

**Regulation of glucose kinetics during exercise by the glucagon-like peptide-1 receptor.**

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**Table of Contents Category:** Skeletal muscle and exercise

## KEY POINTS

- Glucagon-like peptide-1 (Glp1) regulates hepatic glucose production (HGP) and muscle glucose uptake (MGU) via its ability to stimulate insulin secretion.
- Using exercise as a means to stimulate glucose flux independently of insulin secretion, we observed that Glp1 receptor (Glp1r) knockout (Glp1r<sup>-/-</sup>) mice become hyperglycemic but display normal rates of MGU.
- Since euglycemia during exercise is typically maintained by matching rates of HGP to rates of MGU, we hypothesize that exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice is due to excessive HGP.
- Using innovative catheterization and isotope dilution techniques, we demonstrate that hyperglycemia in exercising Glp1r<sup>-/-</sup> mice is associated with excessive HGP and glucagon secretion.
- These results suggest an essential and novel role for basal Glp1r signaling in the suppression of alpha cell secretion during exercise.

(128 Words)

## **ABSTRACT**

In response to oral glucose, glucagon-like peptide-1 receptor (Glp1r) knockout (Glp1r<sup>-/-</sup>) mice become hyperglycemic due to impaired insulin secretion. Exercise also induces hyperglycemia in Glp1r<sup>-/-</sup> mice. In contrast to oral glucose, exercise decreases insulin secretion. This implies that exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice results from the loss of a non-insulinotropic effect mediated by the Glp1r. Muscle glucose uptake (MGU) is normal in exercising Glp1r<sup>-/-</sup> mice. Thus, we hypothesize that exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice is due to excessive hepatic glucose production (HGP). Wild-type (Glp1r<sup>+/+</sup>) and Glp1r<sup>-/-</sup> mice implanted with venous and arterial catheters underwent treadmill exercise or remained sedentary for 30 min. [3-<sup>3</sup>H]glucose was used to estimate rates of glucose appearance ( $R_a$ ), an index of HGP, and disappearance ( $R_d$ ). 2[<sup>14</sup>C]deoxyglucose was used to assess MGU. Glp1r<sup>-/-</sup> mice displayed exercise-induced hyperglycemia due to an excessive increase in  $R_a$  but normal  $R_d$  and MGU. Exercise-induced glucagon levels were ~2-fold higher in Glp1r<sup>-/-</sup> mice, resulting in a~2-fold higher glucagon:insulin ratio. Since inhibition of the central Glp1r stimulates HGP, we tested whether intracerebroventricular (ICV) infusion of the Glp1r antagonist exendin(9-39) (Ex9) in Glp1r<sup>+/+</sup> mice would result in exercise-induced hyperglycemia. ICV Ex9 did not enhance glucose levels or HGP during exercise, suggesting that glucoregulatory effects of Glp1 during exercise are mediated via the pancreatic Glp1r. In conclusion, functional disruption of the Glp1r results in exercise-induced hyperglycemia associated with an excessive increase in glucagon secretion and HGP. These results suggest an essential role for basal Glp1r signaling in the suppression of alpha cell secretion during exercise.

(250 words)

## **ABBREVIATIONS LIST**

Glp1, glucagon-like peptide-1; Glp1r, glucagon-like peptide-1 receptor; HGP, hepatic glucose production; MGU, muscle glucose uptake; ICV, intracerebroventricular; Ex9, exendin(9-39).

## INTRODUCTION

Glucagon-like peptide-1 (Glp1) is an incretin hormone that enhances the secretion of insulin in response to glucose intake. Following a meal, Glp1 is secreted from intestinal L cells and activates a Glp1 receptor (Glp1r) expressed in pancreatic beta cells (Kreymann et al. 1987; Scrocchi et al. 1996; Gromada et al. 1998). Glp1 also regulates glucose excursions via its ability to suppress glucagon secretion, delay gastric emptying and reduce food intake (Orskov et al. 1988; Tang-Christensen et al. 1996; Turton et al. 1996; Nauck et al. 1997).

The ability of Glp1 to regulate hepatic glucose production (HGP) and muscle glucose uptake (MGU) has been primarily attributed to its effects on pancreatic insulin secretion (Orskov et al. 1996; Toft-Nielson et al. 1996; Ryan et al. 1998; Ahren and Pacini 1999; Vella et al. 2002). However, it has been suggested that Glp1 can modulate HGP and/or MGU independently of its insulinotropic effect. Gutniak and colleagues (Gutniak et al. 1992) showed that the rate of glucose disappearance increases with Glp1 infusions under controlled insulinemic and glycemic clamp conditions in type 1 diabetic subjects. Other studies have subsequently demonstrated insulin secretion-independent regulation of HGP and/or MGU in response to Glp1r agonists (D'Alessio et al. 1994; D'Alessio et al. 1995; Shalev et al. 1998; Meneilly et al. 2001; Egan et al. 2002; Prigeon et al. 2003; Azuma et al. 2008). We have shown that mice with a functional disruption of the Glp1r (Glp1r<sup>-/-</sup> mice) display increased HGP during a hyperinsulinemic-euglycemic clamp, indicative of impaired hepatic insulin action (Ayala et al. 2009). Conversely, insulin-stimulated MGU is enhanced in Glp1r<sup>-/-</sup> mice (Knauf et al. 2005; Ayala et al. 2009). Since the Glp1r is not expressed in hepatocytes or in skeletal muscle, non-insulinotropic regulation of HGP and MGU under controlled hyperinsulinemic conditions is not mediated via direct activation of a hepatic or muscle Glp1r. Instead emerging evidence suggests a role for the central Glp1r in the regulation of HGP and MGU.

