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Voluntary physical activity protects against olanzapine-induced hyperglycemia

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Abstract

Olanzapine (OLZ) is used in the treatment of schizophrenia and a growing number of "off-label" conditions. While effective in reducing psychoses, OLZ causes rapid impairments in glucose and lipid homeostasis. The purpose of this study was to investigate if voluntary physical activity via wheel running (VWR) would protect against the acute metabolic side effects of OLZ. Male C57BL/6J mice remained sedentary or were provided with running wheels overnight, prior to treatment with OLZ either at the beginning of the light cycle, or 7 or 24 hours following the cessation of VWR. Prior VWR protected against OLZ-induced hyperglycemia immediately and 7 hours following a bout of overnight wheel running. Protection against, hyperglycemia immediately following VWR was associated with increased insulin tolerance and an attenuated OLZ-induced increase in the serum glucagon:insulin ratio. The protective effect of VWR against OLZ-induced increases in hyperglycemia and glucagon:insulin ratio was maintained in high fat fed, and AMPK β1 deficient mice, models which display a potentiated OLZ-induced increase in blood glucose. Repeated OLZ treatment did not impair VWR performance and protection against the acute effects of OLZ on blood glucose was present after 1 week of daily OLZ treatment in mice given access to running wheels. In contrast to the effects on glucose metabolism, VWR, for the most part, did not impact OLZ induced perturbations in lipolysis, liver triglyceride accumulation or whole-body substrate oxidation. Collectively our findings demonstrate the efficacy of voluntary physical activity as an approach to protect against OLZ-induced impairments in glucose metabolism.

New and Noteworthy The antipsychotic medication olanzapine causes rapid and large increases in blood glucose. We demonstrate that a prior bout of voluntary overnight wheel running can protect against this harmful side effect and is likely mediated by reductions in olanzapine

induced increases in the circulating glucagon to insulin ratio. This study highlights the powerful effects of voluntary activity in conditions of treatment with antipsychotic medications.

Introduction

Second-generation antipsychotic (SGA) drugs, such as olanzapine (OLZ), are used in the treatment of schizophrenia, a severe mental illness affecting about 1% of the population (47). In recent years the use of SGAs in the management of, "off-label" conditions (45) such as anxiety, dementia, attention-deficit, bipolar and sleep disorders (50) have increased dramatically. SGAs act through binding dopamine (D_2), serotonin (HT_{2A}) and muscarinic (M_3) receptors (23). Although effective at reducing psychosis, the use of SGAs is associated with serious metabolic side effects occurring in ~70% of individuals prescribed these medications (21). Metabolic complications of SGAs include weight gain (8), dyslipidemia (54), and impairments in glucose homeostasis (13, 41, 43, 58).

A plethora of studies have demonstrated that SGAs cause rapid and direct disturbances in glucose metabolism. This occurs in the absence of weight gain and has been reported in both preclinical rodent models (37, 40, 49, 57) and in humans (32). The mechanisms behind the acute effects of OLZ-induced glucose dysregulation are multifactorial, including: impairments in insulin secretion (16, 17), decreases in insulin sensitivity (16), reductions in carbohydrate oxidation (40) and increases in hepatic glucose production (HGP) (16, 36, 37). Recently, our laboratory has provided strong evidence that HGP, mediated via increased circulating glucagon, is a crucial factor in OLZ-induced hyperglycemia. Castellani et al., (15) found that acute OLZ treatment increased serum glucagon concentrations and that OLZ-induced hyperglycemia was prevented in glucagon receptor knockout mice. Likewise, pharmacological approaches that prevent OLZ-induced increases in the glucagon to insulin ratio, such as liraglutide, a glucagon

like peptide-1 (GLP-1) receptor agonist (6) can attenuate OLZ-induced hyperglycemia (48). Interestingly, the acute effects of SGAs on glucose metabolism are potentiated in conditions of pre-existing obesity (60), as would often be seen in individuals with schizophrenia prior to treatment with SGAs (4, 59).

With increasing evidence of the adverse metabolic effects of OLZ, the development of approaches to alleviate the metabolic complications of these drugs have been investigated (11). Commonly prescribed anti-diabetic drugs including metformin, thiazolidinediones (TZDs) and sulfonylureas alone, or in combination, are only partially effective in treating acute SGA-induced disturbances in glucose metabolism (10, 11) thus highlighting the difficulty in identifying treatments to offset the numerous metabolic effects of SGAs.

Exercise has profound effects in regulating glucose metabolism. Seminal studies have shown that a single bout of exercise increases skeletal muscle insulin sensitivity (53) and insulin-independent skeletal muscle glucose uptake (34). A previous study reported that regularly performed exercise for 9 weeks in the form of wheel running protected against SGA-induced weight gain and glucose intolerance in rats (12) . Similarly, clinical investigations have shown that the combination of exercise prescription and alterations in diet leads to reductions in adiposity (28) and fasting blood glucose concentrations (31) in patients being treated with SGAs. Previously we found that exhaustive, but not moderate, exercise protected against OLZ-induced hyperglycemia (14). While this particular finding provides intriguing evidence that exercise could be effective in protecting against acute SGA-induced disturbances in glucose metabolism, the clinical translatability is limited as exercise adherence in those taking SGAs is poor (5) and prescribing daily, exhaustive exercise prior to treatment is likely an unrealistic option. Given this, we wanted to determine if a less stressful and voluntary form of exercise could confer

protection against the acute metabolic side effects of SGAs. To do this we used a model of voluntary wheel running (VWR), which has been previously shown to be an effective model to study the effects of physical activity in mice (46). Compared to forced treadmill running, VWR offers several advantages. First, the physical activity pattern is similar to natural running behavior of mice; performed under stress free conditions, according to the rhythmicity of the animal (46). Second, VWR requires no direct interference from the researchers and removes the additional stress of animals being handled. Lastly, mice run spontaneously when given access to running wheels. Within this framework we sought to determine if 1) an overnight bout of VWR would be sufficient to protect against acute SGA-induced disturbances in glucose metabolism and if so, how long this protective effect would last, 2) potential mechanisms that could explain the ability of VWR to protect against SGA-induced perturbations in glucose metabolism and 3) if the ability of VWR to protect against SGA-induced excursions in blood glucose is maintained under conditions of a potentiated SGA-response.

Methods

All experimental procedures were approved by the University of Guelph Animal Care Committee and followed the guidelines of the Canadian Council on Animal Care. 8-12-week old C57BL/6J male mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Breeding pairs of wild-type and AMPK $\beta1^{-/-}$ mice on a C57BL/6 background, generated as previously described by Dzamko et al. (24), were used to establish a colony at the University of Guelph. Male wild-type and AMPK $\beta1^{-/-}$ mice were studied at ~16-24 week of age. All animals were individually housed in clear polycarbonate shoebox-style cages (dimensions: 7 1⁄2" x 11 1⁄2" x 5") with wire lids. Rooms were kept at an ambient temperature of 22°C with 45% humidity and a 12:12 h light dark cycle. Mice were given free access to water and standard rodent chow (7004-Teklad S-2335 Mouse Breeder Sterilizable Diet; Teklad Diets Harlan Laboratories, Madison WI).

Materials: OLZ (cat. no. 11937) was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Dimethylsulfoxide (DMSO) was purchased from Wako Pure Chemical Industries (Richmond, VA). Freestyle Lite blood glucose test strips and a handheld glucometer were acquired from Abbott Diabetes Care Inc. (Alameda, CA, USA). Insulin was purchased from Eli Lilly (Toronto, ON, CAN). Kolliphor EL (cat. no C5135) was purchased from Millipore Sigma (Etobicoke, ON, CAN). Injections were carried out using 25-gauge needles purchased from ThermoFisher Scientific (Mississauga, ON, CAN; cat. no. BD B305122). Glucagon (cat. no. 10-1281-01) and insulin (cat. no. 10-1247-01) enzyme-linked immunosorbent assay (ELISA) kits were obtained

from Mercodia Inc. (Winston-Salem, NC 27103, USA). GLP-1 ELISA kits were obtained from Millipore Sigma (Etobicoke, ON, CA; cat no. EZGLP1–36K).

