Pioglitazone Acutely Reduces Energy Metabolism and Insulin Secretion in Rats

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Our objective was to determine if the insulin-sensitizing drug pioglitazone acutely reduces insulin secretion and causes metabolic deceleration in vivo independently of change in insulin sensitivity. We assessed glucose homeostasis by hyperinsulinemic-euglycemic and hyperglycemic clamp studies and energy expenditure by indirect calorimetry and biotelemetry in male Wistar and obese hyperinsulinemic Zucker diabetic fatty (ZDF) rats 45 min after a single oral dose of pioglitazone (30 mg/kg). In vivo insulin secretion during clamped hyperglycemia was reduced in both Wistar and ZDF rats after pioglitazone administration. Insulin clearance was slightly increased in Wistar but not in ZDF rats. Insulin sensitivity in Wistar rats assessed by the hyperinsulinemic-euglycemic clamp was minimally affected by pioglitazone at this early time point. Pioglitazone also reduced energy expenditure in Wistar rats without altering respiratory exchange ratio or core body temperature. Glucose-induced insulin secretion (GIIS) and oxygen consumption were reduced by pioglitazone in isolated islets and INS832/13 cells. In conclusion, pioglitazone acutely induces whole-body metabolic slowing down and reduces GIIS, the latter being largely independent of the insulin-sensitizing action of the drug. The results suggest that pioglitazone has direct metabolic deceleration effects on the β-cell that may contribute to its capacity to lower insulinemia and antidiabetic action. ***Diabetes*** **62:2122–2129, 2013**

Major drugs developed to treat type 2 diabetes aim at either increasing insulin secretion or reducing insulin resistance (1–4). Two classes of insulin-sensitizing agents are currently used, the biguanides (metformin) and the thiazolidinediones (TZDs), of which the only one still recommended for use in some countries is pioglitazone (5). TZDs are peroxisome proliferator–activated receptor-γ (PPARγ) agonists. They stimulate adipocyte differentiation, relieving other tissues from fat excess, thereby reducing their resistance to insulin (6,7). The beneficial effects of TZDs are not limited to increased insulin sensitivity and also include preservation of β-cell function (8). It is thought that the beneficial effect of TZDs on β-cell function in vivo is indirect and occurs via a relief of the need for insulin hypersecretion because of their insulin sensitizing action. We should, however, consider the possibility that the classical antidiabetic insulin sensitizers, pioglitazone and metformin, might also have beneficial effects on glucose homeostasis via direct reduction of insulin hypersecretion independently of insulin resistance.

We previously demonstrated in vitro that pioglitazone acutely slows down glucose and lipid metabolism in the β cell and inhibits glucose-induced insulin secretion (GIIS) primarily at submaximal and much less at maximal glucose concentrations (right shift in the glucose dose response) via a PPARγ-independent mechanism (9). These acute effects of pioglitazone are likely attributable to complex I inhibition of the electron transport chain (10) and involve reduced glucose oxidation, decreased ATP levels, and increased AMPK activation (9). Interestingly, metformin causes similar effects (J.L. and M.P., unpublished data). Hence, we proposed the novel concept of "metabolic deceleration" as a mode of action of some antidiabetic drugs and suggested that the action of pioglitazone to reduce glucose metabolism and insulin secretion in the β-cell may partly explain its beneficial effects (9). The concept that metabolic deceleration protects the β-cell from both oxidative and endoplasmic reticulum stress has recently been reviewed (11,12).

In the current study we performed in vivo experiments in normal Wistar and obese Zucker diabetic fatty (ZDF) rats to better understand how acute treatment with pioglitazone alters glucose homeostasis, with particular focus on how it reduces hyperinsulinemia. The following questions were asked: *1*) Can we confirm in vivo our previous in vitro findings in isolated rat islet and β-cell line that pioglitazone acutely reduces insulin secretion? *2*) Is this acute effect of pioglitazone on insulin secretion independent of its effects on insulin sensitivity? and *3*) Does pioglitazone acutely slow down whole-body energy metabolism?

RESEARCH DESIGN AND METHODS

Materials. Pioglitazone-HCl was suspended either at 5 mg/mL in 0.5% methylcellulose prepared in autoclaved tap water or at 10 mmol/L in DMSO for in vivo and in vitro experiments, respectively. Stock solutions of GW1929 (Alexis Biochemicals, San Diego, CA) and CAY10599 (Cayman Chemical, Ann Arbor, MI) were prepared in DMSO at 1.2 and 15 mmol/L, respectively. Glycemia was monitored using a glucometer. Total plasma insulin, total insulin content, and media insulin concentrations were determined by radioimmunoassay using human insulin standards (Linco Research, St. Charles, MO). ELISA was used to determine rat plasma C-peptide concentrations (both I and II; Mercodia AB, Uppsala, Sweden).

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Animals and pioglitazone administration. All procedures were approved by the Centre de Recherche du Centre Hospitalier de l'Université de Montréal Institutional Committee for the Protection of Animals; 300- to 350-g male Wistar rats (Charles River, St. Constant, Quebec, Canada) and 10-week-old male obese ZDF rats (Charles River, Kingston, NY) were housed under controlled temperature on a normal 12-h light–dark cycle with unrestricted access to water and standard chow. For glucose clamp procedures, animals underwent catheterization of the jugular vein and carotid artery as described (13) and recovered for 6 days. For indirect calorimetry studies, rats were acclimatized for 36 h in acrylic chambers bedded with hardwood chips. For biotelemetry studies, C50-PT probes (Data Sciences International, St. Paul, MN) were implanted with a catheter inserted through the femoral artery up to the abdominal aorta and the telemetry device body fixed to the muscle wall as described (14). Rats were allowed 10–13 days to recover and received 5 mg/kg carprofen subcutaneously during the first 3 days. On experimental days, food was withdrawn at 0700 h and pioglitazone (30 mg/kg) or vehicle was administered by gavage (6 mL/kg) at 0900 h. All animals were killed at the end of procedures by pentobarbital overdose.