Intracerebroventricular (ICV), but not peripheral, infusions of the Glp1r antagonist exendin(9-39) (Ex9) impair hepatic glycogen storage but stimulate MGU during hyperinsulinemic-hyperglycemic clamps in mice (Knauf et al. 2005; Cabou et al. 2008; Knauf et al. 2008; Burmeister et al. 2012). Conversely activation of the central Glp1r enhances the suppression of HGP by insulin but attenuates glucose disappearance during hyperinsulinemic-euglycemic clamps in rodents (Sandoval et al. 2008; Burmeister et al. 2012). Taken together, these observations suggest that the Glp1r can regulate HGP and MGU independently of its ability to stimulate insulin secretion. Furthermore, such a role is mediated, at least in part, via the central Glp1r.

We have previously used exercise as a means to explore an insulin secretion-independent glucoregulatory role for the Glp1r (Ayala et al. 2009). Exercise stimulates glucose flux even as insulin levels decrease (Wasserman 1995). As such, this is a valuable experimental tool for the assessment of insulin-independent mechanisms that regulate glucose metabolism. During moderate intensity exercise, increased glucose utilization by the working muscle is matched by increased HGP, resulting in little change in arterial glucose levels (Wasserman 1995). While this euglycemia is observed in wild-type (Glp1r<sup>+/+</sup>) mice during exercise, Glp1r<sup>-/-</sup> mice display exercise-induced hyperglycemia (Ayala et al. 2009). This is not due to a defect in MGU since glucose uptake by hindlimb skeletal muscle is normal compared to Glp1r<sup>+/+</sup> mice (Ayala et al. 2009). This raises the possibility that loss of functional Glp1r expression results in excessive stimulation of HGP during exercise resulting in hyperglycemia.

In the present studies, an infusion of [3-<sup>3</sup>H]glucose was used to test the hypothesis that exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice is due to an excessive rate of glucose appearance. Since the Glp1r is not expressed in hepatocytes, we hypothesized that an excessive increase in glucose appearance is secondary to the loss of Glp1r action in a non-hepatic organ. Given the fact that Glp1r activation suppresses

glucagon secretion (Orskov et al. 1988) and glucagon is a key stimulus of HGP during exercise (Wasserman et al. 1989; Hirsch et al. 1991; Wasserman and Cherrington 1991; Wasserman 1995; Lavoie et al. 1997) we hypothesized that exercise stimulates an excessive increase in glucagon in *Glp1r*<sup>-/-</sup> mice. Alternatively, since the central *Glp1r* has been implicated in the regulation of peripheral glucose metabolism, we also tested the hypothesis that the exercise-induced hyperglycemia observed in *Glp1r*<sup>-/-</sup> mice would be recapitulated by antagonizing the central *Glp1r* in *Glp1r*<sup>+/+</sup> mice. Experiments were performed in chronically catheterized mice whereby catheters were implanted in the jugular vein and carotid artery for infusions and sampling, respectively. The carotid artery catheter allows for the acquisition of blood samples for the assessment of glucose kinetics during the exercise bout.

## METHODS

All procedures performed were approved by the Animal Care and Use Committees at Vanderbilt University and at the Sanford-Burnham Medical Research Institute at Lake Nona. Glp1r<sup>+/+</sup> and Glp1r<sup>-/-</sup> mice on a C57Bl/6 background were studied at 4-5 months of age. Mice were maintained on a standard light:dark cycle (0600-1800h light) and a rodent chow diet (Purina 5001).

**Surgical Procedures.** Catheters were implanted in the left common carotid artery and right jugular vein for sampling and infusions, respectively, as previously described (Ayala et al. 2006; Ayala et al. 2007; Ayala et al. 2008; Ayala et al. 2009; Ayala et al. 2010). In some Glp1r<sup>+/+</sup> mice, intracerebroventricular (ICV) cannulae were implanted using a stereotaxic apparatus (David Kopf Instruments) as previously described (Burmeister et al. 2012). Verification of cannula position in the lateral cerebroventricle was made by observing spontaneous flow of cerebrospinal fluid from the tip of the cannula after removal of the obturator. Animals were individually housed after surgery and allowed to recover for 5 days, during which time body weight was recorded daily. ICV cannula placement in the lateral cerebroventricle was confirmed by observing increased drinking behavior following administration of angiotensin-II (Ang-II) on day 3 of recovery (Roesch et al. 2001; Ahima et al. 2002; Unger et al. 2010). Mice whose weight did not return to within 10% of pre-surgery weight or that did not respond to Ang-II were excluded.

**Exercise experiments.** Following 5 days of recovery, mice were acclimated to treadmill running with a single 10-min exercise bout ( $15.5 \text{ m} \cdot \text{min}^{-1}$ , 0% grade). Exercise experiments were performed 2 days after this acclimation trial. Two hours prior to the exercise bout ( $t = -120 \text{ min}$ ), mice were placed in the treadmill for acclimation. At  $t = -90 \text{ min}$  a primed-continuous infusion (3  $\mu\text{Ci}$  prime,  $0.05 \mu\text{Ci} \cdot \text{min}^{-1}$  continuous) of HPLC-purified [ $3\text{-}^3\text{H}$ ]glucose (Perkin-Elmer) was begun and maintained throughout the experiment. In animals with ICV cannulae, a continuous infusion ( $0.1 \mu\text{g} \cdot \text{min}^{-1}$ , 0.25

