GIPR Agonism Enhances TZD-Induced Insulin Sensitivity in Obese IR Mice

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Recent studies have found that glucose-dependent insulinotropic polypeptide receptor (GIPR) agonism can enhance the metabolic efficacy of glucagon-like peptide–1 receptor agonist treatment by promoting both weight-dependent and -independent improvements on systemic insulin sensitivity. These findings have prompted new investigations aimed at better understanding the broad metabolic benefit of GIPR activation. Herein, we determined whether GIPR agonism favorably influenced the pharmacologic efficacy of the insulin-sensitizing thiazolidinedione (TZD) rosiglitazone in obese insulin-resistant (IR) mice. Genetic and pharmacological approaches were used to examine the role of GIPR signaling on rosiglitazone-induced weight gain, hyperphagia, and glycemic control. RNA sequencing was conducted to uncover potential mechanisms by which GIPR activation influences energy balance and insulin sensitivity. In line with previous findings, treatment with rosiglitazone induced the mRNA expression of the GIPR in white and brown fat. However, obese GIPR-null mice dosed with rosiglitazone had equivalent weight gain to that of wild-type (WT) animals. Strikingly, chronic treatment of obese IR WT animals with a long-acting GIPR agonist prevented rosiglitazone-induced weight-gain and hyperphagia, and it enhanced the insulin-sensitivity effect of this TZD. The systemic insulin sensitization was accompanied by increased glucose disposal in brown adipose tissue, which was underlined by the recruitment of metabolic and thermogenic genes. These findings suggest that GIPR agonism can counter the negative consequences of rosiglitazone treatment on body weight and adiposity, while improving its insulin-sensitizing efficacy at the same time.

ARTICLE HIGHLIGHTS

- Body weight gain resulting from treatment with the insulin-sensitizing thiazolidinedione rosiglitazone does not require the glucose-dependent insulinotropic polypeptide receptor (GIPR).
- Treatment with a GIPR agonist prevents the hyperphagic and obesogenic activity of rosiglitazone.
- Activation of the GIPR improves the insulin-sensitizing actions of rosiglitazone.

Type 2 diabetes (T2D), is a chronic, heterogenous disease, characterized by hyperglycemia, insulin resistance (IR), and impaired pancreatic β-cell function, that affects millions of people worldwide (1). Common approaches used to combat T2D include lifestyle modification (diet and exercise), pharmacotherapy, and a combination thereof (2). However, these strategies often fail owing to poor compliance, suboptimal efficacy, tolerability, and safety issues (3,4). Hence, there continues to be a need to develop medicines that deliver safe, effective, and durable efficacy for the management of T2D and its associated comorbidities.

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Incretin-based therapeutics offer safe and effective treatment options for people with obesity and T2D (5). Indeed, the incretin mimetic tirzepatide targets the glucose-dependent insulinotropic polypeptide receptor (GIPR) and glucagon-like peptide-1 receptor (GLP-1R) that provide superior glycemic control and weight loss when compared with standard of care in patients with T2D and obesity (6–8). These clinical findings have led to major interest in better understanding of how the GIPR axis contributes to the superior therapeutic profile of multireceptor agonism (9–11). Beyond stimulating glucose-dependent insulin secretion (12,13), GIPR agonism has been shown to result in reduced food intake and body weight (14,15), to alleviate nausea and emesis (16,17), and to enhance systemic and adipose insulin action in preclinical models of obesity-induced IR (18). In particular, the consequences of activating the GIPR in adipose tissue is of interest because of the known contribution of adipocyte dysfunction to the pathophysiology of obesity and its associated comorbidities (19,20). From a therapeutic standpoint, it is well established that the adipocyte is the primary target of the insulin-sensitizing thiazolidinedione (TZD) class of medicines, which includes pioglitazone and rosiglitazone (21,22). The TZDs are ligands for the master regulator of adipogenesis, the peroxisome proliferator-activated receptor-γ (PPARγ), and although treatment with these compounds causes weight gain and an array of additional safety issues (e.g., fluid retention and cardiovascular risk), TZDs were once widely used to treat T2D (22). Interestingly, the promoter region of the GIPR gene contains functional PPARγ response elements (23,24), and activation of PPARγ with TZDs increases the mRNA expression of the GIPR in mouse and human adipocytes (23,24).

Therefore, considering the apparently similar insulin-sensitizing effects of GIPR and PPARγ activation (18,22, 25), while noting the contrasting difference in energy balance (10,26–28), we conducted a series of studies investigating whether activation and/or inhibition of GIPR signaling would influence the pharmacological profile of rosiglitazone on body weight and glycemic control in obese IR mice. Taking this approach allowed us to explore further the potential mechanism(s) by which GIPR activation improves insulin sensitivity. The key findings of this study are that treatment of high-fat-fed obese IR mice with a long-acting GIPR agonist (LAGIPRA) blocked rosiglitazone-induced weight gain and improved the insulin-sensitizing efficacy. These results may offer a new approach for improving the therapeutic index of the TZD class of medicines.

RESEARCH DESIGN AND METHODS

Peptide Synthesis
The LAGIPRA (17) and mouse selective GIPR antagonist antibody (29) were synthesized by Eli Lilly and Company.

Animals
All experiments were performed in accordance with protocols approved by the Eli Lilly and Company Institutional Animal Care and Use Committee. All animals were individually housed in a temperature-controlled (23°C–28°C) facility with a 12-h light/dark cycle. Wild-type (WT) and germline Gipr-null mice ($Gipr^{-/-}$) (30) on a C57BL/6 background were from Taconic Contract Breeding. Male WT and $Gipr^{-/-}$ mice were given ad libitum access to food (a high-fat diet [HFD]: 60% fat, 20% carbohydrate, and 20% protein; D12492, Research Diets) and water.

Body Composition
Body composition was assessed using quantitative nuclear magnetic resonance (EchoMRI, Echo Medical Systems).

Diet-Induced Obesity
WT and $Gipr^{-/-}$ mice were housed in a thermal neutral environment (28°C) (31) and fed the 60% HFD for a minimum of 12 weeks. Body weight and food intake were recorded weekly. Body composition was assessed before and after HFD feeding, via quantitative nuclear magnetic resonance.

In Vivo Metabolic Analysis
Metabolic rate and substrate utilization rates were determined via indirect calorimetry using an open respirometer system (PhenoMaster/LabMaster System, TSE Systems). Animals were placed in the calorimeter for 5 days of acclimation at 28°C and had ad libitum access to food and water. Treatments were administered to ad libitum–fed obese mice 30 to 90 min before the onset of dark cycle daily for 14 days. Daily body weight and food intake were measured throughout the study. VO_2 and VCO_2 (both measured in mL/kg/h) were measured throughout the 14-day dosing period. VO_2 and VCO_2 were used to calculate energy expenditure and the respiratory exchange ratio (VCO_2/VO_2).

In Vivo Pharmacology Studies in WT and $Gipr^{-/-}$ Mice
Male mice were maintained on an HFD and had free access to food and water before randomization by weight. Animals were dosed with either vehicle (saline); LAGIPRA 10 nmol/kg subcutaneously (s.c.) (17); rosiglitazone (3, 30, or 100 mg/kg, via oral gavage [per os (p.o.)]); a combination of LAGIPRA (10 nmol/kg) and rosiglitazone (3, 30, or 100 mg/kg); or a GIPR antagonist antibody (30 mg/kg s.c.) (29) for 14 days. Body weight and food intake were recorded daily. Mice were sacrificed, plasma collected in EDTA-coated tubes, and tissues collected and snap frozen in liquid nitrogen.

Glucose Tolerance Tests
Mice were fasted overnight (16 h), after which they received 3 g/kg 50% dextrose p.o. or an intraperitoneal injection of glucose (2 g/kg 50% dextrose). Blood glucose concentration was determined by tail clip at 0, 15, 30, 60, and 120 min after injection.

Insulin Tolerance Test

Animals were fasted for 4 h and then received an intraperitoneal injection of insulin (0.5 units/kg; Humilin R, Eli Lilly and Company, Indianapolis, IN). Blood glucose concentration was determined via glucometer at 0, 15, 30, 60, and 120 min after the injection of insulin.