Hyperglycemic clamps. Conscious rats were subjected to one-step (ZDF) or two-step (Wistar) hyperglycemic clamp (HGC) followed by an arginine bolus in a technique modified from another study (13). The clamp procedure was started 45 min after the rats were gavaged with pioglitazone or vehicle. Blood glucose was clamped either at 16.7 mmol/L for 120 min (one-step) or at 8.3 mmol/L for 30 min and 16.7 mmol/L for another 30 min (two-step). A bolus of arginine (174 mg/kg) was injected at 121 min to assess total β-cell secretory capacity. Plasma samples (150 μL blood, 11 samples) were collected from the carotid artery for insulin and C-peptide determinations before glucose infusion, during hyperglycemia, and 1 and 10 min after arginine injection. Insulin clearance was estimated by the C-peptide/insulin molar ratio (15). HGC with ZDF rats experiments were all performed in 10-week-old animals.

Hyperinsulinemic-euglycemic clamps. On the day of the experiment, the jugular vein catheter was connected to two syringes containing either insulin (Humulin-R; Eli Lilly) or 20% dextrose installed on a Harvard 33 Twin Syringe Pump. Insulin was prepared as follows: 20 μL insulin 100 units/mL was added to 20 μL rat plasma and diluted to 200 mU/L with saline. The clamp procedure started 45 min after the gavage of pioglitazone or vehicle in conscious animals by an insulin bolus (time 0 min) of 75 mU/kg/min for 45 s and 37.5 mU/kg/min for another 45 s, and the insulin infusion was then reduced to 5 mU/kg/min for the rest of the procedure. After 5 min, a blood sample was collected and a 20% dextrose infusion was started to clamp blood glucose at preclamp level (6.5 mmol/L). Glycemia was monitored from samples collected every 5 min. Plasma samples (150 μL of blood) were collected from the carotid artery for insulin determination at 0 (before any infusion), 60, 90, and 120 min. An index of insulin sensitivity (M/I index) was calculated by dividing the average glucose infusion rate during the last 60 min of the clamp ("M" expressed in μmol/kg/min) by the average circulating insulin value ("I" expressed in pmol/L) during the same time period (15).

Indirect calorimetry. Gas exchanges were measured for 6 h after gavage by pioglitazone or vehicle in an open-circuit calorimeter with ambulatory activity monitoring using PhysioScan and VersaMax systems (AccuScan Instruments, Columbus, OH). Oxygen and carbon dioxide concentrations were analyzed in each cage by sequential readings from gas samples continuously withdrawn from cages at a rate of 2.0 L/min. Gas samples were compared with ambient air composition for VO_2 and VCO_2 calculations. The oxygen analyzer was calibrated before each experiment by adjusting respiratory quotient (VCO_2/VO_2) readings to 0.7 using two rats fasted for 24 h (not included in the study). Ambulatory activity was monitored based on infrared beam interruptions in horizontal and vertical positions. Gas fractions and flow rate were used to calculate energy expenditure as follows: energy expenditure = $[(4.33 * VO_2) + (0.67 * VCO_2)] * BW$ (in kg) * 60, in which gas fractions are expressed in mL/kg/min (16).

Biotelemetry. The C50-PT probe–implanted rats had their core body temperature, heart rate, and blood pressure values recorded every minute using Data Sciences system and they were averaged over 30 or 60 min for clarity. This was a cross-over study with all animals receiving pioglitazone and placebo in an alternating order, with a 10-day washout period in between. For each experimental period, data were collected for a total of 68 h from 1900 h, with food being removed after 36 h, pioglitazone or vehicle being administered by gavage after 38 h, and food being returned after 46 h.

Cell culture. INS832/13 cells (17) were cultured as described (9). Experiments were conducted in Krebs-Ringer bicarbonate buffer containing 10 mmol/L HEPES (KRBH; pH 7.4) except during measurement of the oxygen consumption rate (OCR), where bicarbonate was omitted.

Islet isolation and ex vivo insulin secretion measurement. Islets were isolated from Wistar rats as previously described (9,18). For ex vivo insulin secretion measurement, the islet isolation procedure was started 90 min after