Overnight Voluntary Wheel Running: Mice were weight matched into a sedentary (SED) or voluntary wheel running (VWR) group following the acclimation period. Mice had timed access to 8-inch wire mesh rodent wheels (Prevue Pet Products Inc, Chicago, IL) overnight, with free access to food and water. The access to running wheels was given 3 hours prior to the start of the dark cycle, and wheels were locked at the beginning of the light cycle (~ 0800 hours). Running distance was recorded daily (Mountain Equipment Co-Op, Vancouver, BC, CAN). The intraperitoneal (I.P.) OLZ tolerance test (OTT) was conducted immediately post-wheel lock.

Intraperitoneal OLZ tolerance test (OTT): A powdered stock of OLZ was dissolved in DMSO at 1 mg/100 µl, and then added to a sterile solution of saline (0.9% NaCl) and Kolliphor. The final solution concentrations consisted of 90% saline, 5% Kolliphor, and 5% DMSO/OLZ solution, with a final OLZ concentration of 0.5 mg/ml. Vehicle (VEH) solution was made using the same reagent quantities, except OLZ stock was replaced with an equivalent volume of DMSO. Mice remained either sedentary overnight or were physically active with access to a running wheel. Immediately after overnight VWR, or 7- or 24-hours following wheel lock, while having ad libitum access to food and water, blood glucose was measured with a handheld glucometer using a small drop of blood from the end of the tail. Mice were then injected I.P. with a weight-adjusted bolus of OLZ (5 mg/kg BW) or an equivalent amount of vehicle solution and blood glucose determined at 30, 60, 90- and 120-minutes post treatment.

AMPK beta1 KO experiments: AMPK $\beta1^{-/-}$ male mice were weight matched into a SED or VWR group and given access to 8-inch wire mesh rodent wheels overnight, with free access to food and water. The access to running wheels was given 3-4 hours prior to the start of the dark cycle, and wheels were locked at the beginning of the light cycle. Running distance was recorded the next morning. The OTT was conducted immediately post-wheel lock.

High fat diet experiments: After acclimation, mice were switched to a high-fat diet (HFD; 60% kcal from fat, Research Diets D12492) for 4 weeks. After 4 weeks mice were weight matched into SED or VWR group and given access to 8-inch wire mesh rodent wheels overnight, with free access to food and water. The access to running wheels was given 3-4 hours prior to the start of the dark cycle, and wheels were locked at the beginning of the light cycle (~ 0800 hours). Running distance was recorded the next morning. The OTT was conducted immediately post-wheel lock.

Tissue harvest: Following all experiments a weight adjusted bolus of sodium pentobarbital (~60 mg/kg) was injected intraperitoneally (IP) and the liver was freeze clamped *in situ* and snap-frozen in liquid nitrogen. Blood was collected via cardiac puncture of the right ventricle with a 25-gauge needle, allowed to clot for 30 min at room temperature, and then centrifuged at 5,000 *g* for 10 min with the serum being aliquoted and frozen at -80 °C until further analysis.

Insulin tolerance Test: SED or VWR mice were treated with OLZ or vehicle as described above in the overnight VWR protocol. 60 minutes following treatment with OLZ, blood glucose was determined and mice were then injected with a weight-adjusted bolus of insulin (Eli Lilly) (0.5

U/kg bw). Blood glucose was measured 20 and 30 minutes following the insulin injection. As described in detail previously, blood glucose levels were expressed relative to the initial values prior to insulin injections within each animal (61).

Comprehensive lab animal monitoring system (CLAMS): At the beginning of the animal's light cycle, after overnight exercise, and immediately post wheel lock, mice were injected with OLZ (5mg/kg) or vehicle and placed into CLAMS caging. Respiration and activity were measured over the next 2 h. The mean values following OLZ or vehicle treatment were determined for respiratory exchange ratio (RER) (VCO2/VO2) and activity.

Repeated overnight VWR and OTT: The overnight VWR procedure was repeated daily for 7 days. SED and VWR mice were injected every day with fresh drugs prepared daily. An OTT was performed on days 1 and 7 immediately post wheel lock. The initial rise in blood glucose (60-minute post injection), after the first and seventh night of wheel running was measured from the tail vein following injections. An abbreviated OTT on days 1 and 7 only was completed in order to minimize the stress of repeated blood sampling over the course of the 7-day treatment.

Measurement of circulating hormones and metabolites: Serum non-esterified fatty acid (NEFA) (Wako Bioproducts, Richmond, VA, USA), glycerol (F6428; Millipore Sigma), and triglycerides (cat. No. 10010303, Cayman chemical, Ann Arbor, MI, USA) were measured on 96-well plates using commercially available kits. Serum concentrations of insulin, glucagon and GLP-1 were measured using ELISAs. All assays were conducted in accordance with manufacturer's instructions, in duplicate with an average CV of <10%. Plates were read using Biotek Synergy Mx Multi Format Microplate Reader.

Liver TAGs: Snap frozen liver was chipped, weighed, and used to quantify triglyceride (TAG)

content. Liver chips were homogenized in 1 ml of methanol:chloroform (1:2), and agitated overnight at 4°C (9). One ml of 4 mM $MgCl_2$ was added the following day, vortexed, and centrifuged for 1 hour at 1,000 g at 4°C. The organic infranatant was transferred into a new tube, evaporated overnight in a fume hood, and reconstituted in a 3:2 1-butanol-Triton X-114 mix. TAG content was measured with a commercially available kit (Sigma-Aldrich, cat. No. F6428) in duplicate and read using Biotek Synergy Mx Multi Format Microplate Reader.

Western blotting: ~ 30 mg of liver samples were homogenized (TissueLyser LT; Qiagen) with NP40 cell lysis buffer (ThermoFisher, #FNN0021; Waltham, MA, USA) supplemented with protease inhibitor cocktail and phenylmethylsulfonyl fluoride (PMSF). Homogenized samples were centrifuged (10 min at 5000 *g* at 4°C) and protein content was determined in the supernatant. Equal amounts of proteins were separated on 10% SDS-PAGE gels and transferred onto nitrocellulose membranes. Membranes were blocked for 1 hour and incubated overnight with phospho-PKA substrates primary antibodies (Cell Signaling Techonology, #9624; Danvers, MA, USA) diluted (1:1000) in Tris-buffered saline with Tween (TBST)/5%bovine serum albumin at 4°C with gentle agitation. Afterward, membranes were washed with TBST and incubated for 1 hour at room temperature with horseradish peroxidase–conjugated secondary antibodies (Cell Signaling Technology Cat #7074; 1:2000; Danvers, MA, USA), and signals were detected using enhanced chemiluminescence and quantified with ImageJ software. Phosphorylated proteins were expressed relative to a within-gel ponceau stain (3, 22, 29, 55).