Insulin Euglycemic Clamp

Catheters were surgically placed in the left carotid artery and the right jugular vein 7–8 days before performing the assay. Animals were fasted overnight and, after 2 h of study-box acclimation, a continuous intravenous (i.v.) infusion of 3-^{3}H-glucose (PerkinElmer; 6 μCi bolus; basal period, 0.05 μCi/min; clamp period, 0.125 μCi/min) was maintained throughout the experiment. Glucose was measured from arterial blood via a glucometer (Accu-Chek, Roche) every 5 min during the experiment. An i.v. infusion of 3 mU/kg/min Humulin R and 22.5% glucose was started and periodically adjusted to maintain blood glucose concentration at 115–125 mg/dL. Somatostatin (5 μg/kg/min; Bachem) was administered i.v. to inhibit endogenous insulin secretion, and washed erythrocytes were infused to maintain blood volume. Three blood samples were collected at the end of the basal and clamp periods to determine endogenous glucose production (EGP). A bolus dose of 2-[1-^{14}C] deoxy-D-glucose (PerkinElmer; 5 μCi) was administered i.v. to measure tissue glucose uptake under steady-state glucose concentrations. Mice were euthanized, plasma was collected in EDTA-coated tubes, and tissues were collected and snap-frozen in liquid nitrogen. The glucose infusion rate (GIR), EGP, rate of disappearance, and tissue glucose uptake values were calculated as previously described (18,32,33).

Plasma Metabolites and Circulating Factors

Blood samples were collected on ice before storage of plasma at −80°C. Plasma triglyceride and free fatty acid levels were measured using a Hitachi 912 Clinical Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Insulin (Crystal Chem Inc., Downers Grove, IL; or the ALPCO mouse ultrasensitive insulin assay that measures both mouse and human insulin, with respective mouse and human standard curves), Adiponectin (BioVendor Inc., Asheville, NC), insulin-like growth factor binding protein 2 (R&D Systems, Minneapolis, MN), and leptin (Crystal Chem Inc.) were measured by ELISA.

RNA Isolation, RT-PCR, and Real-Time qPCR

Total RNA was isolated using TRIzol reagent (Thermo Fisher Scientific), and total RNA purification was performed using a RNeasy mini kit (Qiagen). Total RNA was used for reversed transcription using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific), and cDNA levels were measured by qPCR or by qPCR-based TaqMan open array (ThermoFisher). Data were normalized to the housekeeping gene β-actin, and fold changes were calculated using the comparative cycle threshold method relative to vehicle.

RNA Sequencing and Gene Set Enrichment Analysis

Raw FastQ files were quality trimmed using cutadapt (cutadapt1.9.1) and aligned using GSNAP (1) (v2013–11–27; command-line parameters: -B 5 -A sam -N 1 -t 8 -s splice-sites –quality-protocol = sanger –gunzip –sam-multiple-primaries –maxsearch = 1000 – npaths = 100) to build 38.p3 of the mouse genome. Read counts were quantified using a custom Perl script and summarized at the gene level (National Center for Biotechnology Information m38o annotation). Normalization and differential expression were done using the limma/voom R package (version 3.46.0) (34). Genes are noted as differentially expressed if the adjusted *P* value (default adjustment method = "BH") from limma/voom was less than 0.05. Pathway analyses were done using rank-based FGSEA (version 1.16.0) (35) with MSigDB (version c2.cp.v7.2).

Statistical Analyses

Data are presented as mean ± SEM. Statistical analyses performed included Student unpaired *t* test, one-way ANOVA, two-way ANOVA, or three-way ANOVA with Tukey–Kramer or Dunnett's multiple-comparison tests as appropriate. Differences were considered significant when $P < 0.05$.

Data and Resource Availability

The data sets generated during or analyzed during this study are available from the corresponding author on reasonable request.

RESULTS

GIPR Agonism Is Not Required for Rosiglitazone-Induced Weight Gain in Mice

Compounds of the TZD antidiabetic drug class increase the mRNA expression of the GIPR in murine and human adipocytes (36). This has led to the hypothesis that activation of the GIPR may account for the accretion of body fat that is typical of TZD-mediated activation of the key adipogenic factor PPARγ (24). Therefore, to determine whether GIPR agonism is required for rosiglitazone to drive excess adiposity, we implemented a loss-of-function approach using both mouse genetics and in vivo pharmacology. First, we examined whether administration of rosiglitazone promotes the mRNA expression of the *Gipr* in adipose tissue collected from obese IR mice (Fig. 1). In agreement with previous findings in isolated white adipocytes (23,24), rosiglitazone treatment increased transcript levels of the *Gipr* after 72-h of treatment in white and brown fat (Fig. 1*A–C*).

To establish whether the GIPR is necessary for TZDs to increase body weight, we compared the propensity of rosiglitazone to promote weight gain in obese WT and *Gipr*$^{−/−}$ mice. Germline *Gipr*$^{−/−}$ mice are protected from HFD-induced obesity (DIO) when housed at room temperature (37). Thus, to circumvent this issue, we exposed *Gipr*$^{−/−}$ animals to an HFD (60% of calories from fat) for 12 weeks while they were housed in a thermal neutral
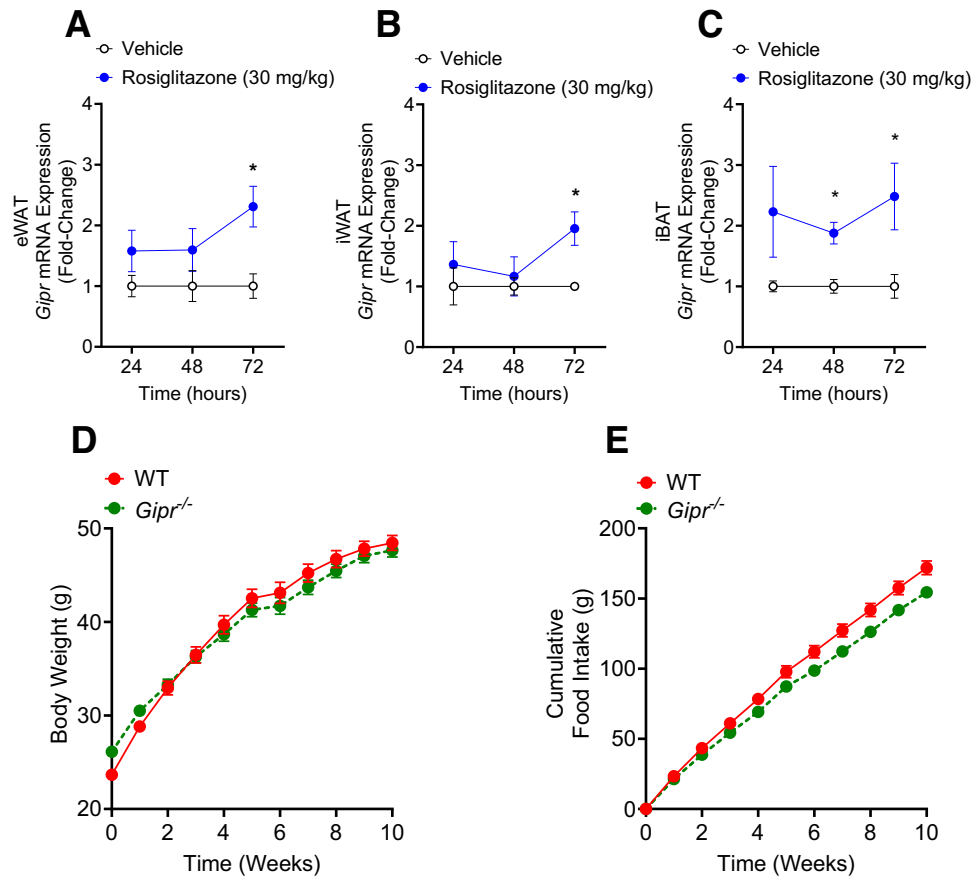
Figure 1 — Rosiglitazone increases *Gipr* mRNA expression in adipose tissue. *Gipr* mRNA expression in epididymal WAT (eWAT; *A*) and inguinal WAT (iWAT; *B*), and interscapular BAT (iBAT; *C*) collected from obese mice treated with rosiglitazone. Body weight (*D*) and food intake (*E*) for WT (*n* = 12) and germline whole-body *Gipr*$^{-/-}$ mice (*n* = 12) fed an HFD (60% of calories from fat) for 10 weeks housed at thermoneutrality (28°C). Data are presented as mean ± SEM. *$P < 0.05$ compared with vehicle. Statistical analyses included the Student unpaired *t* test or a two-way ANOVA, followed by Dunnett's multiple comparisons test where appropriate.