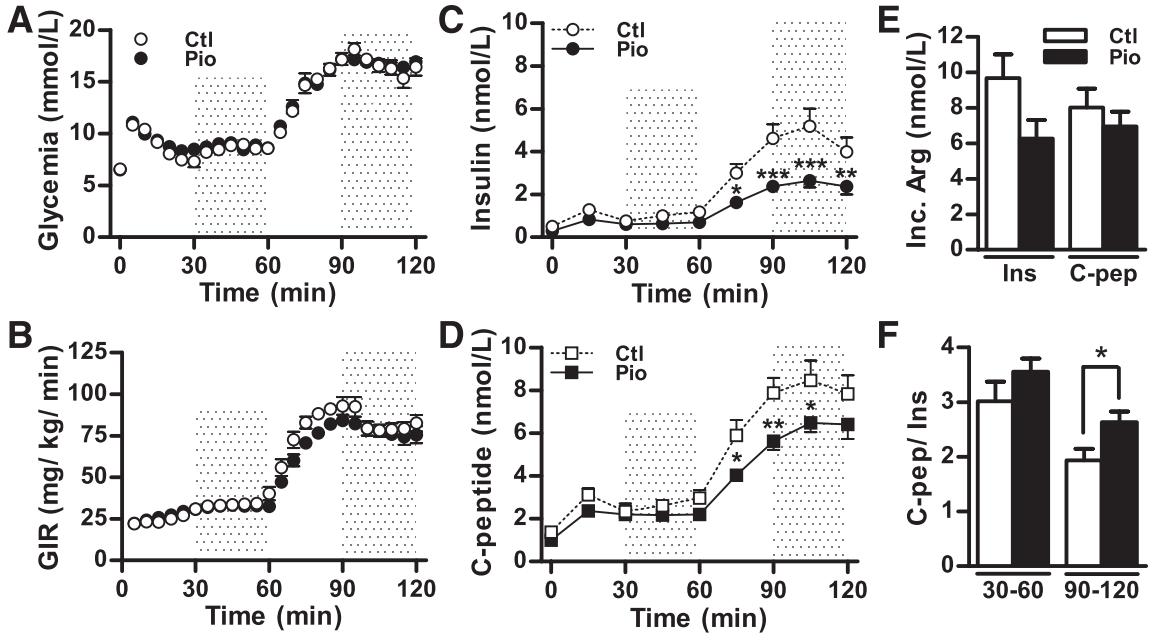

FIG. 1. Pioglitazone administration acutely inhibits insulin secretion and increases insulin clearance in Wistar rats. Two-step HGCs were performed in animals 45 min after pioglitazone (black symbols or bars; 30 mg/kg) or vehicle (control; white symbols or bars) administration by gavage. Glycemia was clamped consecutively at 8.3 mmol/L (first step, from min 30 to 60) and 16.7 mmol/L (second step, from min 90 to 120) as represented by dotted areas, and then an arginine bolus (174 mg/kg) was administered (min 120, after last sampling). Plasma glucose values (*A*), glucose infusion rate (GIR) (*B*), plasma insulin (*C*), and plasma C-peptide concentrations (*D*) during the procedures. Two-way repeated-measures ANOVA post hoc analyses: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. vehicle-treated group. *E*: Increment in plasma insulin and C-peptide concentrations 1 min after arginine administration. *F*: Insulin clearance estimated via C-peptide over insulin/molar ratio for first (30–60 min) and second (90–120 min) steps of the clamps. Unpaired *t* test: *$P < 0.05$ vs. vehicle-treated group. Data expressed as means ± SEM, n = 7–8 animals. Inc, increment; Pio, pioglitazone; Ctl, control; Ins, insulin; C-pep, C-peptide; Arg, arginine.

gavage. Batches of 10 islets were used for static insulin secretion measurements in KRBH as previously described (9).

Oxygen consumption and insulin secretion in vitro. OCR in vitro was measured using a XF24 respirometer (Seahorse Bioscience, Billerica, MA). INS832/13 cells were seeded 1 day before the experiments at 7×10^4 cells/well in XF24 microplates. Media were changed to RPMI 1640 supplemented as described in another study (9) and containing 3 mmol/L glucose 2 h before the experiments. Isolated islets were hand-picked onto XF24 islet capture microplates, 3 h before the experiments, 75 islets/well in RPMI 1640 supplemented as described in another study (9) and containing 3 mmol/L glucose. Cells or islets were then washed and incubated for 1 h (cells) or 25 min (islets) in KRBH containing no bicarbonate, 2 (cells) or 3 mmol/L (islets) glucose, and 0.07% BSA (KRBH-XF) at 37°C under atmospheric CO_2 concentrations. After basal measurement for 20 min, pioglitazone or DMSO was added by three successive automatic injections of KRBH-XF to reach 25 (cells) or 50 μmol/L (islets) pioglitazone or equivalent DMSO volume. After a period of preincubation (20 min for cells and 1 h for islets), glucose was added by a fourth injection of medium to reach 8 or 25 mmol/L. OCR was measured for another 45 min, after which media were collected to measure accumulated insulin secretion over the time of the total incubation (~150 min).

Testing of PPARγ agonists on insulin secretion in INS832/13 cells and isolated rat islets were performed as previously described for pioglitazone (9).

Statistical analysis. Statistical analysis was performed using Prism version 5.01 and InStat version 3.06 (GraphPad Software, San Diego, CA) with two-tailed unpaired Student test or, for multiple comparisons, Kruskal-Wallis test followed by Dunn post hoc test, one-way ANOVA followed by Tukey post hoc test, two-way repeated-measures ANOVA, or two-way ANOVA followed by Bonferroni post hoc tests. Differences between groups were considered significant at $P < 0.05$.

RESULTS

Pioglitazone acutely inhibits glucose-induced insulin secretion. We assessed the acute effect of pioglitazone on in vivo insulin secretion using HGC in Wistar rats (Fig. 1*A*). Pioglitazone-treated animals showed lower insulinemia starting from the 75th min and throughout the second step of the clamp (Fig. 1*C*). During the latter, insulin levels in pioglitazone-treated rats were only ~50% of those of the vehicle-treated rats. The effect of the arginine bolus on insulinemia was not significantly changed by pioglitazone treatment (Fig. 1*E*). Similarly, plasma C-peptide was markedly reduced during the HGC (Fig. 1*D*) but not in response to arginine (Fig. 1*E*). This indicates an acute in vivo inhibitory effect of pioglitazone on GIIS. The glucose infusion rate during the HGC was the same in both groups (Fig. 1*B*).

The C-peptide/insulin molar ratio, an index of insulin clearance (19), was slightly elevated in pioglitazone-treated animals in comparison with control rats in the second step of the clamp (Fig. 1*F*), suggesting that increased insulin clearance could contribute to the lowered insulinemia observed in the pioglitazone-treated animals.

Islets from pioglitazone-treated and control animals were isolated 90 min after gavage and GIIS was assessed ex vivo after isolation. GIIS was unaltered by previous in vivo treatment with pioglitazone (data not shown). This lack of pioglitazone effect on ex vivo GIIS could be attributable to efflux of the drug from islets during their isolation, preincubation, and incubation periods (~5 h altogether).

Acute pioglitazone administration has minimal effect on in vivo insulin sensitivity. TZDs enhance insulin sensitivity with long-term treatment (20). We therefore performed hyperinsulinemic-euglycemic clamp to measure insulin sensitivity in vivo in Wistar rats. Both control and pioglitazone-treated groups required similar rates of glucose infusion (Fig. 2*B*) to maintain euglycemia (Fig. 2*A*). However, the achieved insulinemia during the 60-min clamp period was slightly lower in the acute pioglitazone-treated rats (Fig. 2*C*) such that the glucose infusion rate over insulinemia ratio, known as the M/I index of insulin sensitivity, trended higher in the pioglitazone group, although the difference was not significant (Fig. 2*D*). The mildly reduced insulinemia achieved in pioglitazone-treated rats (at 90 and 120 min) (Fig. 2*C*) occurred despite equivalent rates of insulin infusion. This could be attributable to increased insulin clearance in the pioglitazone treated Wistar rats (Fig. 1*D*).