$\mu\text{l}\cdot\text{hr}^{-1}$ ) of the Glp1r antagonist Ex9 (Tocris) was begun at  $t = -60$  min (Knauf et al. 2005; Cabou et al. 2008; Burmeister et al. 2012). Artificial cerebrospinal fluid (ACSF; Harvard Apparatus) was used as vehicle. At  $t = -15$  and  $-5$  min arterial samples were taken for the measurement of baseline blood glucose and plasma [ $3\text{-}^3\text{H}$ ]glucose. Additional blood was taken at  $t = -5$  min for the measurement of baseline insulin and glucagon. Beginning at  $t = 0$  min mice either remained sedentary or ran on the treadmill for 30 min at  $15.5 \text{ m}\cdot\text{min}^{-1}$ , 0% grade. This is the speed used in our previous study (Ayala et al. 2009) and reflects a work intensity that is ~75% of maximal oxygen consumption in Glp1r $+/+$  mice (Fernando et al. 1993; Ayala et al. 2009). However, based on our previous findings that Glp1r $-/-$  mice exhibit lower exercise capacity, for this absolute speed, Glp1r $-/-$  mice exercise at a higher relative work intensity (~88% of maximal oxygen consumption (Ayala et al. 2009)). Mice were encouraged to run with the use of an electric grid placed at the back end of the treadmill (1.5 mA, 200 ms pulses, 4 Hz). At  $t = 5$  min, a 12  $\mu\text{Ci}$  bolus of  $2[^{14}\text{C}]$ deoxyglucose ( $2[^{14}\text{C}]$ DG; Perkin Elmer) was administered via the jugular vein catheter. At  $t = 7, 10, 15$  and  $20$  min, arterial samples were taken to determine blood glucose and plasma [ $3\text{-}^3\text{H}$ ]glucose and  $2[^{14}\text{C}]$ DG. At  $t = 30$  min, an arterial sample was taken for the measurement of blood glucose and plasma insulin, glucagon, [ $3\text{-}^3\text{H}$ ]glucose and  $2[^{14}\text{C}]$ DG. Mice were then anesthetized with sodium pentobarbital. The soleus, gastrocnemius and superficial vastus lateralis (SVL) were excised, immediately frozen, and stored at  $-80^\circ\text{C}$  until analyzed.

**Processing of plasma and tissue samples.** Arterial plasma insulin was determined by ELISA (Millipore). Arterial plasma glucagon and corticosterone were determined by radioimmunoassay at the Vanderbilt Mouse Metabolic Phenotyping Center Hormone Assay and Analytical Resources Core. Plasma [ $3\text{-}^3\text{H}$ ]glucose and  $2[^{14}\text{C}]$ DG and tissue  $2[^{14}\text{C}]$ DG-6-phosphate ( $2[^{14}\text{C}]$ DGP) radioactivity were determined as previously

described (Ayala et al. 2007; Ayala et al. 2008; Ayala et al. 2009; Ayala et al. 2010; Burmeister et al. 2012). The accumulation of 2[<sup>14</sup>C]DGP was normalized to tissue weight.

**Calculations.** Whole-body glucose appearance ( $R_a$ ) and disappearance ( $R_d$ ), were determined using Steele nonsteady-state equations (Steele et al. 1956; Debodo et al. 1963). Glucose metabolic index ( $R_g$ ) was calculated as previously described (Kraegen et al. 1985; Ayala et al. 2007; Ayala et al. 2008; Ayala et al. 2009; Ayala et al. 2010; Burmeister et al. 2012).

**Statistical analysis.** Data are presented as means  $\pm$  SEM. Differences between groups were determined by repeated measures ANOVA followed by Tukey's post hoc tests or by two-tailed t test as appropriate. The significance level was  $P < 0.05$ .

## RESULTS

### Arterial glucose during exercise in Glp1r<sup>-/-</sup> mice

As shown in our previous studies (Ayala et al. 2009), Glp1r<sup>-/-</sup> mice display exercise-induced hyperglycemia (**Fig. 1**). Glucose levels were not different between genotypes in sedentary mice.

### MGU during exercise in Glp1r<sup>-/-</sup> mice

A bolus of 2[<sup>14</sup>C]deoxyglucose was administered to assess the tissue glucose metabolic index ( $R_g$ ), an index of tissue glucose uptake. There were no differences in hindlimb skeletal muscle  $R_g$  between genotypes in sedentary mice (**Figs. 2A-C**). Exercise stimulated hindlimb skeletal muscle  $R_g$  equally in both genotypes (**Figs. 2A-C**). These results also support previous findings (Ayala et al. 2009) and confirm that exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice is not due to impaired MGU.

### Glucose turnover during exercise in Glp1r<sup>-/-</sup> mice

We next explored the possibility that exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice is due to excessive HGP. A primed-continuous infusion of [3-<sup>3</sup>H]glucose was used to estimate whole body rates of glucose appearance ( $R_a$ ) and disappearance ( $R_d$ ).  $R_a$  and  $R_d$  values were not different in sedentary mice (**Figs. 2D and E**). As expected, exercise significantly stimulated  $R_a$  and  $R_d$  in both genotypes.  $R_a$  and  $R_d$  were equally increased in Glp1r<sup>+/+</sup> mice throughout the exercise bout (**Fig. 2D**). Contrasting this,  $R_a$  increased to a greater degree than  $R_d$  in Glp1r<sup>-/-</sup> mice during the first 15 min of exercise (**Fig. 2E**). This would be expected to produce the increase in arterial glucose levels observed in Glp1r<sup>-/-</sup> mice (**Fig. 1**). Taken together, these data demonstrate that exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice results from an excessive HGP response compared to the response in Glp1r<sup>+/+</sup> mice.

### **Arterial insulin, glucagon and corticosterone during exercise in Glp1r<sup>-/-</sup> mice**

Insulin levels decreased to an equivalent concentration in Glp1r<sup>-/-</sup> and Glp1r<sup>+/+</sup> mice in response to exercise (**Fig. 3A**). Since fasting insulin levels tended to be lower ( $P=0.11$ ) in Glp1r<sup>-/-</sup> mice (**Fig. 3A**), this resulted in a tendency for a smaller decrease in insulin levels during exercise in these mice ( $0.74\pm0.32$  and  $0.35\pm0.13$  ng·mL<sup>-1</sup> in Glp1r<sup>+/+</sup> and Glp1r<sup>-/-</sup>, respectively;  $P=0.13$ ). In contrast, the exercise-induced increase in arterial plasma glucagon levels was ~2-fold higher in Glp1r<sup>-/-</sup> mice compared to Glp1r<sup>+/+</sup> mice (**Fig. 3B**). This resulted in a ~2-fold higher glucagon:insulin ratio in Glp1r<sup>-/-</sup> mice ( $230.4\pm56.0$  and  $565.1\pm107.2$  pg·ng<sup>-1</sup> in Glp1r<sup>+/+</sup> and Glp1r<sup>-/-</sup>, respectively). There were no significant differences in fasting or exercise-induced corticosterone levels between genotypes (**Fig. 3C**). Interestingly, there was a tendency for the exercise-induced increase in corticosterone levels to be lower in Glp1r<sup>-/-</sup> mice ( $302.6\pm117.7$  and  $124.4\pm58.3$  ng·mL<sup>-1</sup> in Glp1r<sup>+/+</sup> and Glp1r<sup>-/-</sup>, respectively;  $P=0.09$ ). The combination of a smaller decrease in plasma insulin, an exaggerated glucagon response and the subsequently higher glucagon:insulin ratio provides a hormonal basis for the excessive increase in exercise-induced HGP in Glp1r<sup>-/-</sup> mice.