(28°C) environment, with the aim of minimizing thermal stress for maximizing weight gain (31) Fig. 1). Taking this approach, we found that *Gipr*$^{-/-}$ mice became obese and had a similar body weight to WT animals (Fig. 1*D* and *E*). Importantly, chronic (14-day) administration of rosiglitazone (30 mg/kg) resulted in increased body weight and fat mass to the same extent in obese WT and *Gipr*$^{-/-}$ mice housed at thermoneutrality (effect of treatment: *P* < 0.05; Fig. 2*A–F*). However, although rosiglitazone numerically increased food intake in both WT and *Gipr*$^{-/-}$ mice, the orexigenic activity of rosiglitazone did not reach significance in *Gipr*$^{-/-}$ mice (Fig. 2*F*).

Next, to complement studies conducted in the germline *Gipr*$^{-/-}$ mice, we used a mouse-selective GIPR antagonist antibody that effectively blocks GIPR activation (29) (Supplementary Fig. 1*A* and *B*). In accordance with previous studies (29), treatment of obese IR mice with the GIPR antagonist significantly reduced body weight, fat mass, and food intake (Fig. 3*A–F*). Nevertheless, treatment of obese WT mice (dosed with the GIPR antagonist) with rosiglitazone increased body weight, fat mass, and

food intake (Fig. 3*A–F*); reduced fasting glucose levels; and improved insulin tolerance (indicated via an insulin tolerance test) both in the absence and presence of the mouse GIPR antagonist (Fig. 3*G–J*). Collectively, these findings indicate that the oral insulin-sensitizing agent rosiglitazone does not require activation of the GIPR to drive weight gain or improve insulin tolerance in obese IR mice.

GIPR Agonism Prevents Rosiglitazone-Induced Weight Gain

GIPR agonism has become an attractive cotherapy for the management of T2D and its associated comorbidities (18,30,38–40). Therefore, we sought to determine whether activation of the GIPR could influence the antidiabetic pharmacological profile of rosiglitazone. To address this question, we used a potent and selective LAGIPRA (WO2018/181864) that effectively promotes GIPR agonism in preclinical models of obesity and diabetes (41). As expected, rosiglitazone treatment increased body weight, fat mass, and food intake in obese IR mice (Fig. 4). However, intriguingly,
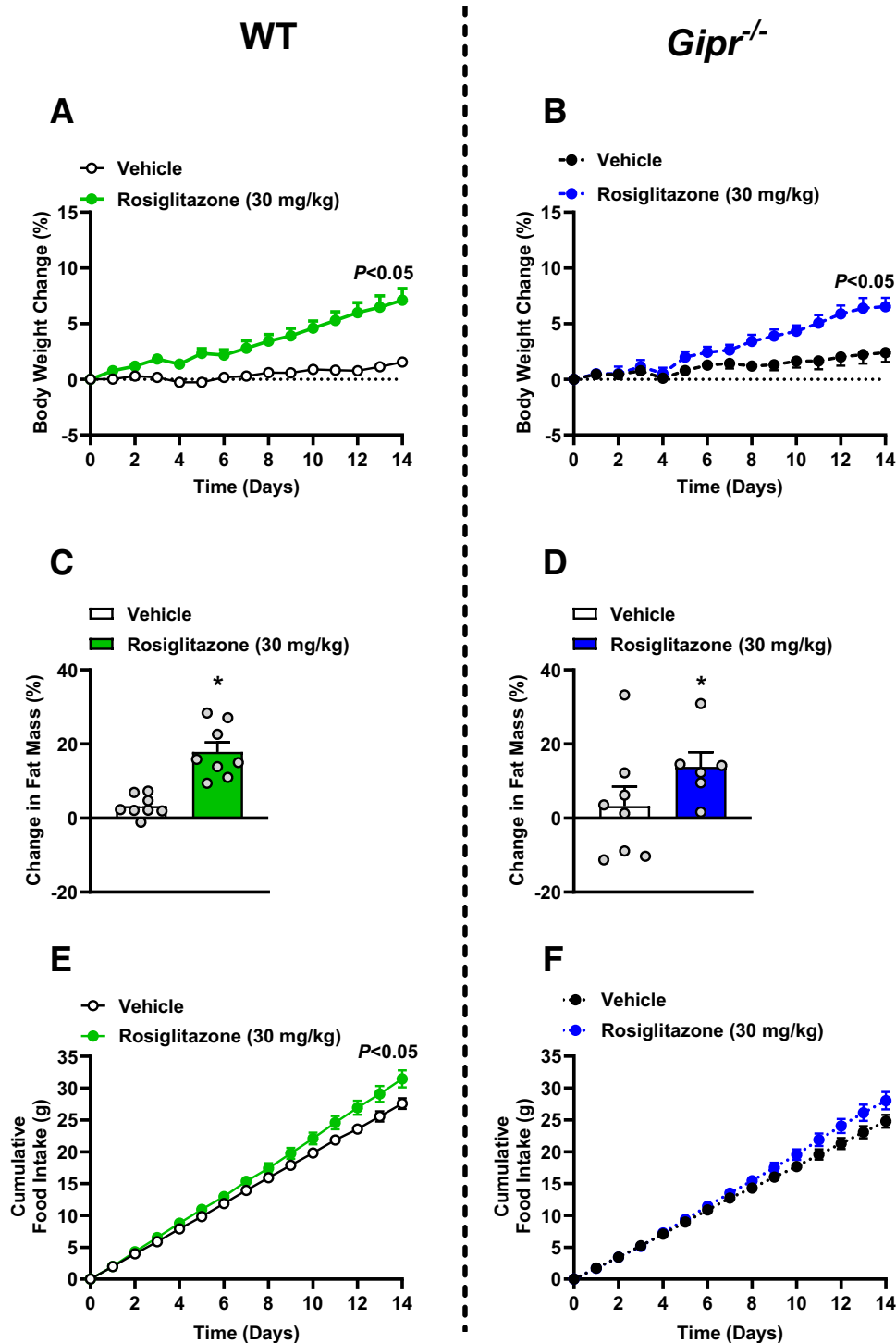
Figure 2—Rosiglitazone promotes obesity in WT and Gipr $^{-/-}$ mice (n = 6 per each treatment group) housed at thermoneutrality (28°C). Graphs report data for body weight (*A* and *B*), fat mass (*C* and *D*), and food intake (*E* and *F*). Data are presented as mean ± SEM. *P < 0.05 compared with vehicle. Rosiglitazone increased body weight in both WT and Gipr $^{-/-}$ animals (P < 0.05). There was no effect of genotype (genotype by treatment interaction, P > 0.05) on the obesogenic action of rosiglitazone. Statistical analyses performed included the Student unpaired t test or three-way ANOVA (effect of treatment, effect of genotype, and treatment by genotype interaction), followed by a multiple comparisons test.