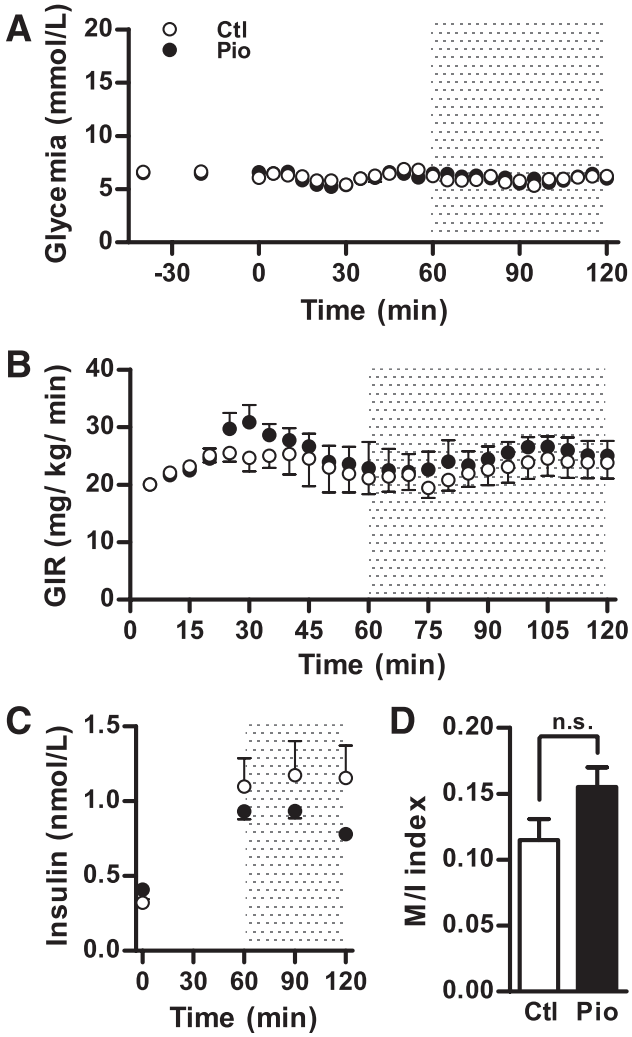

FIG. 2. Pioglitazone administration does not acutely change insulin sensitivity in Wistar rats. Hyperinsulinemic-euglycemic clamps were performed in animals 45 min after pioglitazone (black circles or bar; 30 mg/kg) or vehicle (control; white circles or bar) administration by gavage. Glycemia was clamped at 6.5 mmol/L. *A*: Plasma glucose values during the procedures. *B*: Glucose infusion rate (GIR) during the procedures. *C*: Total (human plus endogenous) plasma insulin measured from samples collected at the indicated time points. *D*: Insulin sensitivity expressed as GIR (M) over total plasma insulin (I). Unpaired *t* test was not significant vs. vehicle-treated group. Data expressed as means ± SEM, *n* = 5–6. Pio, pioglitazone; Ctl, control; n.s., not significant.

Pioglitazone acutely inhibits glucose-induced insulin secretion in ZDF rats. With the objective to determine if pioglitazone can also reduce GIIS in an animal model of type 2 diabetes, we performed HGC in young obese ZDF rats at their transition phase to diabetes (Fig. 3*A*). At 10 weeks of age, ZDF rats show mild hyperglycemia, insulin resistance, and hyperinsulinemia. The glucose infusion rate was the same in both the pioglitazone and control groups

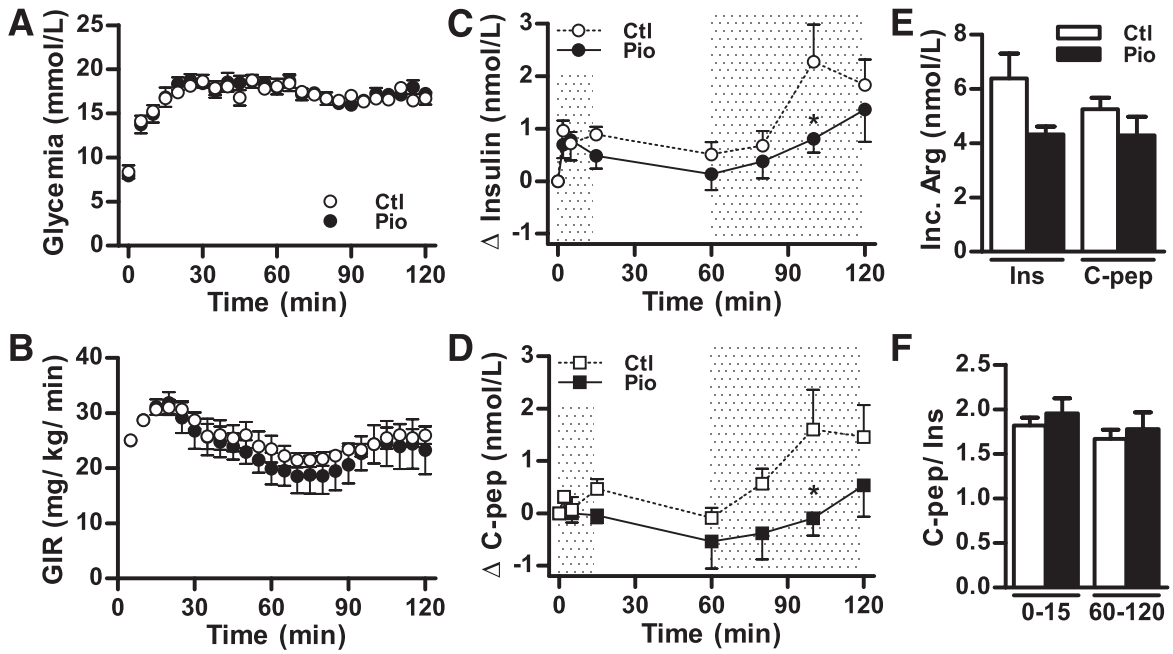

FIG. 3. Pioglitazone administration acutely inhibits glucose-induced insulin secretion in ZDF rats. One-step HGCs were performed in rats 45 min after pioglitazone (black symbols or bars; 30 mg/kg) or vehicle (white symbols or bars) administration by gavage. Glycemia was clamped at 16.7 mmol/L and an arginine bolus (174 mg/kg) was administered at min 120, after last sampling. Plasma glucose values (*A*), glucose infusion rate (GIR) (*B*), increase in plasma insulin (*C*), and C-peptide (*D*) concentrations were above baseline during the procedures. Basal insulin concentrations were 1.8 ± 0.2 and 2.1 ± 0.5 nmol/L for control and pioglitazone groups, respectively. Basal C-peptide concentrations were 4.0 ± 0.4 nmol/L and 4.4 ± 0.9 nmol/L for control and pioglitazone groups, respectively. Two-way repeated-measures ANOVA post hoc analyses: *$P < 0.05$ vs. vehicle-treated group. *E*: Increment in plasma insulin and C-peptide concentrations 1 min after arginine administration. *F*: Insulin clearance estimated via C-peptide over insulin molar ratio for first (0–15 min) and second phases (60–120 min) of insulin secretion. Data expressed as means ± SEM, $n = 6$ animals. Ctl, control; Pio, pioglitazone; Arg, arginine; Inc, increment; C-pep, C-peptide; Ins, insulin.

