each pair of groups at each time point, with Bonferroni adjustment for multiple comparisons, demonstrated significantly higher GINF for the Veh/Veh group compared to all other groups throughout the clamp phase, and significantly higher values in the Met 150/Ola group compared to Veh/Ola at the 150- and 170-min time points (Fig. 3C). Examining glucose production, the mixed effect model demonstrated a significant time by group interaction ($F_{24,203} = 1.84$; $P = 0.012$), driven by differences of group effect during the basal phase (Fig. 4A). Contrasts were used to look at the average group effect during the clamp phase accounting for group effects at the basal phase (defined as the difference in R_a between clamp and basal phases). Table 1 summarizes statistical testing comparing the effect in R_a between pairs of groups, with adjustments for multiple comparisons using the Bonferroni method. The decrease in hepatic glucose production (HGP) from basal to clamp phase was significantly greater in both the Veh/Veh and Met 150/Ola groups as compared to Veh/Ola. Comparison of Veh/Ola with Met 400/Ola demonstrated a borderline significant difference ($P = 0.0540$) in between group decreases in R_a . Similar to analyses for R_a , a significant time by group effect was noted for R_d ($F_{24,203} = 3.13$; $P < 0.0001$). To control for differences in basal R_d , comparison of treatment effect between groups was defined as the change from basal to clamp rates in R_d (Fig. 4B and Table 1). Table 1 summarizes statistical testing

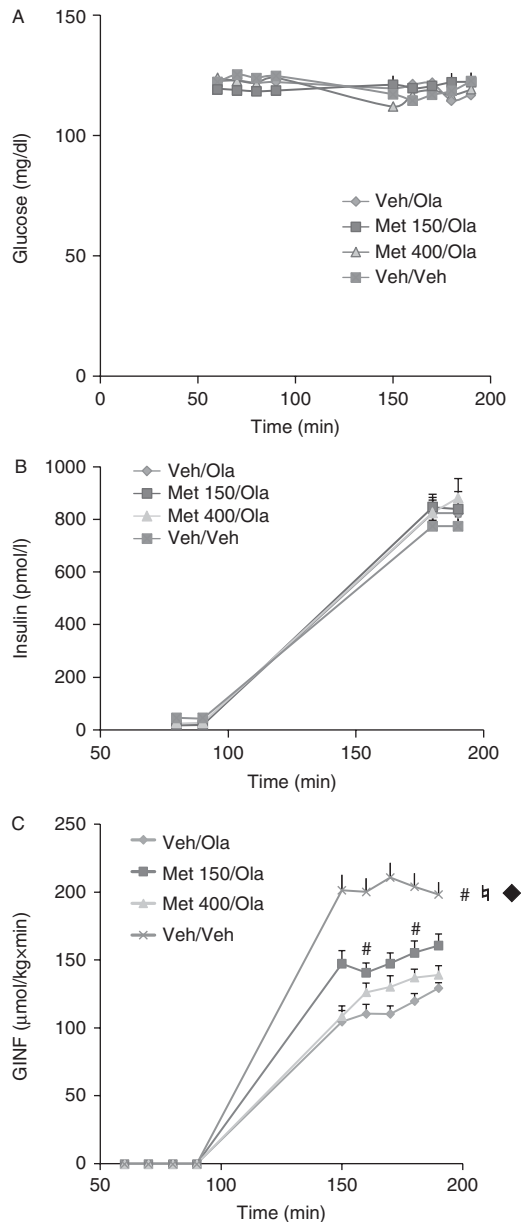


Figure 3

Effect over time, and by treatment group: vehicle+vehicle (Veh/Veh) ($n=11$); vehicle+olanzapine (Veh/Ola) ($n=11$); metformin 150 mg/kg + olanzapine (Met 150/Ola) ($n=13$); metformin 400 mg/kg + olanzapine (Met 400/Ola) ($n=11$) on plasma glucose (A), insulin (B) and glucose infusion rate (GINF) (C) during a hyperinsulinemic-euglycemic clamp procedure in male, Sprague-Dawley rats. A significant time \times group interaction was found during the hyperinsulinemic clamp phase ($P < 0.05$) for GINF with *post-hoc* comparisons and adjustments using the Bonferroni method demonstrating significantly higher GINF in Veh/Veh animals vs all other groups across all time points, and Met 150/Ola differing from Veh/Ola at 150 and 170 min. # $P < 0.05$ vs Veh/Ola; $^{\circ}P < 0.05$ vs Met 150/Ola; $^{\bullet}P < 0.05$ vs Met 400/Ola. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JOE-15-0074>.

comparing the effect in R_d between pairs of groups, with adjustment for multiple comparisons using the Bonferroni method. Veh/Veh group increases in R_d were found to be significantly larger than in all other groups, whereas the other groups did not differ significantly from each other.