chronic administration of the LAGIPRA prevented rosiglitazone from promoting weight gain and excess adiposity (Fig. 4*A–F*), and the combination of the LAGIPRA and rosiglitazone resulted in increased lean mass (Fig. 4*H* and *I*). Importantly, protection from excess adiposity appeared to be

driven by GIPR agonism negating the orexigenic activity of rosiglitazone treatment. Specifically, chronic treatment with the LAGIPRA blocked the hyperphagic activity of rosiglitazone (Fig. 4*J, K,* and *L*) and increased whole-body fat oxidation rates during the first 5 days of treatment
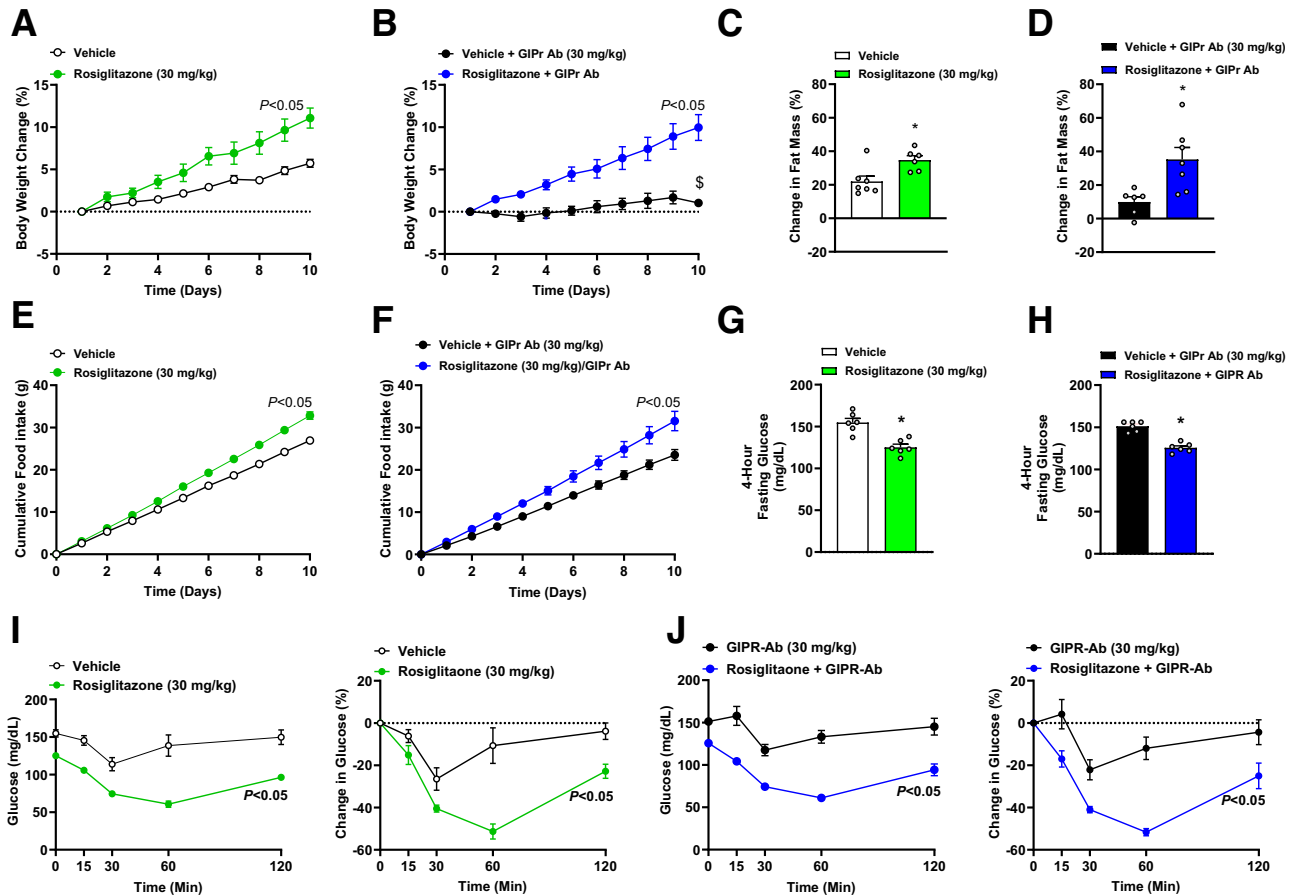
Figure 3—Rosiglitazone promotes obesity in obese WT mice (n = 6–8 per treatment group) in the presence and absence of a mouse GIPR antagonist antibody (Ab) while being housed at thermoneutrality (28°C). Graphs report data for body weight (*A* and *B*), fat mass (*C* and *D*), food intake (*E* and *F*), and insulin tolerance (*G* and *H*) after 10 days of treatment. Rosiglitazone significantly increased body weight and fat mass in both the presence and absence of the GIPR antagonist monoclonal Ab (mAb) ($P < 0.05$). There was no effect of the GIPR antagonist mAb (GIPR antagonist mAb by rosiglitazone treatment interaction, $P > 0.05$) on the obesogenic activity of rosiglitazone. Data are presented as mean ± SEM. *$P < 0.05$ compared with vehicle. Statistical analyses performed included Student unpaired t test or three-way ANOVA (effect of treatment [GIPR antagonist] by treatment [rosiglitazone] interaction), followed by Dunnett's or Tukey's multiple comparisons test where appropriate.

(Supplementary Fig. 2*B* and *C*), but had no effect on daily energy expenditure (Supplementary Fig. 2*A*). Together, our results indicate that chronic activation of the GIPR counters rosiglitazone-mediated promotion of a positive energy balance in obese IR mice. These findings may have important clinical implications because weight gain is a well-established side effect associated with the administration of the TZD class of medications.

GIPR Agonism Enhances Rosiglitazone-Induced Insulin Sensitization

Historically, the obesogenic activity of rosiglitazone has been suggested to be intimately tied to its insulin-sensitizing efficacy (26). Therefore, because GIPR agonism negated the effect of rosiglitazone on adiposity, and because of the recent discovery that GIPR agonism improves insulin sensitivity in obese IR mice (18), we examined whether activation of the GIPR would prevent the negative consequences of rosiglitazone treatment on energy balance, while, at the same time,

augmenting its insulin-sensitizing efficacy. In line with our previous studies, chronic administration of the LAGIPRA prevented the effect of rosiglitazone on body weight and food intake (data not shown). Furthermore, indicative of improved glycemic control, the LAGIPRA and rosiglitazone treatments both reduced fed and fasted blood glucose levels and fed insulin levels, but neither agent decreased fasting insulin levels (Fig. 5*A–D*). Administration of the LAGIPRA alone and in combination with rosiglitazone boosted circulating levels of the insulin sensitizer IGFBP-2 (42) (Fig. 5*E*), whereas only rosiglitazone augmented plasma levels of adiponectin, a biomarker of enhanced adipose tissue health (43) (Fig. 5*F*).