### **Arterial glucose levels during exercise in response to inhibition of the central Glp1r**

Previous studies have shown that inhibition of the central Glp1r results in increased HGP (Knauf et al. 2005; Burmeister et al. 2012). We, therefore, tested whether pharmacological inhibition of the central Glp1r in Glp1r<sup>+/+</sup> mice results in exercise-induced hyperglycemia as observed in whole-body Glp1r<sup>-/-</sup> mice. Glucose levels did not vary appreciably during exercise in mice receiving ICV infusions of ACSF, demonstrating that the ICV infusion itself does not cause a stress response or affect glucose kinetics

during exercise (**Fig. 4**). ICV infusion of the Glp1r antagonist Ex9 at a dose we have previously shown to stimulate HGP (Knauf et al. 2005; Burmeister et al. 2012) did not affect arterial glucose levels in either sedentary mice or mice undergoing treadmill exercise (**Fig. 4**). In both mice receiving ICV ACSF or Ex9, hindlimb muscle  $R_g$  and glucose  $R_a$  and  $R_d$  were equally stimulated in response to exercise (**Figs. 5A-E**). These results show that the loss of central Glp1r action does not recapitulate the exercise-induced hyperglycemia observed in whole-body Glp1r<sup>-/-</sup> mice.

## DISCUSSION

We have previously demonstrated a role for the Glp1r in the regulation of glucose homeostasis during exercise (Ayala et al. 2009). Mice lacking functional expression of the Glp1r display modest hyperglycemia during treadmill exercise. This is not due to a defect in skeletal muscle glucose uptake, a finding that was confirmed in the present studies. In our previous studies, we did not directly address whether exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice was due to an excessive increase in HGP. In the present studies, we employed [ $3\text{-}^3\text{H}$ ]glucose isotope dilution methods to determine the contribution of the rate of arterial glucose appearance to the exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice. Based on the important role of glucagon in stimulating HGP during exercise (Wasserman et al. 1989; Hirsch et al. 1991; Wasserman and Cherrington 1991; Wasserman 1995; Lavoie et al. 1997), we further hypothesized that elevated levels of glucagon contribute to the excessive increase in HGP observed in Glp1r<sup>-/-</sup> during exercise. Both the rate of glucose appearance and the glucagon response to exercise were elevated in Glp1r<sup>-/-</sup> compared to Glp1r<sup>+/+</sup> mice. The absolute decrease in insulin during exercise was also attenuated in Glp1r<sup>-/-</sup> mice, resulting in an exaggerated glucagon:insulin ratio. Taken together, these findings extend the essential islet actions of the Glp1r by demonstrating that the Glp1r regulates HGP independently of its ability to stimulate pancreatic insulin secretion.

The ability of nutrient intake to stimulate Glp1 secretion from intestinal L cells is well known (Drucker 2006). Glp1 levels have also been shown to increase in response to exercise (Adam and Westerterp-Plantenga 2004; Martins et al. 2007; Chanoine et al. 2008; Ueda et al. 2009; Ellingsgaard et al. 2011). Ellingsgaard and colleagues (Ellingsgaard et al. 2011) showed that increased Glp1 levels in response to exercise are dependent upon interleukin-6 (IL-6) production by the contracting skeletal muscle. IL-6 knockout mice and mice injected with antibodies specific to IL-6 did not exhibit an

increase in Glp1 levels following an exercise bout. Interestingly, stimulation of Glp1 secretion by IL-6 was observed not only from intestinal L cells but also from pancreatic alpha cells. This was associated with increased expression of proglucagon and prohormone convertase 1/3, the latter being the proteolytic enzyme that releases Glp1 from proglucagon (Ellingsgaard et al. 2011). This proposes a novel crosstalk mechanism between skeletal muscle, the gut and the pancreas in the regulation of glucose homeostasis during exercise. This also introduces a seemingly paradoxical situation in which Glp1, known for its insulinotropic effect, is secreted during exercise, a physiological condition characterized by decreased insulin secretion. A likely explanation for this finding is that the magnitude of the insulinotropic effect mediated by Glp1 is dependent on the prevailing circulating glucose concentration. Infusion of Glp1 in the presence of fasting glucose levels only modestly stimulates insulin secretion, but this insulinotropic effect is enhanced with increasing glucose concentrations (Vilsboll et al. 2003). This glucose-dependent feature of pancreatic Glp1 action is a safety mechanism that prevents insulin from being secreted during hypoglycemic conditions. Since glucose levels do not normally increase during moderate intensity exercise (Wasserman 1995), then an exercise-induced increase in Glp1 levels would not be expected to stimulate insulin secretion.