Real-time PCR: RNA was extracted using Trizol and RNeasy Mini Kits (Qiagen, #74106; Hilden, Germany) and genomic DNA removed using DNase-free treatment (ThermoFisher, #AM1906; Waltham, MA, USA). cDNA was produced using a High-Capacity cDNA Reverse Transcript kit (ThermoFisher, #4368814; Waltham , MA, USA), and real-time PCR was run with

Sso Advanced Universal SYBR Green Supermix (Bio-Rad, #1725271; Hercules, CA, USA) using PCR primers on a Bio-Rad CFX Connect system. All genes are expressed relative to PPIB using the $2^{-\Delta\Delta}$Ct method (44). Primer sequences were synthesized by the Genomics Facility at the University of Guelph for *Ppib* (39) (FWR: 5'-GGAGATGGCACAGGAGGAA-3'; REV 5'-GCCCGTAGTGCTTCAGCTT-3') and *G6pc* (30) (FWR 5'-AGGAACGCCTTCTATGTCCTCTTT-3'; REV 5'-GCGTTGTCCAAACAGAATCCACTTG-3').

Statistical Analysis: A two-way or repeated measures two-way ANOVA were used to determine differences between the OLZ and VEH sedentary and exercise groups followed by a Tukey's post hoc analysis where appropriate. All data was expressed as mean ± S.E.M, and statistical significance was determined at $P < 0.05$. Analyses and figures were created using GraphPad (Prism, version 8; La Jolla, CA).

Results

Overnight physical activity protects against OLZ-induced hyperglycemia We first wanted to determine if voluntary physical activity, would be sufficient to offset the negative metabolic side effects of acute OLZ treatment. As female mice are protected against acute OLZ-induced disturbances in glucose metabolism (48, 49) we utilized male C57BL/6J mice. Mice were treated with a weight-matched bolus of OLZ (5 mg/kg) or vehicle and changes in blood glucose were tracked for 120 minutes. When analyzing the blood glucose curves, there was a significant group x time interaction ($p<0.05$) such that blood glucose was increased relative to vehicle within the same group (SED or VWR) at each time point measured in SED-OLZ, while this was only evident at 60 and 120 mins post treatment in VWR mice. Blood glucose concentrations were significantly ($P<0.05$) greater in SED-OLZ compared to VWR-OLZ mice at 90 minutes. While

OLZ significantly increased blood glucose area under the curve (AUC) compared to vehicle (Figure 1 A and B) in both groups, the effects of OLZ were significantly attenuated in VWR compared to sedentary mice (Figure 1 A and B). There was not a significant association between distance run and the blood glucose AUC under OLZ stimulated conditions ($r=-0.064$, $P=0.784$). These findings provide evidence that voluntary physical activity can protect against OLZ-induced hyperglycemia as does exhaustive forced exercise (14).

Next, we wanted to determine how long a protective effect of prior wheel running against OLZ-induced hyperglycemia may last. To this end mice were given access to running wheels overnight, and then at the beginning of the subsequent light cycle the wheels were locked for either 7 or 24 hours. When examining the blood glucose curves for the 7 hours wheel lock there was a group x time interaction ($P < 0.05$) such that blood glucose was increased compared to vehicle at each time point measured following OLZ treatment in sedentary mice. Blood glucose levels were significantly ($P < 0.05$) greater in OLZ treated sedentary compared to VWR mice at 60 and 90 minutes (Figure 1 C). As shown in Figure 1 (D), OLZ-induced increases in the glucose AUC were absent in mice 7 hours following the cessation of voluntary wheel running. Analysis of the glucose curves for the 24 hours wheel lock (Figure 1E) revealed a group x time interaction ($P < 0.001$) such that blood glucose was increased compared to vehicle controls at 30, 60, 90, and 120-minutes post treatment in the VWR group, whereas blood glucose was increased relative to control at 30 and 90 minutes post treatment in sedentary mice. When analyzing the glucose AUC OLZ caused significant increases in both groups of mice (Figure 1 F). Collectively, these findings provide evidence that overnight, voluntary activity is sufficient to protect against OLZ-induced increases in blood glucose, however this effect is somewhat short-lived.

293 *Overnight voluntary wheel running alters OLZ-induced changes in the glucagon to insulin ratio*
294 We have previously shown that OLZ treatment increases the glucagon to insulin ratio in male
295 mice (14, 49, 57) and thus we wanted to determine if prior physical activity would alter this
296 response. A 2-way-ANOVA demonstrated main effects of VWR and OLZ to increase and
297 decrease serum insulin levels, respectively (Figure 2 A). There was a significant interaction
298 between VWR and OLZ such that OLZ treatment increased serum glucagon levels in SED but
299 not VWR mice (Figure 2 B). Consequently, the ratio of glucagon-to-insulin, a measure which
300 has been linked to dysglycemia and markers of pancreatic dysfunction (42), was increased by
301 OLZ under SED conditions while VWR protected against this (Figure 2 C). OLZ treatment
302 increased serum GLP-1 levels in both SED and VWR mice (Figure 2 D) suggesting that GLP-1
303 likely does not mediate the protective effects of VWR against OLZ-induced increases in the
304 glucagon to insulin ratio and blood glucose. Glucagon increases hepatic PKA signaling (38) and
305 leads to the induction of gluconeogenic enzymes such *G6Pase*. Given our previous findings (15)
306 demonstrating a central role for glucagon in mediating OLZ-induced hyperglycemia we assessed
307 indices of glucagon signaling in the liver. As shown in Figure 2 E and F, OLZ significantly
308 increased the phosphorylation of PKA substrates in livers from both SED and VWR mice.
309 Conversely, OLZ induced increases in *G6Pase* expression were absent in livers from VWR mice
310 (Figure 2 G). Taken together with the blunted increase in the glucagon to insulin ratio, these
311 findings suggest a reduction in OLZ -induced liver glucagon signaling with prior wheel running.
312
313 *Voluntary physical activity protects against OLZ-induced insulin resistance* We (15), and others
314 (11, 17), have previously shown that OLZ causes marked insulin resistance. As physical activity
315 confers noted insulin sensitizing effects (33) we wanted to determine if the protective effects of

316 prior VWR could be explained, at least in part, by alterations in peripheral insulin action.
317 Following overnight VWR, mice were immediately treated with OLZ and 60 minutes later
318 challenged with a bolus injection of insulin and changes in blood glucose and AUC determined.
319 When analyzing the blood glucose curves, there was a significant group x time interaction
320 ($p<0.05$) such that blood glucose was greater relative to vehicle within the same group (SED or
321 VWR) at 20 minutes post-OLZ injection. As shown in Figure 3 B, there were main effects of
322 OLZ and VWR on insulin tolerance such that OLZ increased and VWR reduced the glucose
323 AUC.
324
325 *Overnight physical activity protects against OLZ-induced hyperglycemia in mouse models*
326 *susceptible to an exaggerated blood glucose response to OLZ.* We next wanted to determine if
327 VWR would still be effective in protecting against excursions in blood glucose under conditions
328 of an exaggerated OLZ response. To that end we utilized AMPK β1 knockout mice, an animal
329 model that displays a potentiated blood glucose response to OLZ (57). When examining the
330 blood glucose curves there was a group x time interaction ($P<0.05$) where SED OLZ was
331 significantly different than vehicle at each point measured following OLZ treatment.
332 Furthermore, there was a significant difference between SED OLZ and VWR OLZ mice at each
333 time point (Figure 4 A). OLZ treatment resulted in a robust increase in the blood glucose AUC in
334 AMPK β1 KO-mice, while a prior night of VWR completely protected against this (Figure 4 B).
335 There was a significant interaction between VWR and OLZ such that OLZ treatment decreased
336 serum insulin levels in SED but not VWR mice (Figure 4 C). A 2-way-ANOVA demonstrated
337 main effects of both OLZ and VWR to increase and decrease serum glucagon levels, respectively
338 (Figure 4 D). Consequently, there was a main effect of OLZ to increase the ratio of glucagon to

insulin (Figure 4 E). The main effect of OLZ to increase glucagon: insulin ratio was driven by the increase in the SED (P=0.0618) but not in the VWR (P = 0.999) mice. As shown in Figure 4, OLZ significantly increased the phosphorylation of PKA substrates (F and G), and gene expression of G6Pase (H) only in livers from SED but not VWR mice.