To directly investigate whether GIPR agonism augments the insulin-sensitizing action of rosiglitazone, we performed hyperinsulinemic-euglycemic clamp studies (Fig. 6 and Supplementary Fig. 3). Using this approach, we found that chronic treatment of obese IR mice with the LAGIPRA augmented the efficacy of rosiglitazone on peripheral
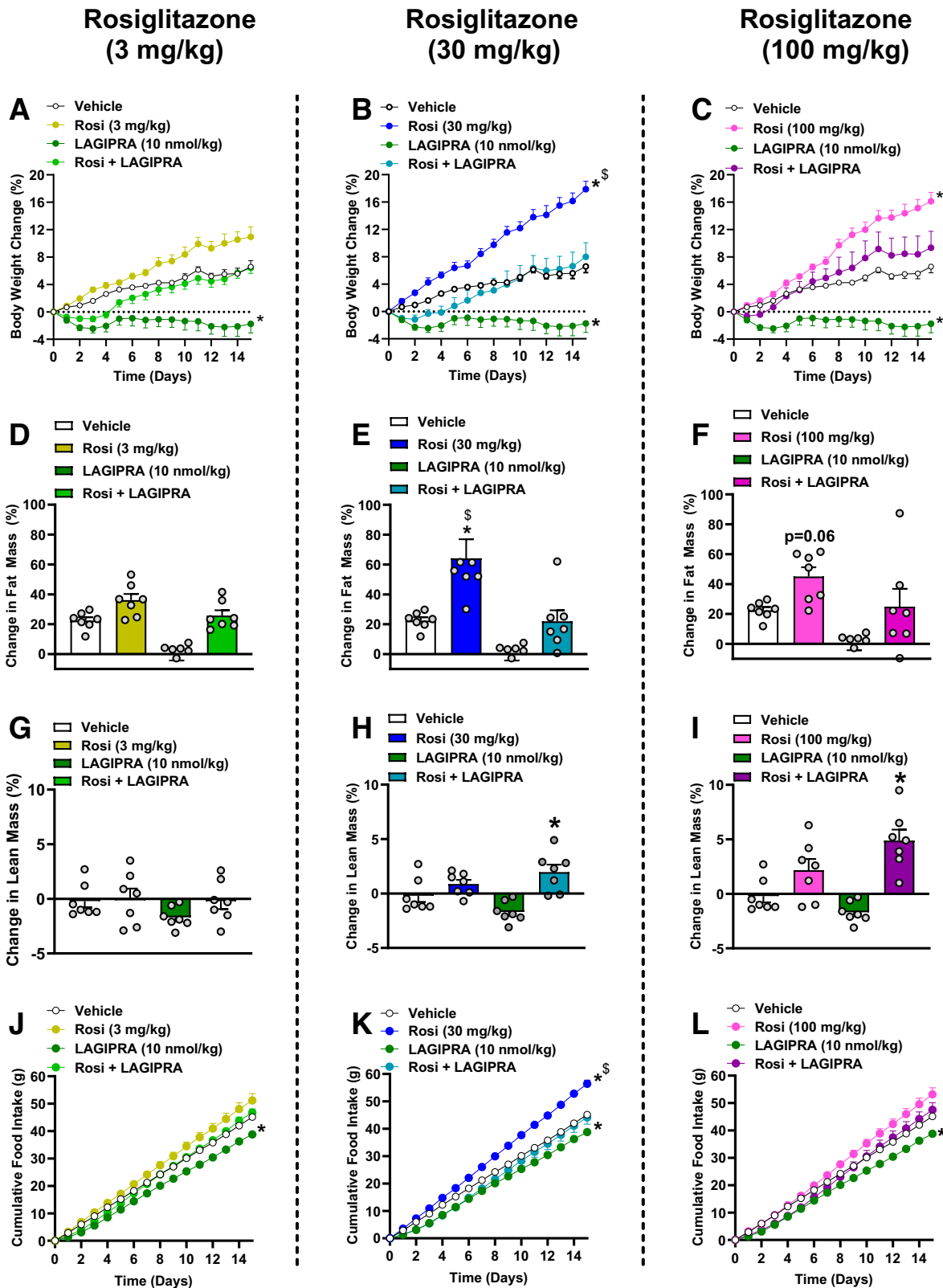
Figure 4—Chronic treatment with an LAGIPRA (10 nmol/kg, s.c.) prevents weight gain induced by rosiglitazone (Rosi; 3, 30, and 100 mg/kg, p.o.) in obese mice (n = 7 per treatment group). Graphs report data for body weight (*A–C*), fat mass (*D–F*), lean mass (*G–I*), and food intake (*J–L*). All studies were conducted in subthermoneutral conditions (24°C). Data are presented as mean ± SEM. *$P < 0.05$ and $P < 0.05$ compared with vehicle and the cotreatment (LAGIPRA and rosiglitazone) group, respectively. Statistical analyses performed included a one-way or two-way ANOVA (effect of treatment), followed by Dunnett's multiple comparisons test where appropriate.
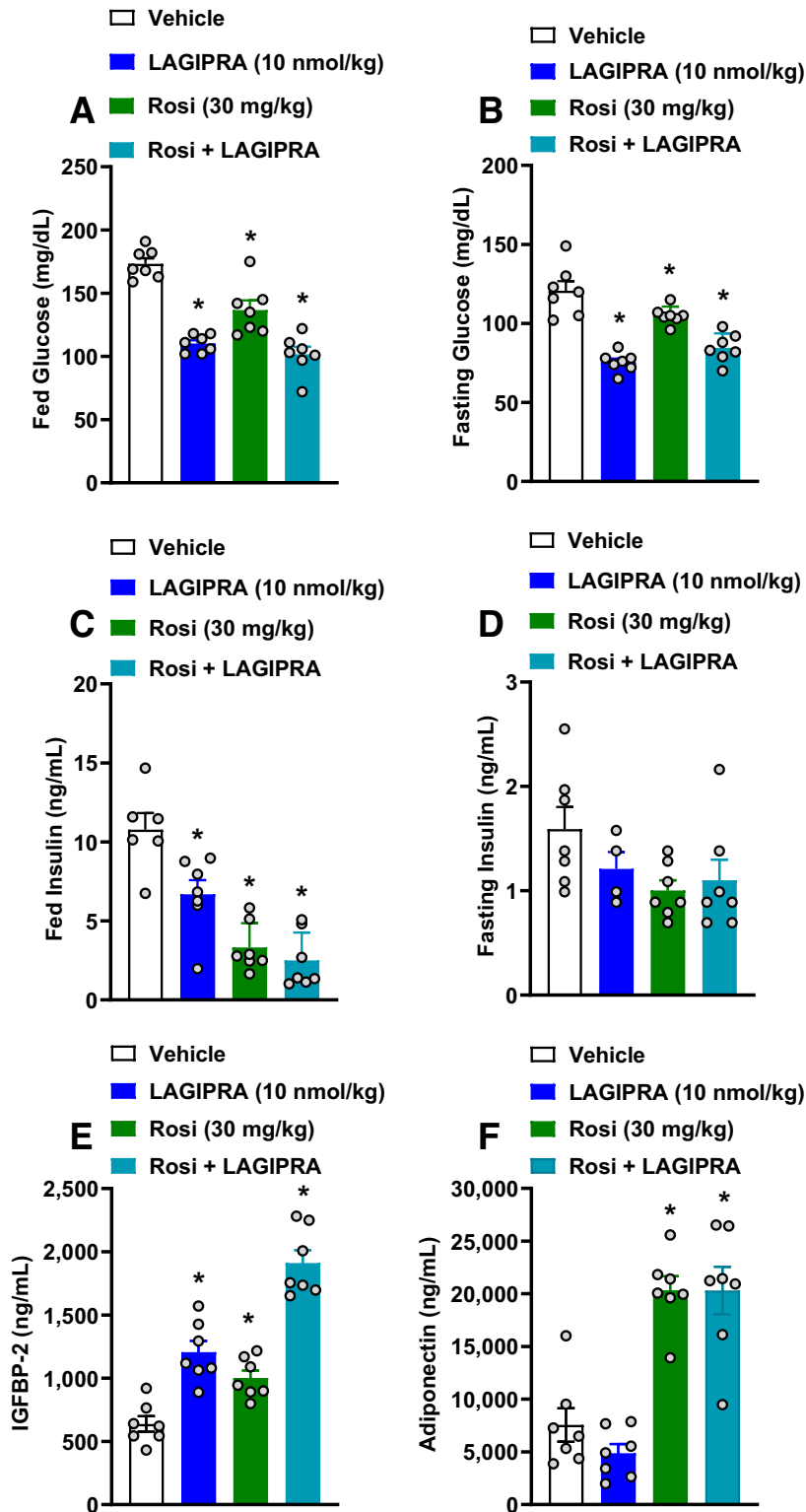
Figure 5—Chronic treatment with an LAGIPRA (10 nmol/kg, s.c.) enhances the insulin-sensitizing activity of rosiglitazone (Rosi; 30 mg/kg, p.o.) in obese IR mice (n = 6–7 per treatment group). Graphs report data on plasma glucose and insulin levels in fed (A and C) and fasted (B and D) mice. Circulating levels of IGFBP2 (E) and adiponectin (F) after 14 days of treatment. All studies were conducted in subthermoneutral conditions (24°C). Data are presented as mean ± SEM. *$P < 0.05$ compared with vehicle. Statistical analyses performed one-way ANOVA, followed by Dunnett's multiple comparisons test where appropriate.
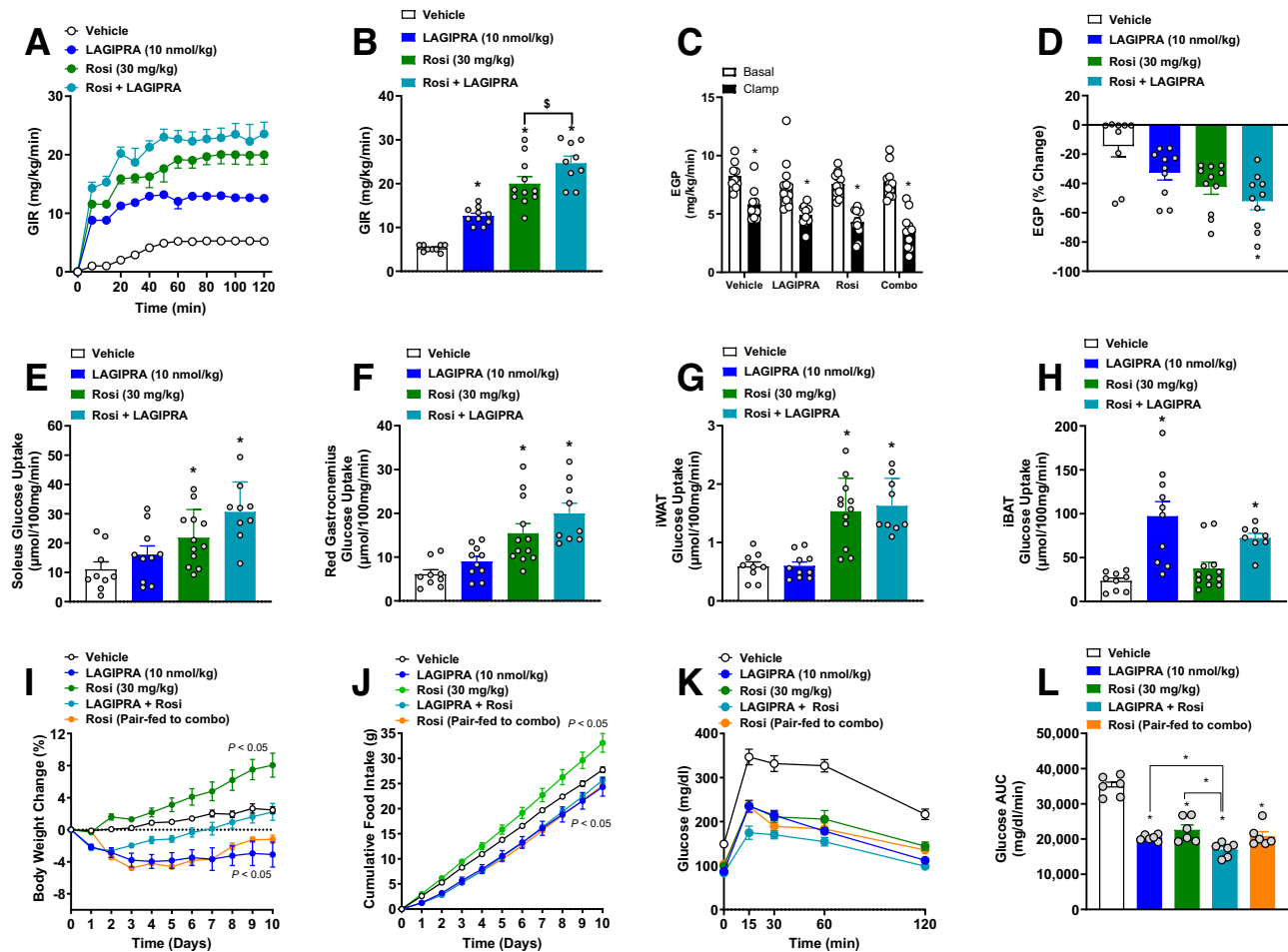
Figure 6—Chronic treatment with an LAGIPRA (10 nmol/kg, s.c.) enhanced the insulin-sensitizing activity of rosiglitazone (Rosi; 30 mg/kg, p.o.) in obese IR mice ($n = 8$–12 per group). Graphs report data on average GIR (*A* and *B*), EGP (*C* and *D*), and insulin-stimulated glucose disposal in soleus (*E*), red gastrocnemius (*F*) skeletal muscle, inguinal white adipose tissue (iWAT; *G*) and interscapular brown adipose tissue (iBAT; *H*) after 14 days of treatment. Chronic treatment with the LAGIPRA (10 nmol/kg, s.c.) enhanced the glycemic benefits of rosiglitazone independent of changes in body weight. Data on body weight change (*I*), cumulative food intake (*J*), and blood glucose levels after an oral glucose challenge (*K* and *L*). All studies were conducted in subthermoneutral conditions (24°C). Data are presented as mean ± SEM. *$P < 0.05$ and $P < 0.05$ compared with vehicle and the cotreatment (LAGIPRA and rosiglitazone) group, respectively. Statistical analyses performed included a one-way ANOVA, or two-way ANOVA, followed by Dunnett's multiple comparisons test where appropriate. Combo, combination.