To explore the unexpected increase in basal rates of glucose production in the high-dose Met group, we measured basal lactate levels prior to insulin infusion and Ola treatment. We found a significant increase in the high-dose Met group relative to Veh (i.e., Veh/Ola and Veh/Veh) or low-dose Met treatment (Fig. 5).

Discussion

In the present study, we employed the HIEC to determine if pre-treatment of healthy, male Sprague-Dawley rats with two Met doses (150 mg/kg or 400 mg/kg) would attenuate impairments in hepatic and peripheral insulin sensitivity following Ola administration. The HIEC was used to assess both peripheral and hepatic insulin sensitivity, and an acute dosing paradigm of Ola was employed to avoid confounding adiposity changes noted under chronic treatment with this agent. The importance of using acute dosing paradigms to study so-called 'direct effects' of AP medications is highlighted by previous findings in healthy rodents showing that Ola can induce increases in adiposity as early as 1 week into treatment (Albaugh *et al.* 2011a). Direct AP-induced disruptions in glucose homeostasis may represent a distinguishing factor for patients treated with APs, supporting preclinical investigation of attenuation/prevention of these distinctive disturbances.

In the pre-clinical literature, at least six other studies have examined the effects of various classes of anti-diabetic agents (e.g., incretins, sulfonylureas, thiazolidinediones, biguanides) in the context of AP-related glucose perturbations; three studies have examined Met. These reports differ from one another with respect to AP treatment dose and duration, as well methods of assessing glucose metabolism (e.g., stand-alone measures of fasting insulin and glucose, GTTs). In general, incretins (Lykkegaard *et al.* 2008, Smith *et al.* 2009) and Met (Adeneye *et al.* 2011, Boyda *et al.* 2012, 2014) appear to improve metabolic indices, whereas the data have been more variable for sulfonylureas and thiazolidinediones (Arulmozhi *et al.* 2006, Boyda *et al.* 2012, 2014).

The first of the aforementioned studies examining Met (Adeneye *et al.* 2011) tested single daily administration (20 mg/kg), comparing treatment with Veh or glibenclamide, during 60 days of administration of

Table 1 Comparison of treatment effect between groups. Treatment effect is defined as change from basal to hyperinsulinemic clamp phase (Δ). *P* values were adjusted using the Bonferroni method

Measure	Label	Estimate \pm s.e.m	DF	t value	Pr > (t)	Adjusted P
R _a	(Δ Veh/Ola) – (Δ Veh/Veh)	5.014 \pm 1.35	203	3.700	<0.0001	0.002
	(Δ Met 150/Ola) – (Δ Veh/Veh)	–1.459 \pm 1.30	203	–1.12	0.264	1.000
	(Δ Met 400/Ola) – (Δ Veh/Veh)	1.453 \pm 1.35	203	1.070	0.284	1.000
	(Δ Met 150/Ola) – (Δ Met 400/Ola)	–2.913 \pm 1.30	203	–2.250	0.026	0.105
	(Δ Met 150/Ola) – (Δ Veh/Ola)	–6.473 \pm 1.30	203	–4.990	<0.0001	<0.0001
	(Δ Met 400/Ola) – (Δ Veh/Ola)	–3.561 \pm 1.35	203	–2.640	0.009	0.054
R _d	(Δ Veh/Ola) – (Δ Veh/Veh)	–10.42 \pm 2.06	203	–5.060	<0.0001	<0.0001
	(Δ Met 150/Ola) – (Δ Veh/Veh)	–12.19 \pm 1.98	203	–6.170	<0.0001	<0.0001
	(Δ Met 400/Ola) – (Δ Veh/Veh)	–11.67 \pm 2.06	203	–5.670	<0.0001	<0.0001
	(Δ Met 150/Ola) – (Δ Met 400/Ola)	–0.525 \pm 1.97	203	–0.270	0.790	1.000
	(Δ Met 150/Ola) – (Δ Veh/Ola)	–1.785 \pm 1.97	203	–0.900	0.367	1.000
	(Δ Met 400/Ola) – (Δ Veh/Ola)	–1.259 \pm 2.05	203	–0.610	0.541	1.000