(Fig. 3*B*), suggestive of similar insulin sensitivity. Acute pioglitazone treatment in ZDF rats reduced glucose-induced increase in insulinemia and almost abolished glucose-induced C-peptide secretion (Fig. 3*C*, *D*). The increase in plasma insulin and C-peptide levels after arginine bolus were not significantly changed by pioglitazone (Fig. 3*E*). Insulin clearance was not changed in pioglitazone-treated ZDF rats (Fig. 3*F*).

Pioglitazone administration acutely reduces whole-body energy expenditure. In our previous study using isolated islets and INS832/13 β cells, we hypothesized that pioglitazone-induced inhibition of GIIS could be attributable to metabolic deceleration of the β-cell (9). We therefore assessed if acute reduction in whole-body energy metabolism occurs in vivo in response to acute pioglitazone administration.

A first approach was to use indirect calorimetry. Energy expenditure (Fig. 4*C*), calculated from VO_2 (Fig. 4*A*) and VCO_2 (Fig. 4*B*) values, was elevated after restraining and gavaging the animals, and slowly decreased and became stable within 2 h. Starting from 1 h after gavage to 5 h after gavage, energy expenditure was significantly reduced in pioglitazone-treated animals by ~10%. During this time period, there was no difference in the respiratory exchange ratio calculated as VCO_2/VO_2 (Fig. 4*D*), indicating a lack of acute impact by pioglitazone administration on fuel selection. Reduced energy expenditure could not be attributed to reduced ambulatory activity (Fig. 4*E*).

Biotelemetry determinations showed that pioglitazone does not acutely change body temperature (Fig. 5*A*, *D*), heart rate (Fig. 5*B*, *E*), and blood pressure (Fig. 5*C*, *F*) during the 4 h immediately after gavage.

On a longer time scale, a single administration of pioglitazone caused a small increase in heart rate (Fig. 5*B*), an effect that becomes statistically significant 4 h after gavage and is sustained for at least 24 h (Supplementary Fig. 1*B*). Blood pressure was also significantly altered on this time scale, with a reduction 24 h after gavage (Supplementary Fig. 1*C*). However, core body temperature was not changed (Supplementary Fig. 1*A*).

Pioglitazone reduces OCR in vitro. Energy metabolism cannot currently be measured in β-cells in vivo. To complement the in vivo experiments on whole-body energy metabolism with in vitro work in β-cells, oxygen consumption was measured in INS832/13 cells and isolated rat islets. In control conditions, an increase in glucose from basal to 8 mmol/L and to 25 mmol/L elicited a similar rapid increase in OCR (Fig. 6*A*, *C*). When pioglitazone was added, the increase in OCR induced by 8 mmol/L glucose was reduced by ~60% in cells and in isolated rat islets. The inhibitory effect of pioglitazone on OCR was partially alleviated at 25 mmol/L glucose, in accordance with the concept of metabolic deceleration. Insulin secreted during these measurements followed the same pattern (Fig. 6*B*, *D*), with the highest glucose concentration relieving, in part, the pioglitazone inhibitory effect.

Pioglitazone effect on insulin secretion is PPARγ independent. Because the effects of pioglitazone in vivo and in vitro in this series of experiments are acute (<4 h after drug), it is unlikely that they are mediated via PPARγ and gene expression changes. In an attempt to further substantiate that the acute effects of pioglitazone on insulin secretion are PPARγ independent, two structurally unrelated non-TZD PPARγ agonists were tested. Treatment of INS832/13 cells and isolated rat islets with GW1929 and