insulin sensitivity. After 14 days of treatment, the LAGIPRA (10 nmol/kg) treatment enhanced the effect of rosiglitazone (30 mg/kg) on fasting glucose and insulin levels before the clamp (Supplementary Fig. 3*A* and *B*) and augmented the GIR (Fig. 6*A* and *B*). The combination of rosiglitazone and the LAGIPRA increased insulin-mediated suppression of EGP when compared with vehicle-treated animals (Fig. 6*C* and *D*). Blood glucose level was maintained at approximately 122 mg/dL during clamp (Supplementary Fig. 3*C*), and there was no effect of treatment on infused (exogenous human) insulin levels at the end of clamp (Supplementary Fig. 3*D*).

Given that both the LAGIPRA and rosiglitazone improved whole-body insulin sensitivity, we next sought to investigate which organs displayed improved insulin-stimulated glucose disposal, by examining 2-[1-^{14}C] deoxy-D-glucose uptake. Rosiglitazone treatment enhanced insulin-stimulated glucose disposal in skeletal muscle (namely, soleus and red

gastrocnemius), and s.c. inguinal white adipose tissue (iWAT), whereas there was no effect of the LAGIPRA (Fig. 6*E*–*G*). However, the LAGIPRA promoted insulin-stimulated glucose disposal in the metabolically active organ brown adipose tissue (BAT) when given either alone or in combination with rosiglitazone (Fig. 6*H*).

To determine whether the LAGIPRA augments the glycemic benefits of rosiglitazone independent of body weight, we examined glycemic control in obese IR mice treated with rosiglitazone, but pair-fed to animals treated with rosiglitazone and the LAGIPRA (Fig. 6). In line with our previous studies, rosiglitazone treatment resulted in increased body weight and food intake, whereas treatment with the LAGIPRA blocked this effect (Fig. 6*I* and *J*). Importantly, although all treatment groups had improved glycemic control, animals dosed with rosiglitazone, but pair-fed to the co-treatment group, had an equivalent glucose tolerance to that of the rosiglitazone-only treated animals (Fig. 6*K* and *L*). In

contrast, the rosiglitazone and LAGIPRA combination group had significantly enhanced glycemic control when compared with the rosiglitazone group (Fig. 6K and L).

Our studies suggest that the LAGIPRA enhances rosiglitazone's glycemic benefits independent of body weight, providing further support to the notion that GIPR agonism augments glycemic control independent of changes in adiposity. Overall, these findings indicate that GIPR agonism counters the negative consequences of rosiglitazone therapy on energy balance, while simultaneously boosting the beneficial effect of rosiglitazone on insulin sensitization. These findings may be clinically relevant because understanding how GIPR signaling blocks rosiglitazone-induced weight gain and further enhances insulin sensitivity could provide insight into how glucose-dependent insulinotropic polypeptide (GIP)-based agents mediate their therapeutic efficacy in the management of obesity and T2D.

GIPR Agonism Augments Metabolic Gene Expression in Brown Fat

To gain insight into the potential mechanism(s) by which the LAGIPRA improved the insulin-sensitizing action of rosiglitazone, we conducted RNA-sequencing profiling in WAT and BAT. In agreement with previous studies, the LAGIPRA and rosiglitazone treatments robustly induced a gene signature indicative of improved metabolic health in both white and brown fat (18,44,45). In BAT, a total of 1,709, 2,733, and 572 genes were differentially regulated (Fig. 7A), with 915, 1,508, and 237 gene transcripts upregulated, and 794, 1,225, and 335 genes downregulated by rosiglitazone, the LAGIPRA, or their combination, respectively. Gene set enrichment analysis indicated that both rosiglitazone and LAGIPRA treatment affect pathways associated with glucose, lipid, and amino acid mitochondrial metabolism (Fig. 7B). Indicative of enhanced metabolic activity, chronic treatment of obese IR mice with the LAGIPRA alone or in combination with rosiglitazone affected the expression of genes involved with substrate delivery (Cd36, Fabp4), lipid metabolism (Plin1, Gpat4, and *Acadsb*), branched-chain amino acid (BCAA) catabolism (Bcat2 and Bckdh), tricarboxylic acid cycle flux (*Suclg1*), oxidative phosphorylation (Ndufv2, *Ndufaf5*, *5uqcrb*, Cox7A, and Cox8b), and thermogenic activity (Ucp1) in BAT (Fig. 7C).