DF, degrees of freedom; Pr, probability; R_a, glucose appearance; R_d, glucose disappearance.

risperidone (0.2 mg/kg once daily). Although significant improvements in all outcome parameters (i.e., weight, fasting glucose, insulin, HbA1c) were observed with Met, failure to take into account Met-related reductions in weight gain could not preclude that improvements in glucose parameters were related to weight loss rather than reversal of direct-risperidone effects on glucose pathways.

Two separate studies by Boyda examined acute Ola (7.5 mg/kg or 15 mg/kg) administration, thus avoiding the confounding effects of changes in adiposity. These studies examined the differential effects of Met (100 mg/kg or 500 mg/kg), rosiglitazone and glyburide, according to single (Boyda *et al.* 2012) or combination treatment (Boyda *et al.* 2014) paradigm, on glucose tolerance and insulin sensitivity (HOMA-IR). Met was given at 1100 h on day 1, and following an overnight fast, rats received (day 2) a single i.p. injection of Ola (7.5 mg/kg or 15 mg/kg). Sixty min later, saphenous blood was drawn for assessment of insulin sensitivity (HOMA-IR), and a second dose of Met was given, followed by an IP-GTT. A similar protocol was repeated for rosiglitazone and glyburide. Findings suggested partial reversal of glucose intolerance by both rosiglitazone and Met, but not glyburide (Boyda *et al.* 2012). In the second study, which followed a similar protocol, Met, as well as glyburide, significantly decreased glucose levels during the IP-GTT, whereas rosiglitazone, in contrast to the earlier study, failed to do so. Examination of dual drug combinations demonstrated unexpectedly that the Met-rosiglitazone combination was associated with increased glucose levels relative to single anti-diabetic drug treatment (Boyda *et al.* 2014).

In keeping with pre-existing studies, we have replicated the beneficial effects of Met on indices of glucose

metabolism following Ola treatment and, in addition, demonstrated several novel findings. While Met is generally understood to enhance both liver and peripheral insulin sensitivity (Wiernsperger & Bailey 1999), our data in Ola-treated animals suggest improvements relative to Veh with 150 mg/kg of Met only in the reduction of glucose production, with no effect on glucose utilization. The lack of effect on glucose disposal might help to explain why the aforementioned studies from Boyda demonstrated only partial reversal of acute Ola-induced glucose intolerance with Met (Boyda *et al.* 2012, 2014).

Failure of Met to impact peripheral insulin resistance linked to antipsychotic treatment in preclinical models has clinical implications. In rodent models of antipsychotic-induced glucose dysregulation, increased HGP is arguably the most commonly observed perturbation; however, whether or not this is the case in humans is unclear. Of the currently published studies in normal-weight healthy volunteers treated with short-term (1–10 days) Ola (Sacher *et al.* 2008, Vidarsdottir *et al.* 2010a,b, Albaugh *et al.* 2011b, Teff *et al.* 2013, Hahn *et al.* 2013), two employed the HIEC and separately assessed peripheral and hepatic insulin sensitivity, and both suggested impairments in glucose uptake, but failed to note increases in glucose production (Vidarsdottir *et al.* 2010a, Teff *et al.* 2013). Although these findings require replication, they suggest that if peripheral insulin sensitivity is indeed most prominently impacted by APs in humans, use of Met for patients on antipsychotic medications who develop diabetes is open to question. It may be that combination therapy is required to optimally target peripheral insulin resistance. Although thiazolidinediones (insulin sensitizers which act through complementary