Besides its insulinotropic effect, Glp1 also inhibits glucagon secretion through direct action on glucagon-producing pancreatic alpha cells and via secretion of islet somatostatin (Holst 2007). Previous findings demonstrated that basal glucagon levels and glucose-induced suppression of glucagon levels are normal in Glp1r<sup>-/-</sup> mice (Scrocchi et al. 1998). Our current results using exercise unmask an essential role for Glp1r signaling in the suppression of glucagon secretion by demonstrating that the normal exercise-induced increase in glucagon is significantly enhanced in Glp1r<sup>-/-</sup> mice. The increased levels of glucagon likely drive the exaggerated increase in HGP during

exercise in Glp1r<sup>-/-</sup> mice (Wasserman et al. 1989; Hirsch et al. 1991; Wasserman and Cherrington 1991; Wasserman 1995; Lavoie et al. 1997). Although the precise role of glucagon as a target for Glp1 during exercise might conceivably be tested using glucagon receptor knockout (Gcgr<sup>-/-</sup>) and Gcgr<sup>-/-</sup>:Glp1r<sup>-/-</sup> mice, these knockout lines exhibit substantial upregulation of intestinal and pancreatic Glp1 secretion and multiple compensatory islet adaptations (Gelling et al. 2003; Ali et al. 2011). This would preclude their utility for elucidating the roles of Glp1 and glucagon during exercise. We must also consider the role of glucagon in the regulation of HGP during exercise in humans. Although glucagon infusions stimulate HGP during exercise in humans undergoing pancreatic clamps (Hirsch et al. 1991; Lavoie et al. 1997), under non-clamp exercise conditions the increase in glucagon levels lags behind the increase in HGP (Mendenhall et al. 1994). However, there may be small yet physiologically significant changes in portal glucagon levels during the early stages of exercise that may have been undetected in the latter study.

Although we focus on glucagon as a mediator of the excessive exercise-induced increase in HGP in Glp1r<sup>-/-</sup> mice, we cannot exclude a potential role for increased catecholamines, especially epinephrine, in mediating this effect (Marker et al. 1991; Wasserman 1995). Due to the requirement of a large blood sample for the measurement of plasma catecholamines (~100µl of blood (Ayala et al. 2006)) and the blood volume limitations of the mouse, we were unable to measure whether an equivalent excessive increase in catecholamine levels was also associated with exercise-induced hyperglycemia in Glp1r<sup>-/-</sup> mice. We did measure plasma corticosterone levels given the stimulatory effect of glucocorticoids on gluconeogenesis (Exton et al. 1972) and the stimulatory effect of Glp1 on the hypothalamic-pituitary-adrenal (HPA) axis (Rinaman 1999; Kinzig et al. 2003; Gil-Lozano et al. 2010). Intriguingly, administration of Glp1r agonists and disruption of Glp1r expression both

result in elevated corticosterone levels especially in response to stress (Rinaman 1999; MacLusky et al. 2000; Gil-Lozano et al. 2010). However, we observed no differences in plasma corticosterone levels between *Glp1r*<sup>-/-</sup> and *Glp1r*<sup>+/+</sup> mice in response to exercise.

Hyperglycemia itself can stimulate MGU during exercise (Zinker et al. 1993). Since *Glp1r*<sup>-/-</sup> mice display exercise-induced hyperglycemia, it is, therefore, surprising that MGU was not enhanced in these mice compared to *Glp1r*<sup>+/+</sup> mice. This could suggest that, for the relative hyperglycemia, MGU is actually impaired in *Glp1r*<sup>-/-</sup> mice. Alternatively, it is possible that under these exercise conditions MGU cannot be further stimulated even by hyperglycemia. Analysis of muscle glucose clearance ( $K_g$ ) in our previous study (Ayala et al. 2009) demonstrated that glucose concentration-independent MGU is not impaired in *Glp1r*<sup>-/-</sup> mice.

The central *Glp1r* has emerged as a regulator of peripheral glucose metabolism. Activation of the *Glp1r* in the arcuate nucleus of the hypothalamus enhances the ability of insulin to suppress HGP (Sandoval et al. 2008). Conversely, antagonism of the central *Glp1r* impairs the effects of insulin on hepatic glucose metabolism (Knauf et al. 2005; Burmeister et al. 2012). Thus, increased HGP during hyperinsulinemic-euglycemic clamps in *Glp1r*<sup>-/-</sup> mice is due, at least in part, to the loss of central *Glp1r* action. We explored whether disruption of central *Glp1r* action could also explain increased HGP in *Glp1r*<sup>-/-</sup> mice during exercise. ICV infusion of the *Glp1r* antagonist Ex9 had no effect on glucose levels or rates of glucose appearance and disappearance during exercise. This demonstrates that the central *Glp1r* does not appear to play a key role in the regulation of hepatic glucose metabolism during exercise. Instead, exercise-induced hyperglycemia in *Glp1r*<sup>-/-</sup> is likely due to the loss of islet *Glp1r* signaling that serves to inhibit alpha cell secretion.

It is important to note that based on our previous findings, Glp1r<sup>-/-</sup> mice have lower exercise capacity (Ayala et al. 2009). Because of this, for the absolute speed used in these studies, Glp1r<sup>-/-</sup> mice exercise at a higher relative intensity compared to Glp1r<sup>+/+</sup> mice (~88% versus ~78% maximal oxygen consumption, respectively (Ayala et al. 2009)). Increasing relative exercise intensity is associated with hyperglycemia due to HGP that outpaces glucose utilization by the working muscle (Wasserman 1995). Thus, in the context of exercise, functional disruption of the Glp1r results in a phenotype associated with increased relative exercise intensity. Glp1r<sup>+/+</sup> and Glp1r<sup>-/-</sup> mice were exercised at the same absolute speed in the present studies in order to retain the same experimental conditions as in our previous study. Testing whether increased glucagon, HGP and glucose levels in Glp1r<sup>-/-</sup> mice is the functional consequence of increased relative exercise intensity will require exercising Glp1r<sup>+/+</sup> and Glp1r<sup>-/-</sup> mice at the same relative rather than absolute intensity.