We next wanted to confirm our findings using AMPK β1 knockout mice and determine if VWR would protect against hyperglycemia in an additional model displaying an exaggerated blood glucose response to SGAs. To this end we completed experiments in mice fed a high fat diet. We have previously shown that acute OLZ-induced hyperglycemia is potentiated in mice fed a high fat diet for 4 weeks (60). When examining the blood glucose curves there was a group x time interaction ($P < 0.05$) where the SED OLZ group was significantly different than vehicle at 60, 90 and 120-minutes following OLZ treatment. Furthermore, there was a significant difference between SED OLZ and VWR OLZ mice at 90-minutes post treatment (Figure 5 A). As shown in Figure 5 B, acute OLZ treatment caused a large increase in the blood glucose AUC and this was prevented by a prior night of wheel running. A 2-way-ANOVA demonstrated a main effect of OLZ to decrease serum insulin levels (Figure 5 C). There was a significant interaction between VWR and OLZ such that OLZ treatment increased serum glucagon levels in SED but not VWR mice (Figure 5 D). Consequently, the ratio of glucagon to insulin, was increased by OLZ (Figure 5 E). Again, the main effect of OLZ to increase glucagon: insulin ratio was driven by the increase in the SED ($P < 0.05$) but not in the VWR ($P = 0.082$) mice. As shown in Figure 5, OLZ significantly increased the phosphorylation of PKA substrates (F and G), and gene expression of G6Pase (H) in livers from SED but not VWR mice.

OLZ induces similar alterations in lipid metabolism in SED and VWR mice. We and others recently demonstrated that OLZ treatment is associated with perturbations in fat metabolism (40, 49, 57) such as increases in lipolysis, liver triglyceride accumulation and fatty acid oxidation. To determine if prior VWR influenced these endpoints, we assessed alterations in circulating/tissue specific metabolites and indices of whole-body substrate oxidation. As shown in Figure 6, there was a main effect of OLZ to increase serum NEFA (A) and glycerol (B). There was a main effect of OLZ to increase and a main effect of VWR to reduce serum triglycerides (Figure 6 C), while there were main effects of OLZ and VWR to increase liver triglyceride concentrations (Figure 6 D). These findings provide evidence that despite protecting against OLZ-induced hyperglycemia, prior VWR does not protect against OLZ-induced perturbations in lipid metabolism.

We next wanted to determine if prior VWR would alter changes in whole body substrate oxidation and physical activity in the immediate, 2-hour period post OLZ treatment where we observed the development of hyperglycemia. As shown in Figure 7 there was an interaction between OLZ and VWR on oxygen consumption (A) and carbon dioxide production (B) such that these were increased in VWR compared to sedentary mice treated with OLZ. Similar to what we, and others have shown (40, 49, 57), there was a main effect of OLZ to reduce RER (Figure 7 C). While OLZ reduced physical activity levels in SED mice (Figure 7D), this effect was absent in mice that had been previously active. Similarly, activity levels were higher in vehicle treated SED compared to VWR mice (Figure 7 D). Collectively, these findings demonstrate that OLZ-induced shifts in substrate oxidation, are not impacted by prior VWR, despite differences in levels of cage activity.

Repeated treatment with OLZ does not alter VWR performance As physical activity levels were reduced following OLZ treatment in VWR mice, and given that previous work (12) has reported decreases in wheel running with SGAs, we wanted to determine if repeated, acute treatment with OLZ, reduced subsequent wheel running performance and if the protective effect of VWR against OLZ-induced hyperglycemia would be maintained following several days of treatment. In order to avoid undue stress to the animals we repeated the VWR experiments for seven consecutive days and measured the initial rise in blood glucose (at 60 min. post injection), after the first and seventh night of wheel running. There was a main effect ($P < 0.05$) of VWR to increase food intake (SED-VEH 21±0.63; SED-OLZ 23±0.87; VWR-VEH 26±1.5; VWR-OLZ 25±1.4g) and a main effect of OLZ ($p<0.05$) (SED-VEH 26±0.38; SED-OLZ 25±0.35; VWR-VEH 26±0.3; VWR-OLZ 24±0.3g) to decrease body weight. As shown in Figure 8 A and B, OLZ treatment at the beginning of the animal's light phase did not impact voluntary physical activity, in the subsequent dark phase as measured by total distance run. Importantly, the protective effect of VWR against acute OLZ-induced increases in blood glucose seen after just 1 bout of VWR (Figure 8 C), was maintained after the seventh bout of VWR (Figure 8 D). Together these findings provide evidence that repeated dosing with OLZ, at least for the duration studied, does not suppress wheel running performance, and the protective effect of voluntary physical activity against excursions in blood glucose is maintained.

Discussion

Voluntary physical activity is a powerful tool with which to modulate glucose and lipid metabolism. In the current study we demonstrate that 1) VWR protects against OLZ-induced hyperglycemia, an effect that is 2) paralleled by improvements in insulin action and a blunting of

406 OLZ-induced disturbances in glucagon and/or insulin, and 3) is maintained under conditions of a
407 potentiated OLZ-response such as seen in mice fed a high fat diet or in mice lacking the AMPK
408 β 1 subunit. Our results build upon work from Barr's laboratory (12) who demonstrated that
409 regularly performed voluntary wheel running blunts chronic SGA-induced increases in weight
410 gain and impairments in glucose homeostasis.

411 Previous findings provide evidence that glucagon is involved in the mechanisms through
412 which OLZ increases blood glucose. In support of this we found that OLZ treatment, as in the
413 current study, increases serum glucagon concentrations in male mice, while the hyperglycemic
414 effects of OLZ are absent in glucagon receptor knockout mice (15). In the present investigation
415 we found that VWR abrogates OLZ-induced increases in the serum glucagon to insulin ratio, and
416 indices of glucagon signaling in the liver, an effect that was maintained in models of exacerbated
417 OLZ response such as obese mice that had been fed a high fat diet.

418 We have recently shown that reductions in AMPK activity potentiates OLZ-induced
419 increases in blood glucose, while the pharmacological activation of this enzyme is sufficient to
420 protect against increases in blood glucose with OLZ treatment (57). In contrast to these data we
421 demonstrate that the protective effect of VWR against OLZ-induced hyperglycemia and changes
422 in the glucagon to insulin ratio are maintained in AMPK β1 knockout -mice, providing evidence
423 that hepatic AMPK is not essential for these beneficial effects of VWR on OLZ-induced
424 hyperglycemia.

425 The insulinotropic hormone GLP-1 can also reduce glucagon secretion (35, 51, 56), and
426 our laboratory recently demonstrated that pharmacological activation of the GLP-1 receptor with
427 compounds such as liraglutide, protects against acute OLZ-induced disturbances in blood
428 glucose in parallel with reductions in serum glucagon (48). Similarly, antagonizing the GLP-1

receptor potentiated OLZ-induced hyperglycemia (48). As prior work has reported that exercise increases serum GLP-1 concentrations via IL-6 in rodents (25) and humans (26) we reasoned that this could be a potential mechanism through which exercise confers protection against OLZ. However, as serum GLP-1 was increased with OLZ treatment to a similar extent in both SED and VWR mice this likely indicates that GLP-1 is not involved in the pathway(s) through which VWR prevents OLZ-induce excursions in blood glucose.