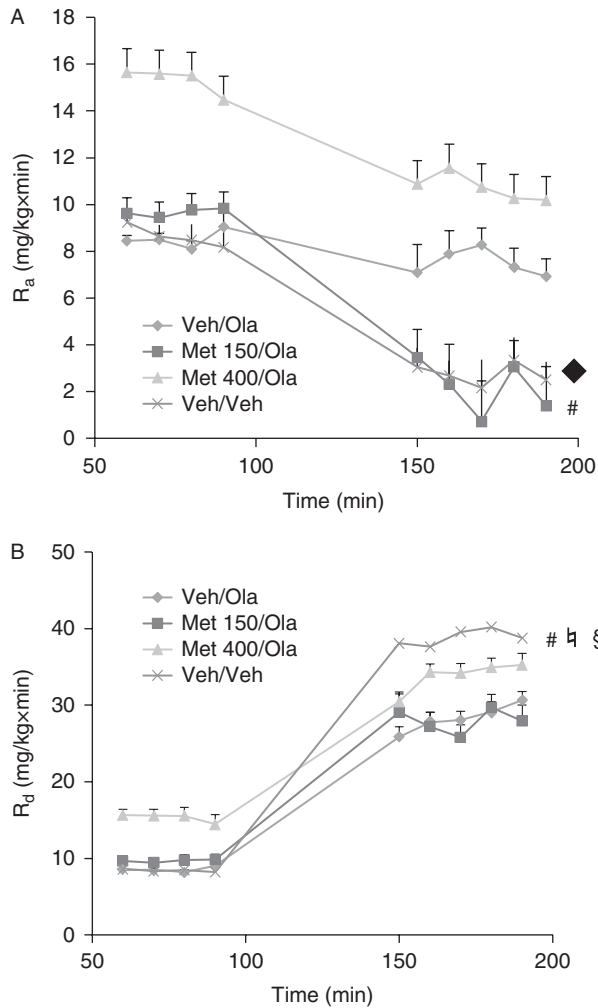


Figure 4 Effect over time, and by treatment group: vehicle+vehicle (Veh/Veh) ($n=11$); vehicle+olanzapine (Veh/Ola) ($n=11$); metformin 150 mg/kg+olanzapine (Met 150/Ola) ($n=13$); metformin 400 mg/kg+olanzapine (Met 400/Ola) ($n=11$) on rate of glucose appearance (R_a)/production (A) and rate of glucose disappearance (R_d)/utilization (B) during a hyperinsulinemic-euglycemic clamp procedure in male Sprague-Dawley rats. Significant time \times group interactions were found for R_a and R_d and were investigated through *post-hoc* contrasts to look at the average treatment group effect during the hyperinsulinemic (HI) clamp phase, accounting for differences during the basal phase. 'Treatment effect' was thus defined as the difference between HI clamp and basal phases (HI clamp-basal) in R_a (A) or R_d (B) and compared between pairs of groups with adjustments using the Bonferroni method. * $P=0.002$ vs Veh/Ola; # $P<0.0001$ vs Veh/Ola; $^{\ddagger}P<0.0001$ vs Met 150/Ola; $^{\S}P<0.0001$ vs Met 400/Ola. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JOE-15-0074>.

pathways to those of Met) would be a rational choice, findings by Boyda *et al.* (2014) involving Ola related glucose intolerance note a lack of beneficial effect for combination therapy with Met and rosiglitazone. Further studies are therefore required.

Contrary to our working hypothesis, the higher dose of Met (400 mg/kg \times two doses) failed to improve insulin sensitivity. Due to an unexpected increase in basal glucose production following high-dose Met, but preceding Ola, as compared to Veh or low-dose Met, we measured plasma lactate. Lactate, a substrate for HGP, was significantly increased in the high-dose Met group relative to both the lower Met dose and Veh. In isolated mitochondria or hepatocytes, Met has been shown to impair mitochondrial respiration by complex 1 inhibition, resulting in increased glycolysis and glucose uptake, decreased liver lactate uptake and decreased use of lactate as a substrate for glucose production (Radziuk *et al.* 1997, Owen *et al.* 2000, Otto *et al.* 2003). However, Met is also known to activate lactate production in the intestine and muscle (Borst & Snellen 2001), which may hypothetically, in an *in vivo* system, lead to an excessive gluconeogenic substrate loading, overriding Met-related reductions in lactate usage. Although speculative, the possibility exists that increased hepatic gluconeogenic loading could have