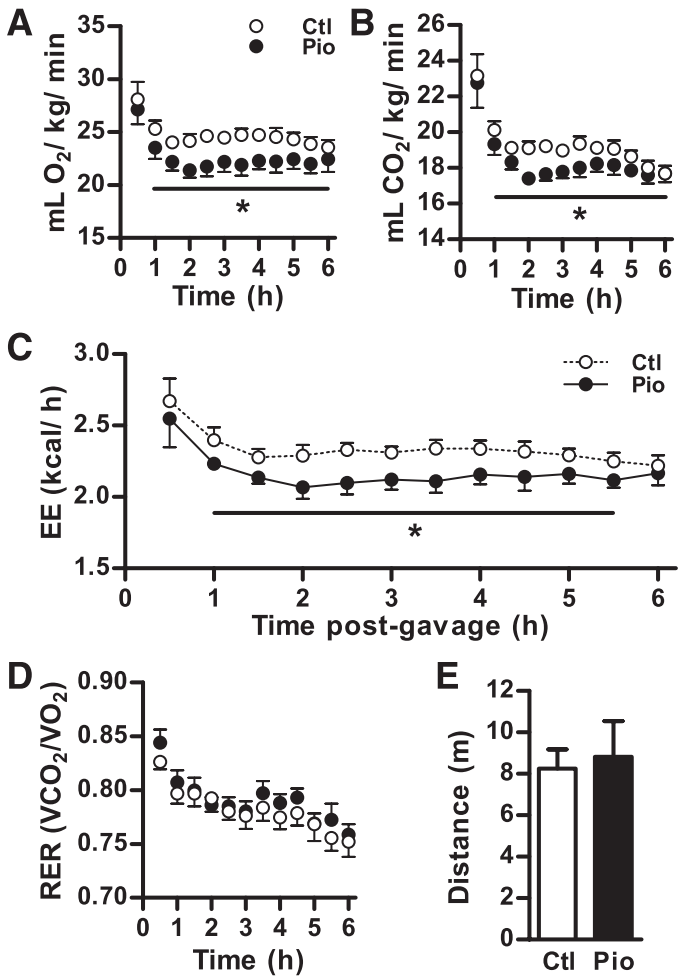

FIG. 4. Pioglitazone administration acutely reduces whole-body energy expenditure in Wistar rats. Pioglitazone (black circles or bar; 30 mg/kg) or vehicle (white circles or bar) was administered 2 h after food withdrawal and data were collected during the next 6 h. *A*: Oxygen consumption (VO_2). *B*: Carbon dioxide production (VCO_2). *C*: Energy expenditure (EE) calculated from VO_2 and VCO_2. *D*: Respiratory exchange ratio (RER) measured as VCO_2/VO_2. *E*: Total horizontal and vertical distances traveled by the animals during the 6-h air sampling. Data expressed as means ± SEM, n = 6–7 for calorimetry and n = 13 for locomotor activity. Two-way repeated-measures ANOVA: $^*P < 0.05$ vs. vehicle-treated group. Pio, pioglitazone; Ctl, control.

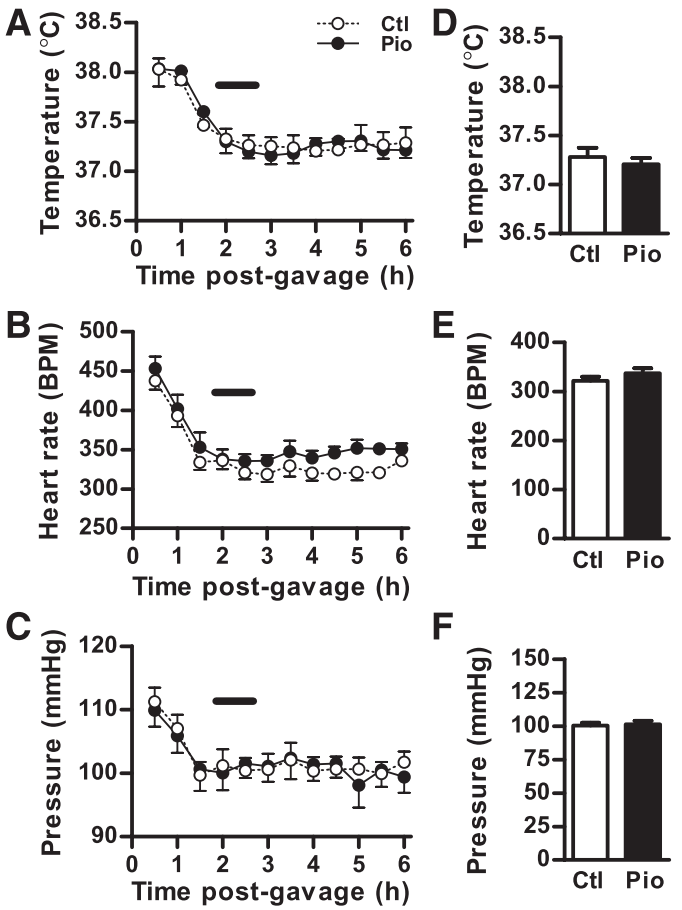

FIG. 5. Short-term assessment of the action of pioglitazone on body temperature, heart rate, and blood pressure in Wistar rats. Core body temperature (*A*), heart rate (*B*), and blood pressure (*C*) were monitored by biotelemetry. Pioglitazone (black circles or bars; 30 mg/kg) or vehicle (white circles or bars) was administered 2 h after food withdrawal. Horizontal bold lines correspond to clamp's last hour (performed with other animals as shown in Figs. 1 and 2). *D–F*: Averaged values of data corresponding to clamp's last hour. Data expressed as means ± SEM, n = 6, in which all animals were used as both treated and control rats alternatively, with 10 days of washout between experiments. Pio, pioglitazone; BPM, beats per minute; Ctl, control.

CAY10599, at a concentration equal to 600 times their half-maximal effective concentration (EC_{50}) for PPARγ transactivation (21,22), did not alter GIIS or KCl-induced insulin release (Fig. 7).

DISCUSSION

Long-term treatment with pioglitazone lowers fasting insulinemia (20). Whether this effect is entirely indirect because of a reduction in insulin resistance or, in part, a consequence of a direct effect on the β-cell is unknown. Here, we report the surprising ability of pioglitazone to acutely inhibit GIIS in vivo before any significant change in insulin sensitivity. Besides inhibiting insulin secretion, pioglitazone also caused a small increase in insulin clearance in healthy Wistar rats but not in ZDF rats. The results provide evidence for a novel acute TZD effect to lower insulinemia, which is not attributable to changes in insulin sensitivity or PPARγ activation. The development of new drugs for treating type 2 diabetes focuses on potential agents to either enhance insulin secretion or reduce insulin resistance. However, here we show an effective antidiabetic agent reducing insulin secretion, a finding of particular interest considering the view that hyperinsulinemia may in some instances drive obesity, insulin resistance, and type 2 diabetes (1,3). Thus, the possibility of a contribution to the beneficial effect of TZDs (23) and metformin (24) on glucose homeostasis via a direct action on the β-cell to reduce insulinemia might have been overlooked.

What evidence indicates that pioglitazone acutely reduces GIIS in vivo? During the HGC, the glucose-induced increase in insulinemia was reduced by 50% in Wistar rats by pioglitazone and by 65% in the hyperinsulinemic ZDF rats. This was quite remarkable considering that pioglitazone was administered as a single oral dose only 135 min earlier. The fact that both insulin and C-peptide levels were reduced is consistent with this effect being a consequence of reduced insulin secretion.