We and others have recently demonstrated that OLZ treatment is associated with perturbations in fat metabolism (40, 49, 57) such as increases in lipolysis, liver triglyceride accumulation and fatty acid oxidation. In the current study, we demonstrate that prior VWR largely does not protect OLZ -induced perturbations in lipid metabolism and thus suggests that the protective effect of VWR against OLZ-induced increases in blood glucose is not secondary to a blunted ability of OLZ to cause perturbations in lipid homeostasis.

OLZ displays potent sedative effects (2, 49, 57, 60), and here we extend this to show that OLZ significantly reduces overall physical activity levels in SED mice, but this effect was absent in mice that had been previously active. Similarly, activity levels were higher in VEH treated SED compared to VWR mice. A caveat to this data is that mice were given ~ 2 hours to acclimatize to the metabolic caging prior to drug treatment. This was a necessity in order to capture the acute metabolic effects of VWR, which we would not have been able to do if mice were acclimatized for ~24-48 hours, as is typically done, prior to drug treatment.

As physical activity levels were reduced following OLZ treatment in VWR mice, and given that previous work (12) has reported decreases in wheel running with SGAs, we wanted to determine if repeated, acute treatment with OLZ, reduced subsequent wheel running performance and if the protective effect of VWR against OLZ-induced hyperglycemia was

maintained. Our results provide evidence that repeated dosing with OLZ, at least for the duration studied, does not suppress wheel running performance, and the protective effect of voluntary activity against excursions in blood glucose is maintained. The discrepancy between Boyda's work (12) and ours in terms of decreased running performance could be due to species related differences (rats compared to mice), sex differences (female rats compared to male mice) and/or the dosage of drug that was used (10 Vs. 5 mg/kg). Regardless of the specific reason for these discrepancies our data provides evidence that prior OLZ treatment at the beginning of the light phase does not suppress wheel running performance during the subsequent dark phase, and that the protective effects of VWR against acute OLZ-induced hyperglycemia are maintained over time.

While our findings of OLZ-induced reductions in body weight with repeated treatment are somewhat surprising, they are consistent with previous studies in the literature (13, 19, 52) especially in male rodents. In this regard, a large body of work has examined the effects of chronic OLZ treatment in rodents and has shown sex-specific effects, with females being more susceptible to OLZ-induced weight gain (1, 18), which would more closely mirror what is seen in clinically.

Castellani et al. (14) demonstrated that exhaustive, but not moderate-intensity, forced treadmill exercise prevented OLZ-induced hyperglycemia. Given these findings it is striking that VWR, which is considered a less strenuous form of exercise, was able to confer a similar degree of protection. When examining voluntary wheel running behavior in C57BL/6J mice it has been reported that mice exercise in ~ 150 second bouts separated by short breaks, with an average running speed of 1.5-3.0 km/h or ~25-50 m/min (7, 20). This running speed is much faster than the moderate intensity (15 m/min) forced treadmill exercise that we previously used, and given

the interspersed rest periods, perhaps could be considered akin to high(er) intensity interval training in humans. As both the intensity and total volume (~ 3 km/night with VWR vs. ~1.1 km with moderate treadmill exercise) of exercise would appear to both be greater with VWR, compared to moderate treadmill exercise, both of these factors are likely important in the design of exercise interventions to protect against the metabolic side effects of SGAs. As prior work has shown the effectiveness of exercise as an adjunct therapeutic treatment in those with schizophrenia (27) it will be important to identify optimal exercise prescriptions that take into account not only the prevention of the metabolic side effects of SGAs, but adherence issues as well.

Competing Interests

The authors have no competing interests to declare.

Author Contributions

HS and DCW designed the experiments and drafted the manuscript. HS, GLM, KDM, and KEA performed some of the experiments. All authors edited and approved the final draft of the manuscript.

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Figure captions

Figure 1. Overnight physical activity protects against OLZ-induced hyperglycemia. SED or VWR mice were injected intraperitoneally with a weight-adjusted bolus of OLZ (5 mg/kg BW, IP) or an equivalent volume of vehicle. Blood glucose was measured over 90-120 min post–OLZ injection and AUC for no wheel lock (A,B), 7 hours wheel lock (C,D), and 24 hours wheel lock (E,F) mice were calculated. Data are presented as means ± SEM for 22 mice/group for (A, B), and 5-7 mice/group for (C-F). Main effects of the 2-way ANOVA are shown above the graph. VWR = main effect of voluntary wheel running, OLZ = main effect of OLZ. A "*" indicates significantly different ($P < 0.05$), than vehicle within the same group (SED or VWR) at the same timepoint; "#" indicates a difference between OLZ treated groups at the same timepoint as determined by repeated measures 2-way ANOVA followed by Tukey post hoc analysis.

Figure 2. Overnight voluntary wheel running alters OLZ-induced changes in the glucagon to insulin ratio. After an overnight session of VWR mice were injected intraperitoneally with a weight-adjusted bolus of OLZ (5 mg/kg BW, IP) or an equivalent volume of vehicle and serum harvested from cardiac blood 120 minutes later for the determination of insulin (A), glucagon (B), the ratio of glucagon:insulin (C) and GLP-1 (D). Hepatic PKA substrate phosphorylation (E, F) and gene expression of G6Pase (G) were measured. Data are presented as means ± SEM for 6-7 mice/group. Main effects of the 2-way ANOVA are shown above the graph. VWR = main

effect of voluntary wheel running, OLZ = main effect of OLZ. *P < 0.05 between indicated groups as determined by Tukey post hoc analysis.

Figure 3. Voluntary physical activity protects against OLZ-induced insulin resistance. SED and VWR mice were injected with a weight-adjusted bolus of OLZ (5 mg/kg, IP) or an equivalent volume of sterile saline. 60 minutes later mice were injected with insulin (0.5 U/kg bw) and blood glucose measured before and 20, and 30 minutes post. Relative changes in blood glucose were plotted (A) and the blood glucose AUC calculated (B). Data are presented as means ± SEM for 13 mice/group. Main effects of the 2-way ANOVA are shown above the graph. VWR = main effect of voluntary wheel running, OLZ = main effect of OLZ. "*" indicates significantly different (P < 0.05), than vehicle within the same group (SED or VWR) at the same timepoint as determined by repeated measures 2-way ANOVA followed by Tukey post hoc analysis.

Figure 4. Overnight voluntary physical activity protects against OLZ-induced hyperglycemia in AMPK beta 1 mice, a model of potentiated blood glucose response. After an overnight VWR session male AMPK $\beta 1^{-/-}$ mice were injected with a weight-adjusted bolus of OLZ (5 mg/kg, IP) or an equivalent volume of sterile saline and blood glucose measured over the following 120 minutes and glucose AUC calculated (A,B). 120 minutes following treatment cardiac blood was collected for the determination of serum insulin (C), glucagon (D), and the glucagon:insulin ratio (E). Hepatic PKA substrate phosphorylation (F, G) and gene expression of G6Pase (H) were also measured. Data are presented as means ± SEM for 4-5 mice/group. Main effects of the 2-way ANOVA are shown above the graph. VWR = main effect of voluntary wheel running, OLZ = main effect of OLZ. "*" indicates significantly different (P < 0.05), than

vehicle within the same group (SED or VWR) at the same timepoint; "#" indicates a difference between OLZ treated groups at the same timepoint as determined by repeated measures 2-way ANOVA followed by Tukey post hoc analysis.