Our results suggest that Glp1 secretion during exercise plays a key role in the regulation of HGP at least in part via control of glucagon secretion. Since increased glucagon and HGP are necessary to prevent hypoglycemia during exercise, Glp1 may be acting to prevent excessive secretion of glucagon and, hence, excessive HGP. We can also not rule out the possibility that Glp1 secretion during exercise regulates processes occurring during the post-exercise recovery period. Nevertheless, the present studies reveal a novel interaction between Glp1 action and glucose metabolism during exercise and indicate a key role for the Glp1r in the maintenance of glucose homeostasis in the absence of increased insulin secretion. The importance of these findings is underscored by the fact that exercise and Glp1-based drugs are both therapeutic interventions for the treatment of type 2 diabetes. Understanding the mechanisms that underlie the beneficial effects of these approaches will ultimately enhance their therapeutic utility.

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## **AUTHOR CONTRIBUTIONS**

Conception and design of the experiments (Burmeister, MA and Ayala, JE); collection, analysis and interpretation of data (Burmeister MA, Bracy DP, James FD, Holt RM, Ayala J, King EM and Ayala JE); drafting/revising the article (Burmeister MA, Ayala J, Wasserman DH, Drucker DJ and Ayala JE).

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**Figure 1. Glp1r-/ mice exhibit exercise-induced hyperglycemia.** Glp1r+/+ (black symbols) and Glp1r-/ (white symbols) mice remained sedentary (diamonds) or underwent treadmill exercise (squares) for 30 min at 15.5 m·min<sup>-1</sup>. Blood glucose measurements were made from samples obtained prior to and during the exercise bout via a surgically implanted arterial catheter. Equivalent blood samples were obtained for mice that remained sedentary. Data are presented as mean ± SEM for n=7-8 mice/genotype for both sedentary and exercise groups. \*P<0.05 vs. Glp1r+/+ Exercise; †P<0.05 vs. Glp1r-/ Sedentary.

**Figure 2. Glp1r-/ mice display normal MGU but excessive HGP during exercise.** (A-C) Tissue-specific glucose uptake ( $R_g$ ) in three skeletal muscles from Glp1r+/+ and Glp1r-/ mice that remained sedentary (white bars) or underwent treadmill exercise (black bars). (D, E) Glucose rates of appearance ( $R_a$ ) and disappearance ( $R_d$ ), measured as indexes of HGP and glucose utilization, respectively, in Glp1r+/+ (D) and Glp1r-/ (E) mice.  $R_a$  (black symbols) and  $R_d$  (white symbols) are shown for exercised (Glp1r+/+, squares; Glp1r-/, diamonds) or sedentary (Glp1r+/+, triangles; Glp1r-/, circles) mice. Data are presented as mean ± SEM for n=7-8 mice/genotype for both sedentary and exercise groups. \*P<0.05 vs.  $R_d$  within genotype and experimental intervention (sedentary or exercise); †P<0.05 vs. Sedentary, same genotype.

**Figure 3. Glp1r-/ mice exhibit increased exercise-induced glucagon levels.** (A) Arterial insulin, (B) glucagon and (C) corticosterone from Glp1r+/+ (white bars) and Glp1r-/ (black bars) mice prior to (Basal) and at the end of treadmill exercise (Exercise). Basal values are from samples obtained at t = -15 min before the onset of the exercise bout. Exercise values are from measurements from samples obtained at t = 30 min after

the onset of the exercise bout. Data are presented as mean  $\pm$  SEM for n=7-8 mice/genotype. \*P<0.05 vs. Glp1r+/+; †P<0.05 vs. Basal, same genotype.

**Figure 4. Inhibition of the central Glp1r does not result in exercise-induced hyperglycemia.** Glp1r+/+ remained sedentary (diamonds) or underwent treadmill exercise (squares) for 30 min at  $15.5 \text{ m} \cdot \text{min}^{-1}$ . Beginning at t = 60 min prior to the onset of exercise, an ICV infusion of ACSF or Ex9 was begun and continued until the end of the experiment. Blood glucose measurements were made from samples obtained prior to and during the exercise bout via a surgically implanted arterial catheter. Equivalent blood samples were obtained for mice that remained sedentary. Data are presented as mean  $\pm$  SEM for n=4 mice/genotype for both sedentary and exercise groups.

**Figure 5. Inhibition of the central Glp1r does not affect MGU or HGP during exercise.** (A-C) Tissue-specific glucose uptake ( $R_g$ ) in three skeletal muscles from Glp1r+/+ mice that remained sedentary (white bars) or underwent treadmill exercise (black bars) and received ICV infusions of ACSF or Ex9. (D, E) Glucose rates of appearance ( $R_a$ ) and disappearance ( $R_d$ ), measured as indexes of HGP and glucose utilization, respectively, in Glp1r+/+ mice receiving ICV ACSF (D) or Ex9 (E).  $R_a$  (black symbols) and  $R_d$  (white symbols) are shown for exercised (ICV ACSF, squares; ICV Ex9, diamonds) or sedentary (ICV ACSF, triangles; ICV Ex9, circles) mice. Data are presented as mean  $\pm$  SEM for n=4 mice/genotype for both sedentary and exercise groups. †P<0.05 vs. Sedentary, same ICV infusion.

Figure 1.