During HGC in both Wistar and ZDF rats, the glucose infusion rate needed to reach and maintain hyperglycemia trended slightly lower in pioglitazone-treated animals, an observation compatible with lower insulin secretion but

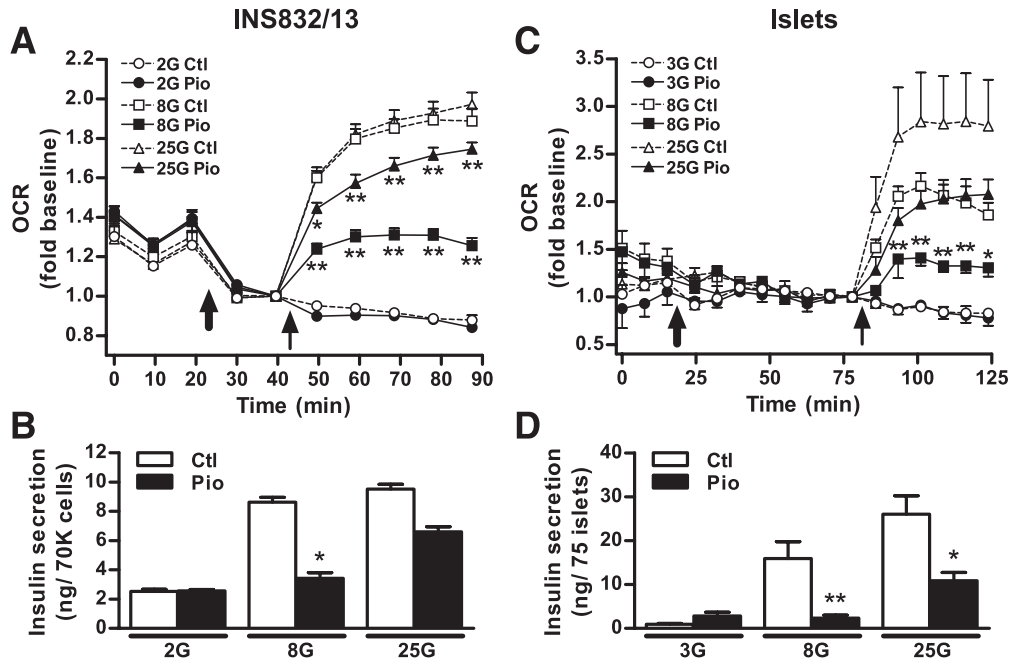

FIG. 6. Pioglitazone reduces OCR in INS832/13 cells and isolated rat islets. *A* and *C*: OCR presented as fold change over the last measurement before glucose addition. Baseline OCRs were, in cells, 174 ± 6 pmol/min and 166 ± 6 pmol/min for control and pioglitazone conditions, respectively, and, in islets, 42 ± 4 pmol/min and 40 ± 5 pmol/min for control and pioglitazone conditions, respectively. Pioglitazone (black symbols or bars; 30 mg/kg) or vehicle (white symbols or bars) was added after basal measurements at the time indicated by the bold vertical arrow, and glucose (2 or 3 mmol/L [2G/3G] = circles; 8 mmol/L [8G] = squares; 25 mmol/L [25G] = triangles) was added after preincubation at the time indicated by the thin vertical arrow. Two-way repeated-measures ANOVA post hoc analyses: *$P < 0.05$, **$P < 0.01$ vs. vehicle-treated wells at same glucose concentration. *B*: Insulin secreted during OCR measurement in cells. Kruskal-Wallis post hoc analyses: *$P < 0.05$ vs. vehicle-treated cells at same glucose concentration. *D*: Insulin secreted during OCR measurement in islets. One-way ANOVA post hoc analyses: *$P < 0.05$, **$P < 0.01$ vs. vehicle-treated islets at same glucose concentration. Data expressed as means ± SEM, $n = 8$ (*A*, *B*; 8 different wells in 2 separate experiments, except for 2 mmol/L glucose conditions where $n = 6$) and $n = 6$–8 (*C*, *D*; 6–8 different wells in 2 separate experiments, except for 3 mmol/L glucose conditions, where $n = 5$). OCR, oxygen consumption rate; Ctl, control; Pio, pioglitazone.

not with improved insulin sensitivity. After acute oral pioglitazone dosing, insulin sensitivity was not significantly altered in Wistar rats, as directly assessed by hyperinsulinemic-euglycemic clamp, and unchanged in ZDF rats, as indirectly shown by similar glucose infusion rates during the HGC with unaltered insulin clearance. In Wistar rats undergoing hyperinsulinemic-euglycemic clamp, there was, however, a trend for a slightly lower insulinemia in the pioglitazone-treated animals, which could have been the result of increased insulin clearance or reduced endogenous insulin secretion. An effect on clearance is supported by the fact that human insulin (measured with a specific antibody) infused at identical rates in both groups also tended to be lower with pioglitazone treatment (not shown). Considering the lower insulin levels in Wistar rats, insulin sensitivity according to the glucose infusion rate over insulinemia ratio was slightly higher in the pioglitazone group, although this difference was not significant. This should not acutely affect the response of pancreatic islets to hyperglycemia and, therefore, the interpretation that pioglitazone has an acute direct effect to reduce in vivo insulin secretion.

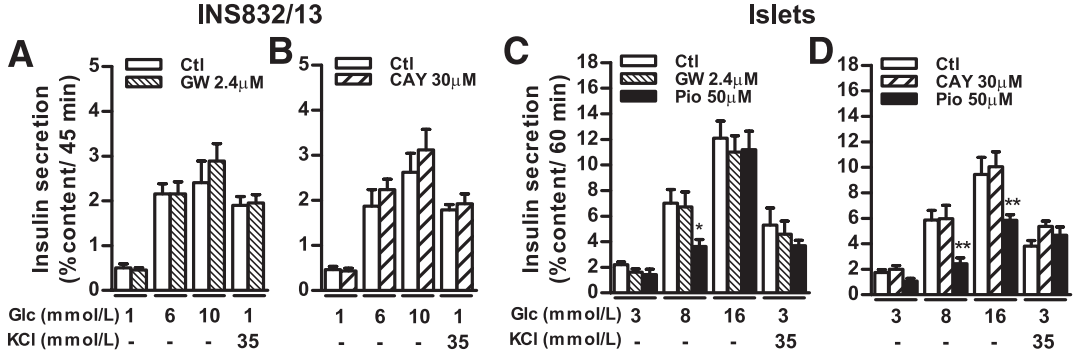

FIG. 7. Lack of effect on insulin secretion of non-TZD PPARγ agonists in INS832/13 cells and isolated rat islets. Cells (*A*, *B*) or islets (*C*, *D*) were incubated in the presence of the indicated glucose concentrations (Glc) and either a non-TZD PPARγ agonist (hatched bars; *A* and *C*, GW1929 [GW]; *B* and *D*, CAY10599 [CAY]) at a concentration equal to 600 times its EC_{50} for PPARγ transactivation, pioglitazone (black bars), or vehicle (white bars). Data were expressed as means ± SEM, $n = 9$ (*A*, *B*; nine different wells in three separate experiments), $n = 8$ (*C*; eight different wells in two separate experiments), and $n = 12$ (*D*; 12 different wells in three separate experiments). Two-way ANOVA post hoc analyses: *$P < 0.05$, **$P < 0.01$ vs. vehicle-treated wells at same glucose concentration. Pio, pioglitazone; Ctl, control.