Figure 5. Overnight voluntary physical activity protects against OLZ-induced hyperglycemia in HFD-fed mice, a model of exacerbated blood glucose response. After an overnight VWR session male mice fed a high fat diet were injected with a weight-adjusted bolus of OLZ (5 mg/kg, IP) or an equivalent volume of sterile saline and blood glucose measured over the following 120 minutes and glucose AUC calculated (A,C). 120 minutes following treatment cardiac blood was collected for the determination of serum insulin (C), glucagon (D), and the ratio of glucagon:insulin (E). Hepatic PKA substrate phosphorylation (F) and gene expression of G6Pase (G) were also measured. Data are presented as means ± SEM for 3-5 mice/group. Main effects of the 2-way ANOVA are shown above the graph. VWR = main effect of voluntary wheel running, OLZ = main effect of OLZ. *$P < 0.05$ between indicated groups as determined by Tukey post hoc analysis. "*" indicates significantly different, than vehicle within the same group (SED or VWR) at the same timepoint; "#" indicates a difference between OLZ treated groups at the same timepoint.

Figure 6. OLZ induces alternations in lipid metabolism to a similar extent in SED and VWR mice. Serum NEFA (A), glycerol (B), TAGs (C), and liver TAGs (D) were measured 2 hours following treatment with either OLZ (5 mg/kg bw) or vehicle. Data are presented as mean ± SEM for 7-8 mice per groups. Main effects of the 2-way ANOVA are shown above the graph. VWR = main effect of voluntary wheel running, OLZ = main effect of OLZ.

Figure 7. OLZ-induces shifts in substrate oxidation to a similar extent in SED and VWR mice, despite differences in levels of cage activity. At the beginning of the animal's light cycle and following overnight exercise, mice were injected with OLZ (5 mg/kg IP) or vehicle and placed into CLAMS caging. Respiration and activity were measured over the next 2 h. The mean values following OLZ or vehicle treatment were determined for oxygen consumption (A), carbon dioxide production (B), respiratory exchange ratio (RER) (VCO_2/VO_2) (C) and activity (D). Data are presented as means ± SEM for 6 mice/group. Main effects of the 2-way ANOVA are shown above the graph. OLZ = main effect of OLZ. *P < 0.05 between indicated groups as determined by Tukey post hoc analysis.

Figure 8. Repeated treatment with OLZ does not alter VWR performance. The overnight VWR procedure was repeated for 7 days. SED and VWR mice were injected daily with OLZ at the beginning of the light cycle and blood glucose determined 60 minutes following OLZ treatment on days 1 and 7. Daily (A) and total (B) running distance was determined and changes in blood glucose after the first (C) and seventh (D) night of wheel running were measured. Data are presented as means ± SEM for 12-14 mice/group. *P < 0.05 between indicated groups as determined by Tukey post hoc analysis.

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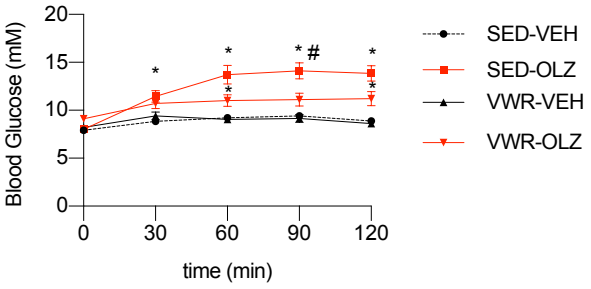
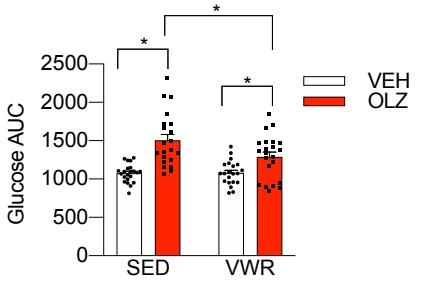
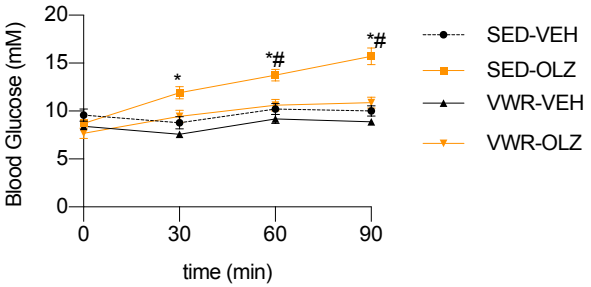
A. No wheel lock