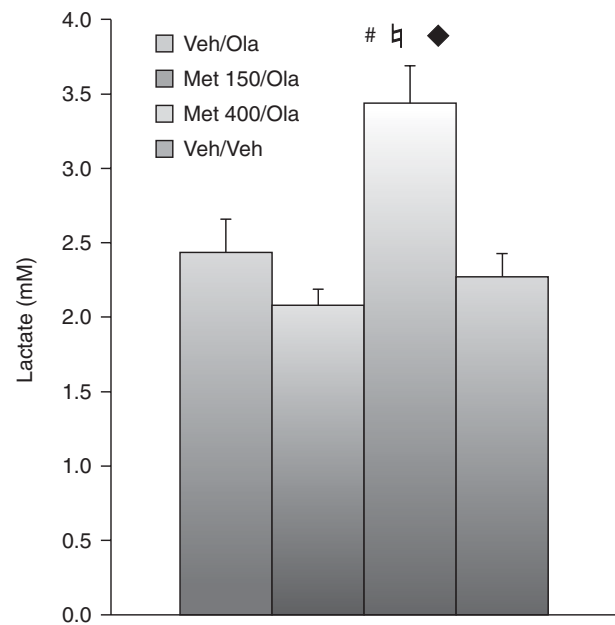


Figure 5 Effect by treatment group: vehicle+vehicle (Veh/Veh) ($n=11$); vehicle+olanzapine** (Veh/Ola) ($n=11$); metformin 150 mg/kg+olanzapine (Met 150/Ola) ($n=13$); metformin 400 mg/kg+olanzapine (Met 400/Ola) ($n=11$) on lactate levels during the basal phase of a hyperinsulinemic-euglycemic clamp procedure; analysis done by ANOVA, followed by Bonferroni adjustments where applicable. # $P<0.05$ vs Veh/Ola; $^{\ddagger}P<0.05$ vs Met 150/Ola; * $P<0.05$ vs Veh/Veh. **Lactate was measured preceding olanzapine administration. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JOE-15-0074>.

contributed to the decreased effect of the high Met dose on suppression of HGP following Ola during hyperinsulinemia. Interestingly, there are at least two *in vivo* studies, administering 300 mg/kg per day or 1000 mg/kg per day of Met in rodents, demonstrating respective increases in, or a lack of inhibition of HGP (Dang *et al.* 2007, Yoshida *et al.* 2009). It remains unclear why, contrary to our findings, Boyda *et al.* (2012) found that both the 100 and 500 mg/kg Met doses significantly decreased glucose intolerance by Ola. However, in their study, the calculated index of insulin resistance in the high-dose Met (HOMA-IR=21.7) appeared to be higher than in the low-dose Met (HOMA-IR=13.9). In addition, the period between administration of the first and second Met doses was longer in the Boyda study (first dose given at 1100 h, vs early evening in the current study). Overall, the clinical relevance of our observation in rodents that a higher Met dose may have masked metabolic benefits in association with an increase in lactate levels is unknown. In humans, the use of higher-range therapeutic doses of Met is unlikely to be of consequence unless elimination of Met is compromised (i.e., renal failure). That said, serious mental illnesses such as schizophrenia and bipolar disorder have been associated with mitochondrial dysfunction and increased lactate levels (Halim *et al.* 2008, Regenold *et al.* 2009, Herberth *et al.* 2011), which raises the unexplored possibility that our patients may require more judicious dosing of Met to obtain maximal benefits. Translation of findings from rodents to clinic may, however, be complicated by genetic factors mediating metabolism and therapeutic action of Met (Shu *et al.* 2007).

To summarize, in keeping with other preclinical studies, we show that Met can partially mitigate effects of Ola-induced glucose dysregulation, attenuating hepatic insulin resistance but failing to impact peripheral insulin resistance. The high prevalence of DM2 in patients with serious mental illnesses highlights the need for clinical trials that extend beyond weight loss to examine prevention or treatment of glucose dysregulation.

Declaration of interest

In the past 3 years, G J Remington has received research support from the Canadian Diabetes Association, the Canadian Institutes of Health Research, Medicare, Neurocrine Biosciences, Novartis, Research Hospital Fund-Canada Foundation for Innovation and the Schizophrenia Society of Ontario. In addition, he has served as a consultant or speaker for Laboratorios Farmacéuticos Rovi, Medicare, Novartis and Synchronuron. M K Hahn has received funding from the Banting Research Foundation, the

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