In s.c. WAT, treatment with the LAGIPRA or rosiglitazone alone and the combination treatment induced differential expression of 5,070, 5,603, and 1,581 genes, respectively (Fig. 8A), with 3,266, 2,922, and 677 gene transcripts upregulated, and 2,337, 2,148, and 9,04 genes downregulated, respectively. Gene set enrichment analysis indicated that both rosiglitazone and the LAGIPRA affect pathways
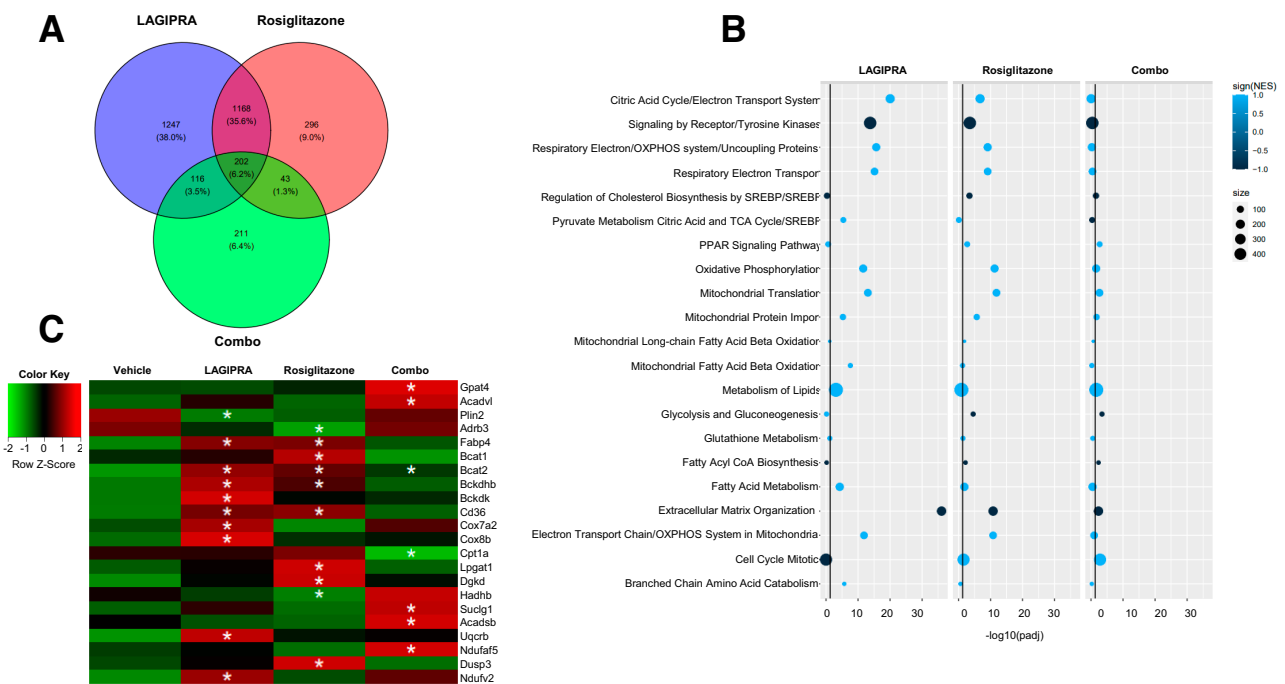

Figure 7—Chronic treatment with an LAGIPRA and/or rosiglitazone induces transcripts levels of proteins that regulate glucose, lipid, and amino acid metabolism in interscapular brown adipose tissue (iBAT). HFD-fed, obese, IR mice were dosed once daily with either vehicle (saline, n = 6), an LAGIPRA (n = 6, 10 nmol/kg, s.c.), rosiglitazone (n = 6, 30 mg/kg), or the combination (Combo) of both treatments (n = 6). After 14 days of treatment, tissue samples were collected for molecular analyses. Venn diagram of differentially (up and down) expressed genes (false discovery rate [FDR] <0.05; A), gene set enrichment analysis of differentially expressed genes (B) and a heat map of RNA-sequencing expression z scores computed for metabolic genes in iBAT (C). *P < 0.05 compared with vehicle. All studies were conducted in subthermoneutral conditions (24°C). Statistical analyses were performed using one-way ANOVA, followed by FDR correction where appropriate. CoA, coenzyme A; NES, normalized enrichment score; OXPHOS, oxidative phosphorylation; padj, adjusted P value.
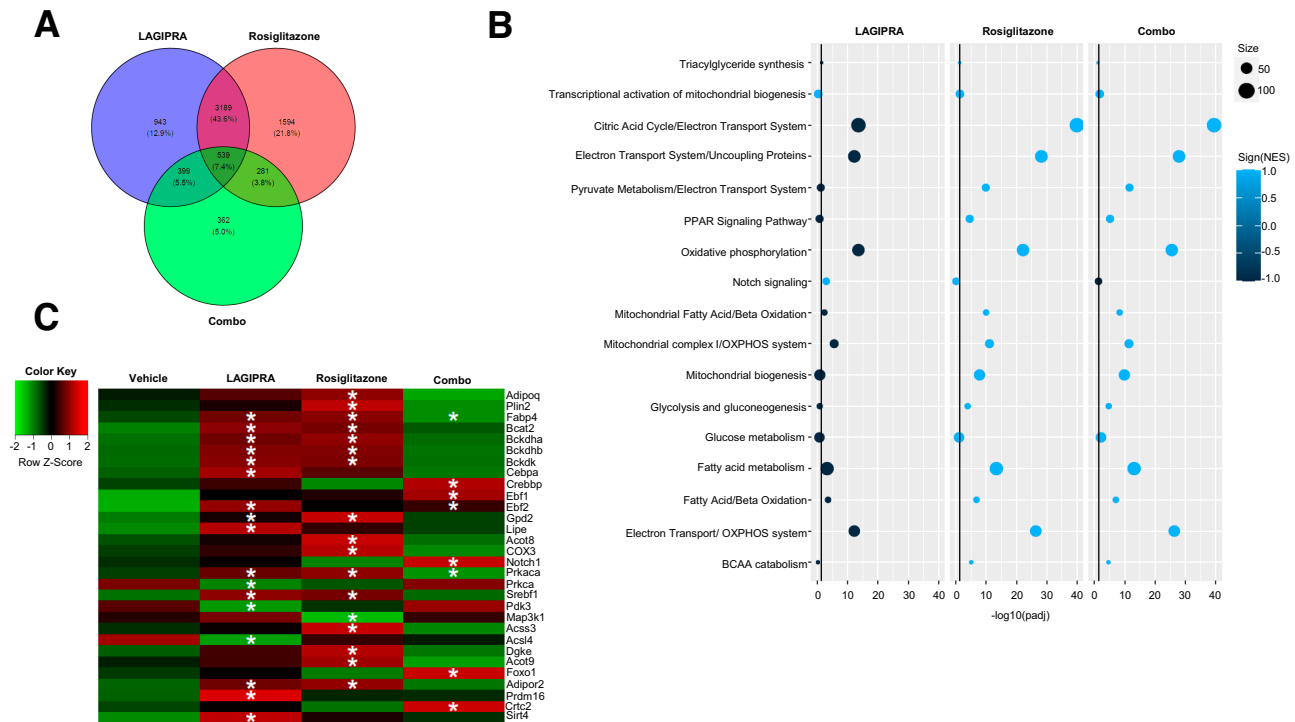
Figure 8—Chronic treatment with an LAGIPRA and/or rosiglitazone induces transcripts levels of proteins that regulate glucose, lipid, and amino acid metabolism in s.c. iWAT. Obese IR mice fed an HFD were dosed once daily with either vehicle (saline, $n = 6$), an LAGIPRA ($n = 6$, 10 nmol/kg, s.c.), rosiglitazone ($n = 6$, 30 mg/kg), or the combination (Combo) of both treatments ($n = 6$). After 14 days of treatment, tissue samples were collected for molecular analyses. Venn diagram of differentially (up and down) expressed genes (false discovery rate [FDR] <0.05; A), gene set enrichment analysis of differentially expressed genes (B) and a heat map of RNA-sequencing expression z scores computed for metabolic genes in iWAT (C). *$P < 0.05$ compared with vehicle. All studies were conducted in subthermoneutral conditions (24°C). Statistical analyses were performed using one-way ANOVA, followed by FDR correction where appropriate.

associated with improved metabolic health (Fig. 8B). Specifically, chronic treatment of obese IR mice with the LAGIPRA and/or rosiglitazone induced transcript levels for proteins promoting white adipocyte health (*Adipoq, Dgke, Lipe, Ebf2,* and *Cebpα*), substrate delivery and breakdown (*Srebf1, Bcat2, Bckdh, Plin1, Acot8,* and *Fabp4*), and improved mitochondrial function (Prdm16, Sirt4, and Cox3), and there was a reduction of gene transcripts indicative of adipocyte dysfunction (*Pdk3, Prkcα,* and *Map3k1*) in s.c. WAT (Fig. 8C). Taken together, these molecular findings suggest that GIPR agonism may enhance the therapeutic efficacy of rosiglitazone by promoting the expression of proteins that drive metabolic health in white and brown fat.