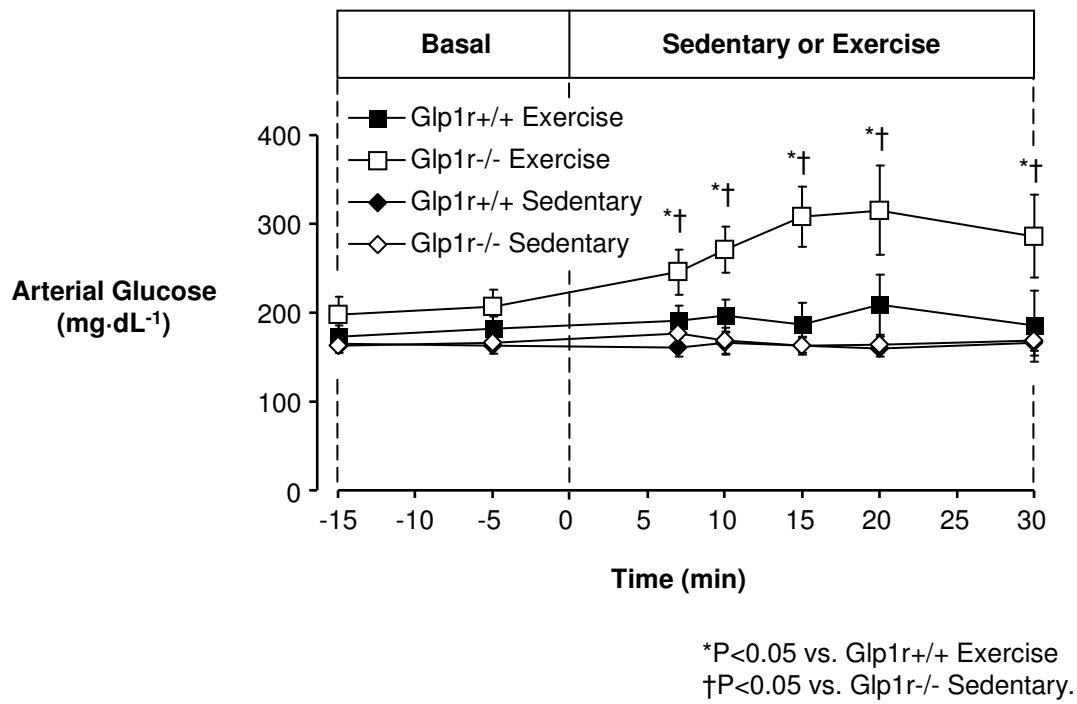
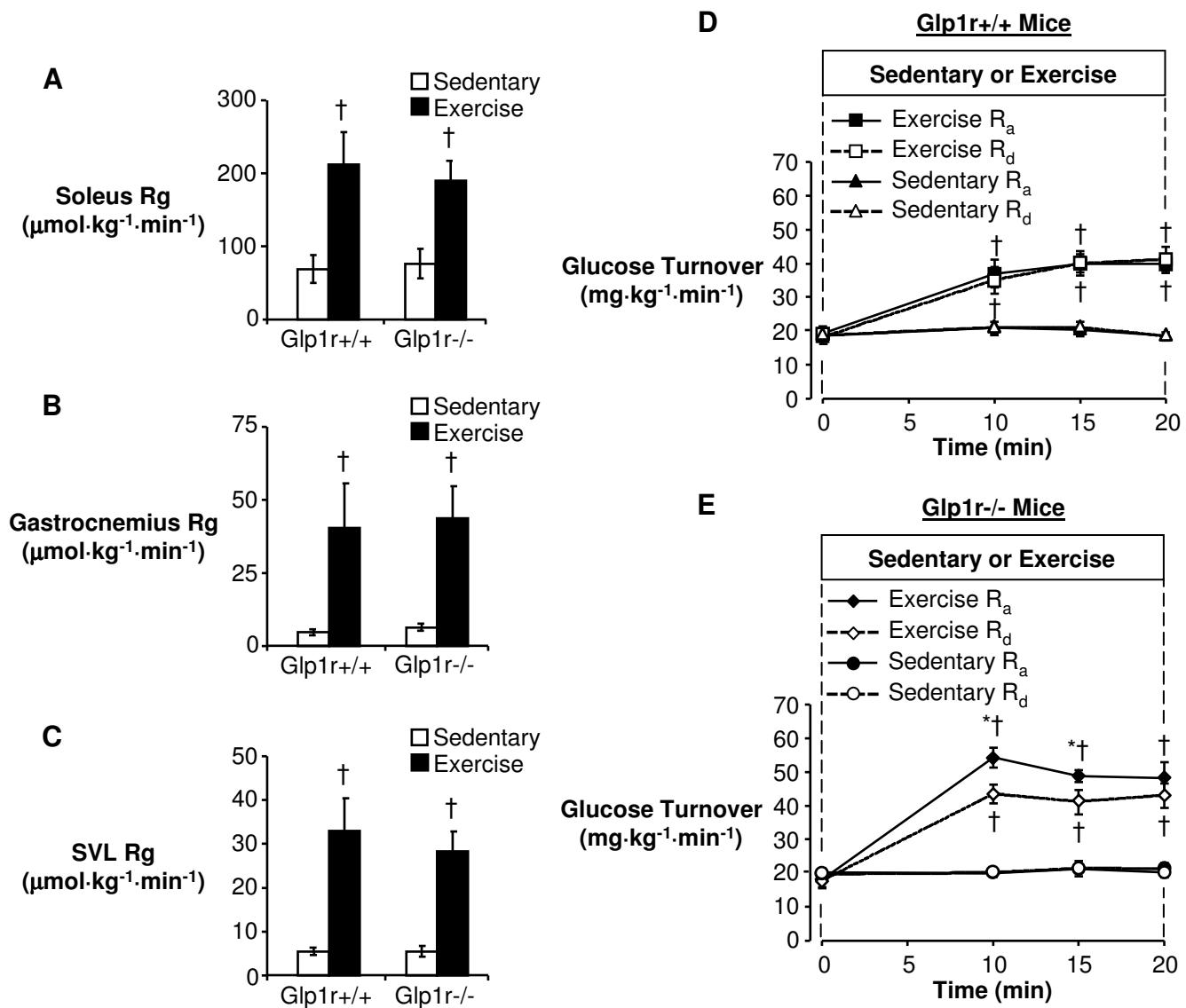


Figure 2.



\*P<0.05 vs. Rd within genotype and experimental intervention (sedentary or exercise)

†P<0.05 vs. Sedentary, same genotype.

Figure 3.