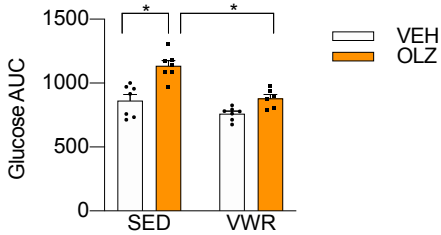
B. AUC no wheel lock



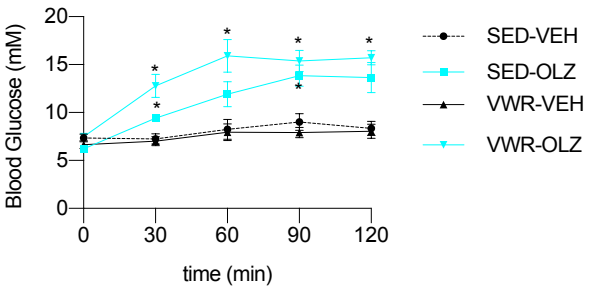
C. 7 hr wheel lock



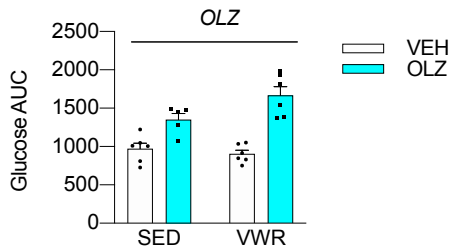
D. AUC- 7hr wheel lock

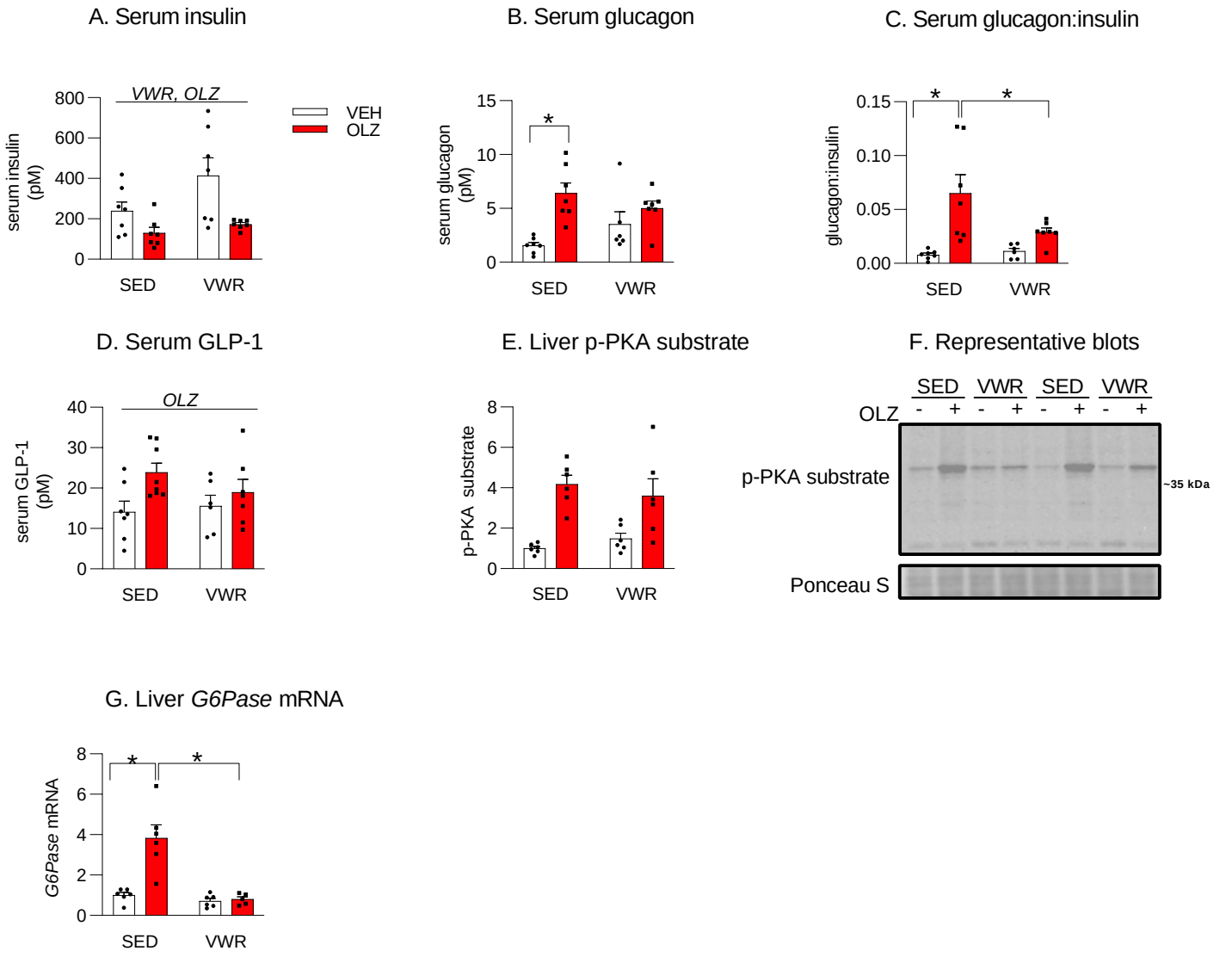


E. 24 hr wheel lock

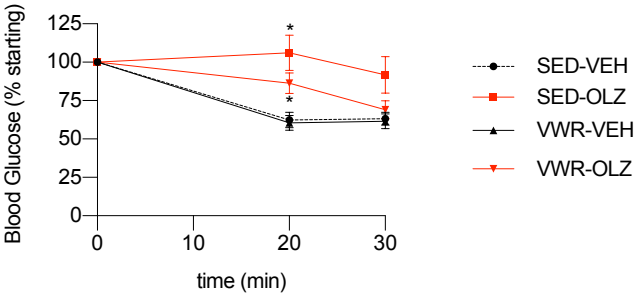


F. AUC- 24hr wheel lock

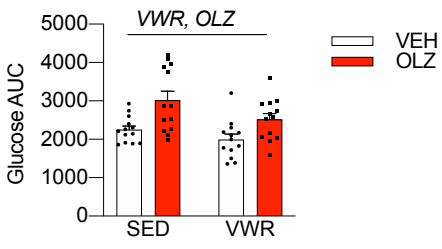



A. Serum insulin
VWR, OLZ
VEH
OLZ
serum insulin (pM)
800
600
400
200
0
SED
VWR
B. Serum glucagon
serum glucagon (pM)
15
10
5
0
*
SED
VWR
C. Serum glucagon:insulin
glucagon:insulin
0.15
0.10
0.05
0.00
*
*
SED
VWR
D. Serum GLP-1
OLZ
serum GLP-1 (pM)
40
30
20
10
0
SED
VWR
E. Liver p-PKA substrate
p-PKA substrate
8
6
4
2
0
SED
VWR
F. Representative blots
SED
VWR
SED
VWR
OLZ
- + - + - + - +
p-PKA substrate
~35 kDa
Ponceau S
G. Liver G6Pase mRNA
G6Pase mRNA
8
6
4
2
0
*
*
SED
VWR