DISCUSSION

A common side effect limiting the use of TZDs as a treatment option for the management of T2D is weight gain (26). Therefore, mechanisms that alleviate the impact of TZDs on adiposity without negatively influencing their insulin-sensitizing efficacy is of therapeutic interest. Recently, GIPR activation has proven to be an effective complementary pathway for enhancing the efficacy of GLP-1R agonism in the management of obesity and T2D (9,46). This finding has inspired new searches for additional therapeutic

partners and major interest in how GIPR agonism functions to mediate the efficacy (47). Here, we have reported on a series of studies we conducted to determine whether GIPR engagement affects the therapeutic outcomes of the common insulin sensitizer rosiglitazone. The major findings of our study are that chronic treatment with an LAGIPRA prevented the obesogenic nature of rosiglitazone while simultaneously augmenting the insulin-sensitizing action in obese IR mice. Mechanistically, we found that GIPR agonism–mediated (9) hepatic and systemic insulin sensitization was associated with increased glucose disposal in BAT and the recruitment of metabolic and thermogenic gene expression in white and brown fat.

There is controversy regarding the cell type expressing the GIPR in WAT in preclinical models and man (48–50), with contemporary findings suggesting that the GIPR is expressed by pericytes, mesothelial cells, and, importantly, a unique subpopulation of fat cells in adult humans (48). Therefore, with GIP-based therapeutics now approved for the management of metabolic diseases (6,9), it is important to understand the impact of modulating GIPR activity in adipose tissue (9). TZDs are among the most effective agents used to alleviate IR in the management of T2D (44). However, although recent findings suggest TZDs may be cardioprotective (51), because of reported cardiovascular

risk (52) and tolerability issues, use of TZDs as oral anti-diabetic agents has been reduced (22). One of the most well-documented unwanted effects associated with the administration of TZDs is weight gain, with clinical (53) and preclinical data (27,28) suggesting that the promotion of a positive energy balance is driven by increased energy intake. Interestingly, the GIPR is a PPARγ target gene, and the TZD analogs rosiglitazone and pioglitazone augment the expression of the GIPR in murine and human adipocytes (23,24). These findings, together with data showing that $Gipr^{-/-}$ mice are protected from DIO (37), have led to the suggestion that GIPR signaling may contribute to the obesogenic nature of TZDs (24). In agreement with previous in vitro findings, we found that chronic treatment of obese mice with rosiglitazone stimulated GIPR expression in both WAT and BAT. However, it is important to note that when fed a mouse fed an HFD, housed at thermoneutrality (~28°C), we found that $Gipr^{-/-}$ mice became obese (>40 g), suggesting that protection from weight gain may be due to thermal stress rather than an obesogenic action of GIP per se. Using both obese $Gipr^{-/-}$ mice and obese WT mice treated with a GIPR antagonist antibody, we investigated whether the GIPR contributes to the effects of rosiglitazone on adiposity. Taking this approach, we found that rosiglitazone increased food intake, body weight, and fat mass and improved glycemic control in WT and $Gipr^{-/-}$ mice, and WT mice treated with a GIPR antagonist antibody, to the same extent. Collectively, our findings imply that GIPR signaling does not contribute to the obesogenic action of rosiglitazone in obese IR mice. However, in keeping with the law of energy balance (54), this is not surprising, because treatment with GIPR agonist analogs does not promote food intake, reduce caloric expenditure, or increase body weight (10).

Over the past decade, GIPR activation has emerged as a compelling therapeutic partner that maximizes desirable, and minimizes undesirable effects of current and novel obesity and T2D medications (16,17,30,40). A key outcome of our studies is that chronic treatment of obese mice with an LAGIPRA blocked rosiglitazone-induced weight gain and adiposity in obese mice, effects that appeared to be due to a suppression of hyperphagic activity of rosiglitazone. In agreement, chemogenetic stimulation of GIPR^{+} cells in the hypothalamus reduces food intake, and administration of LAGIPRA analogs suppresses energy intake and reduces body weight in obese mice (14,15). Mechanistically, PPARγ and the GIPR are expressed in areas of the brain that regulate energy balance and control appetite (17,27,28,55). Furthermore, peripheral administration of rosiglitazone and GIPR agonist analogs induces neuronal activity in the hypothalamus and brainstem, respectively (17,28,56). Additionally, rosiglitazone promotes feeding by downregulating the expression of the neuropeptide POMC in neurons in the ARC (27,28,57), and GIPR agonism suppresses appetite by modulating neuropeptide expression in this area of the brain (14,55,58). Hence, GIPR activation may prevent the hyperphagic action of rosiglitazone and subsequent weight by modulating its influence on anorexigenic and orexiegnic neuropeptides in the mediobasal hypothalamus. Additional mechanistic exploration is required to fully elucidate how GIPR activation prevents rosiglitazone or PPARγ promotion of a positive energy balance.

TZDs cause weight gain of approximately 3–5 kg over a 5-year period, and although fluid retention may contribute, expansion of s.c. WAT is believed to be a key contributing factor (26,44,59). PPARγ is the master regulator of fat cell differentiation (60) and is targeted by TZDs to drive the "healthy" storage of excess calories in s.c. WAT and away from their "unhealthy" deposition in key metabolic organs, thereby improving systemic insulin sensitivity (44). Unfortunately, it is believed that the unwanted side of weight gain is tied to rosiglitazone's adipogenic and insulin-sensitizing activity. However, in accordance with the law of energy balance, the weight gain that accompanies treatment with TZDs is related to their hyperphagic effects and/or a failure of patients to adjust their caloric intake to the proportion of calories no longer lost via the renal threshold, and not their adipogenic or insulin-sensitizing action (27,28). A key finding of our studies is that GIPR agonism not only blocked rosiglitazone-induced weight gain, it also improved its glucose tolerance efficacy independent of changes in body weight. Chronic treatment with the LAGIPRA boosted hepatic and peripheral insulin sensitivity both alone and in combination with rosiglitazone. From a mechanistic perspective, the enhanced systemic and tissue-specific insulin sensitivity following treatment with the LAGIPRA and rosiglitazone alone or in combination was associated with the induction of gene transcripts indicative of improved adipocyte health, the browning of WAT, the uptake and oxidation of lipids, BCAA catabolism, augmented tricarboxylic acid cycle flux, and mitochondrial oxidative phosphorylation in WAT and BAT. The effect of the LAGIPRA and rosiglitazone on genes linked with BCAA breakdown is of particular interest because a dysregulation BCAA and branched-chain keto acid metabolism contributes to the development of systemic IR (61), and activation of both the GIPR and PPARγ may ameliorate IR by promoting the catabolism of BCAAs and branched-chain keto acids in white and brown fat (18,45,62,63).

Summary

In summary, we found that GIPR agonism does not contribute to the obesogenic activity of rosiglitazone. Of note, we report for the first time that treatment of obese IR mice with an LAGIPRA prevents rosiglitazone-induced hyperphagia and weight gain and augments its insulin-sensitizing efficacy. Of note, our results suggest that GIPR activation negates a common side effect of TZD-mediated PPARγ activation while maximizing its most appealing therapeutic attribute of insulin sensitization. These findings provide further support to the notion that GIPR engagement is an attractive partner for combining with mechanisms that improve metabolic health. Our preclinical observations

require clinical validation in adult humans with obesity and T2D.

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