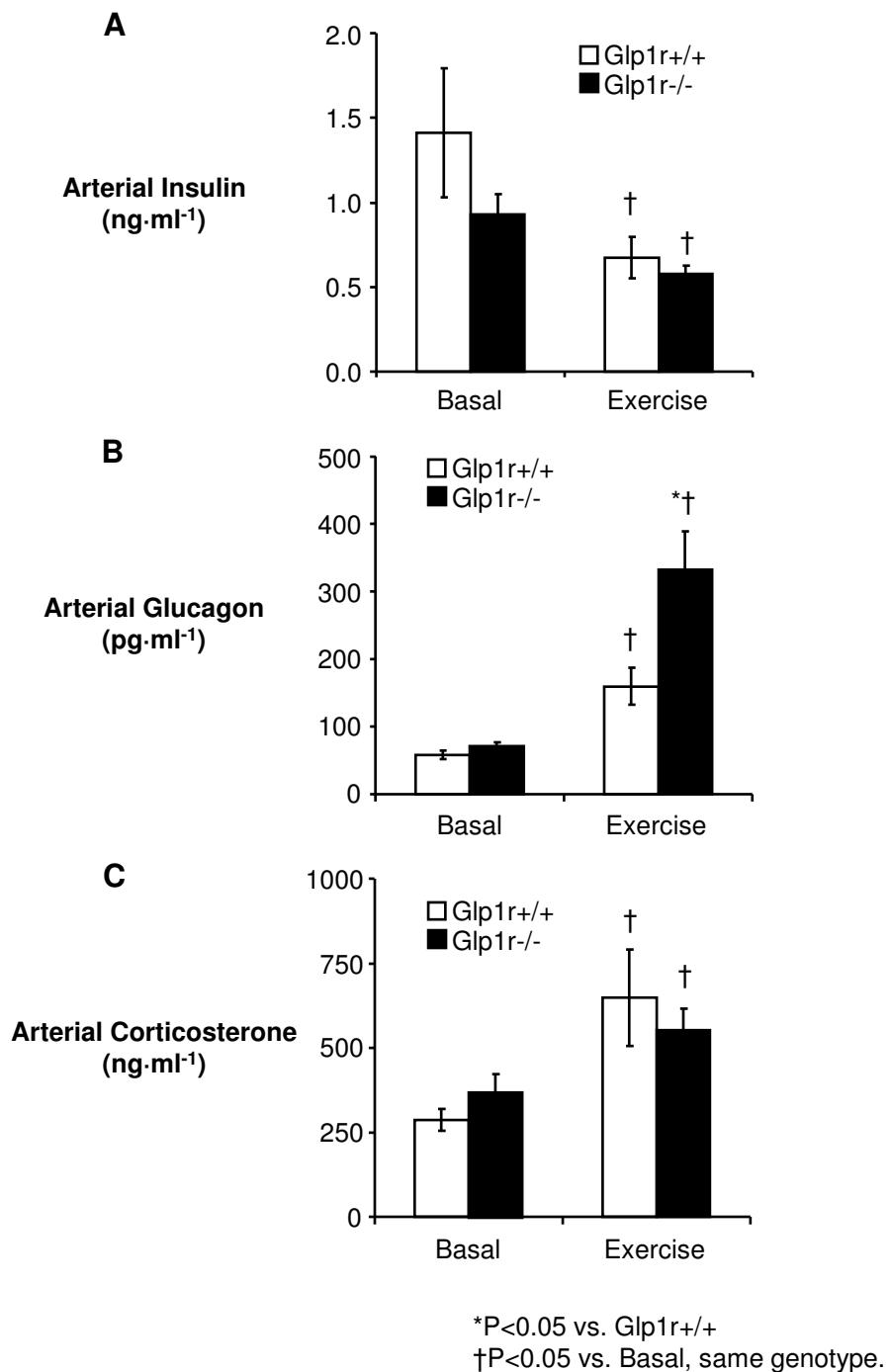


Figure 4.

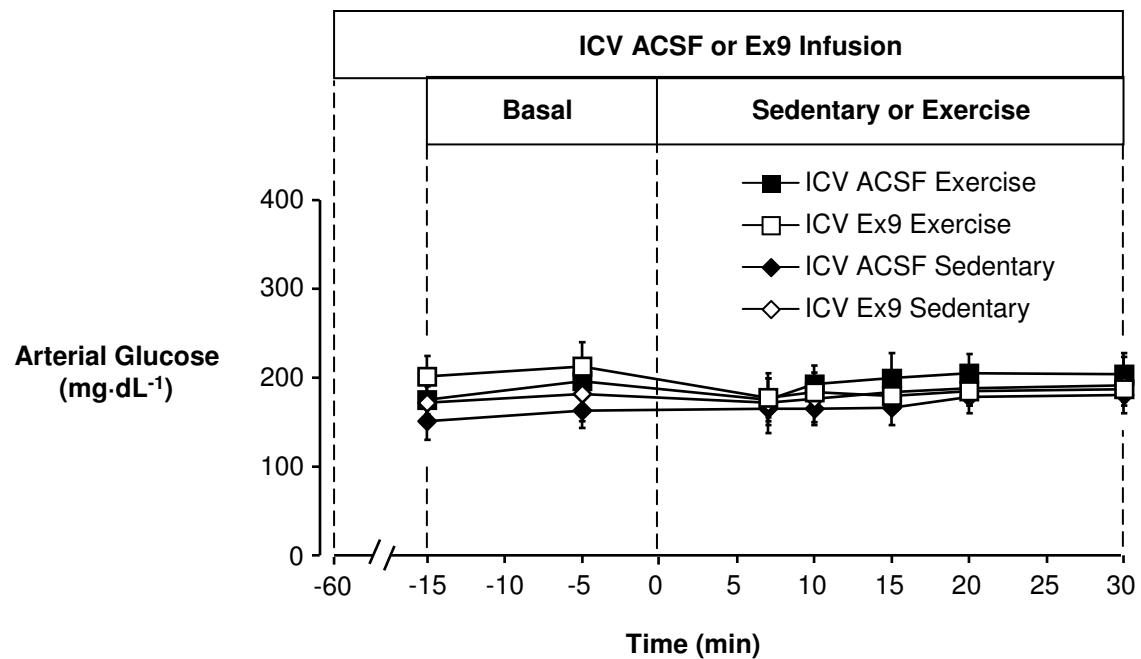


Figure 5.