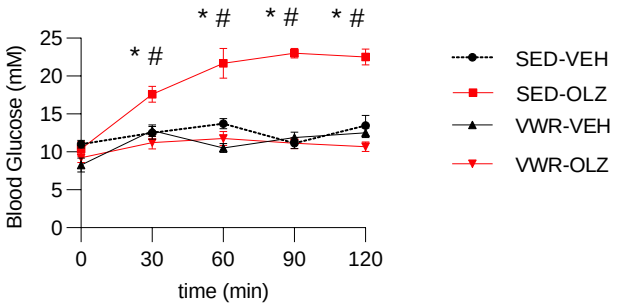
A. Insulin tolerance test



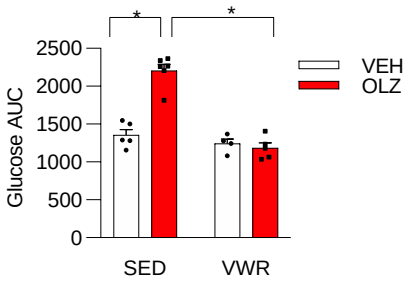
B. Insulin tolerance test AUC



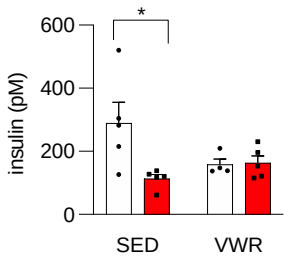
A. AMPK B1 KO



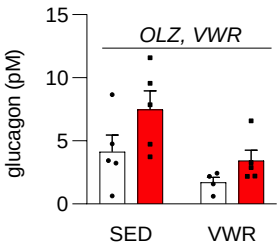
B. AUC AMPK B1 KO



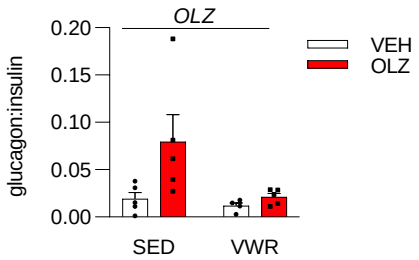
C. insulin AMPK B1 KO



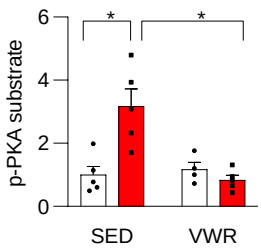
D. glucagon AMPK B1 KO



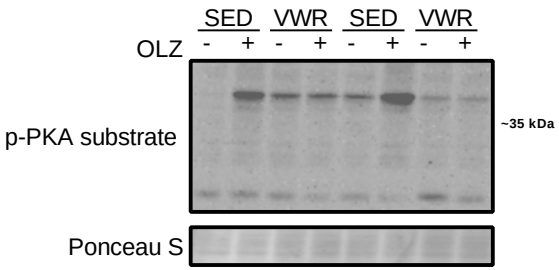
E. glucagon:insulin AMPK B1 KO



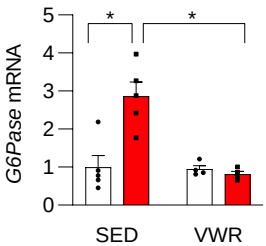
F. Liver p-PKA substrate AMPK B1 KO



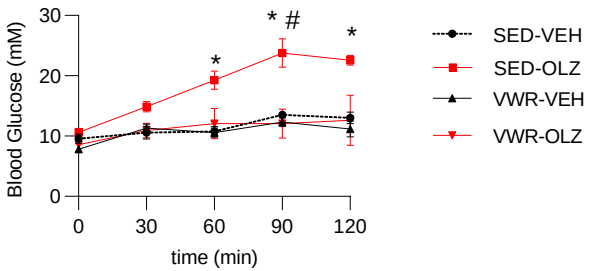
G. Representative blots



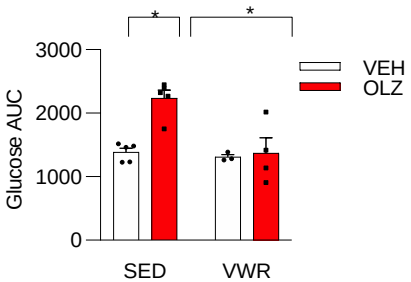
H. Liver *G6Pase* mRNA



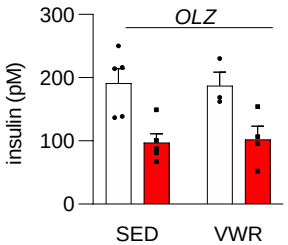
A. HFD



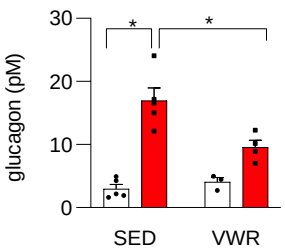
B. AUC HFD



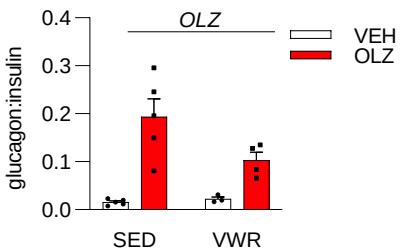
C. insulin HFD



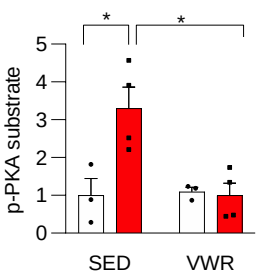
D. glucagon HFD



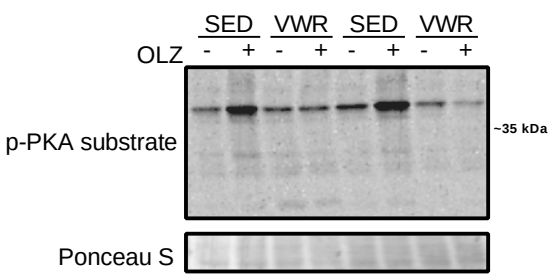
E. glucagon:insulin HFD



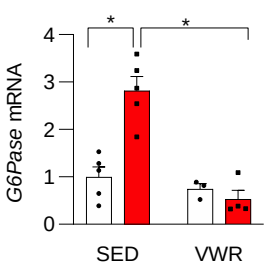
F. Liver p-PKA substrate HFD

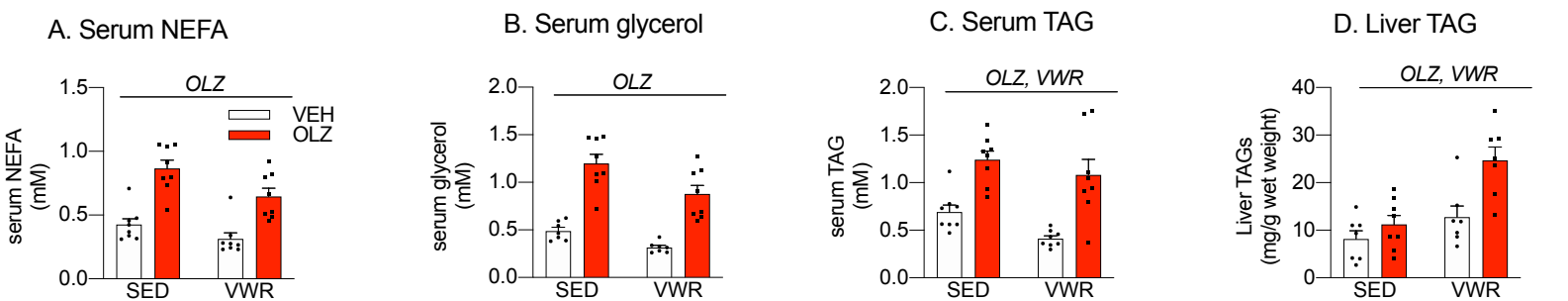


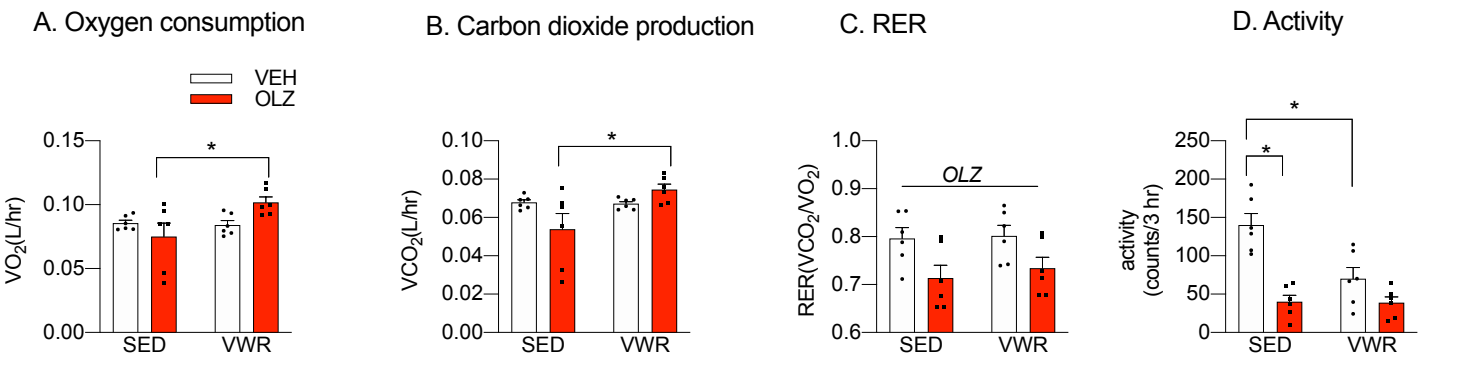
G. Representative blots



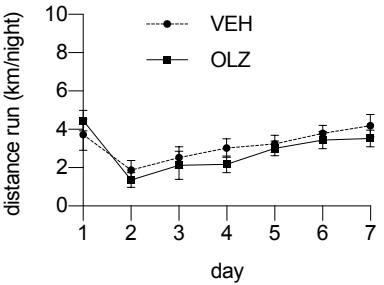
H. Liver *G6Pase* mRNA



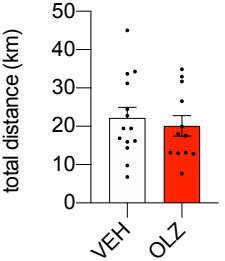

A. Serum NEFA
OLZ
VEH
OLZ
serum NEFA (mM)
1.5
1.0
0.5
0.0
SED
VWR
B. Serum glycerol
OLZ
serum glycerol (mM)
2.0
1.5
1.0
0.5
0.0
SED
VWR
C. Serum TAG
OLZ, VWR
serum TAG (mM)
2.0
1.5
1.0
0.5
0.0
SED
VWR
D. Liver TAG
OLZ, VWR
Liver TAGs (mg/g wet weight)
40
30
20
10
0
SED
VWR


A. Oxygen consumption
VEH
OLZ
0.15
0.10
0.05
0.00
VO2(L/hr)
*
SED
VWR
B. Carbon dioxide production
0.10
0.08
0.06
0.04
0.02
0.00
VCO2(L/hr)
*
SED
VWR
C. RER
1.0
0.9
0.8
0.7
0.6
RER(VCO2/VO2)
OLZ
SED
VWR
D. Activity
250
200
150
100
50
0
activity
(counts/3 hr)
*
*
SED
VWR

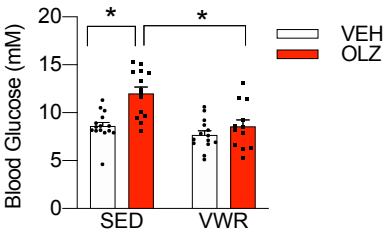
A. Daily distance run



B. Total distance run



C. Day 1



D. Day 7