Long-term and acute TZD treatments increase insulin clearance in humans (25–27). A rapid TZD effect on insulin clearance has been reported by Farret et al. (26) in healthy volunteers undergoing HGC after single-dose rosiglitazone administration. However, the authors could not measure significant changes in C-peptide level in their subjects. A careful look at the C-peptide figure does show a slightly lower (15%) C-peptide level in the rosiglitazone group compared with the control group, suggesting a modest effect of acute rosiglitazone treatment on GIIS in humans. It is also interesting to note that intraperitoneal administration of troglitazone increased AMPK activity in rat liver, muscle, and adipose tissue within 15 min (28), showing an acute effect of TZD in vivo.

The current study was performed with a reasonable dose of pioglitazone for rodents (30 mg/kg body weight). Experiments in rodents with long-term daily administration have been performed by others with pioglitazone doses between 2.3 (29) and 35 mg/kg (30), with most studies using 10 mg/kg. It represents ~50 times what a patient weighing 70 kg would receive with a 45-mg pill. Although it seems disproportionate, TZDs are cleared ~10 times faster by rats when compared with humans because of higher CYP2C expression (31). In addition, our experiments were performed with a single administration of pioglitazone.

What is the biochemical basis of pioglitazone action to reduce GIIS in vivo? Because our previous in vitro results (9) indicated that the reduced GIIS by pioglitazone was related to reduced metabolic activation and ATP production in the β-cells, we examined the possibility that this holds true in vivo. Thus, we assessed the effect of pioglitazone on whole-body energy expenditure. It was found that pioglitazone acutely reduces whole-body energy metabolism, as assessed by oxygen consumption and CO_2 production, without changing fuel selection, as indicated by determination of the respiratory exchange ratio. Pioglitazone did not change rat body temperature or ambulatory activity. The fact that body temperature did not decrease does not preclude an effect of pioglitazone to reduce body heat production, because homeostatic mechanisms could result in improved conservation of body heat.

The demonstration of reduced whole-body energy metabolism fits with the view that pioglitazone may reduce β-cell energy metabolism as well, and may cause its metabolic deceleration in vivo. In accordance with this possibility, results obtained with INS832/13 β-cells and isolated rat islets showed that β-cell oxygen consumption is rapidly reduced by pioglitazone, most efficiently at intermediate glucose concentrations. Similar reduction in oxygen consumption is likely to occur in vivo in the main organs.

What is the significance of this study to our understanding of the mode of action of the main insulin action sensitizers, TZDs and biguanides? First, these compounds also may be direct "insulin secretion decelerators." Second, they may act by being mild restrictors of energy production. Thus, in our previous work (9), we suggested metabolic deceleration as a possible mode of action of pioglitazone characterized by lack of effect at low fuel levels, metabolic inhibition at submaximal substrate concentrations, and the relief of inhibition in the presence of elevated fuel levels.

Interestingly, metformin appears to act on liver gluconeogenesis via reducing the hepatic energy state independently of AMPK (32,33). We also found that metformin, similar to pioglitazone, acutely reduces insulin secretion (9), glucose oxidation, and oxygen consumption, and counteracts the hyperpolarization of the mitochondrial membrane, most efficiently at intermediate glucose concentrations (J.L. and M.P., unpublished data). Thus, our previous results in vitro suggested the possibility that pioglitazone and metformin share a common mode of action by restricting energy production (9). Moreover, certain aspects of this action resemble those of an acute exercise bout in muscle (reduction in ATP, increase in AMP and AMPK activation) (34). Therefore, interventions that cause a mild decrease in cellular energy status may be useful approaches to improve glucose homeostasis in type 2 diabetes by simultaneously and directly increasing insulin sensitivity and reducing hyperinsulinemia. We speculate that this dual action of these drugs that tackle the two characteristic features of type 2 diabetes is the reason why they are so efficient. Reduction of energy status at both cellular and whole-body levels and the pharmacological agents that lead to such effects are of great interest, not only for type 2 diabetes but also for cancer (35), myocardial infarction (36), and life span (37).

In conclusion, we propose that reduction of β-cell energy metabolism helps by protecting the cells from fuel surfeit that causes β-cell dysfunction and possibly death via several factors, including mitochondrial dysfunction, reactive oxygen species production, endoplasmic reticulum stress, and overstimulation of fuel-induced insulin secretion leading to the depletion of insulin stores (38). We report that pioglitazone exerts a direct action on the β-cell to reduce insulin secretion most likely by causing mild metabolic restriction/deceleration. Thus, pharmacologically induced metabolic deceleration represents an attractive avenue to prevent β-cell loss attributable to their exhaustion, to reduce insulin resistance, and to treat type 2 diabetes.

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J.L. performed experiments, contributed to discussions, wrote the manuscript, and reviewed and edited the manuscript. É.J.-A. performed experiments. E.P. performed experiments and reviewed and edited the manuscript. M.-L.P., N.B.R., C.J.N., and E.J. contributed to discussion and edited the manuscript. S.R.M.M. contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. V.P. contributed to discussion and reviewed and edited the manuscript. M.P. contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. M.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. Corkey BE. Banting lecture 2011: hyperinsulinemia: cause or consequence? Diabetes 2012;61:4–13
2. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006;116:1802–1812
3. Shanik MH, Xu Y, Skrha J, Dankner R, Zick Y, Roth J. Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? Diabetes Care 2008;31(Suppl 2):S262–S268
4. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. Lancet 2011;378:169–181
5. Nyenwe EA, Jerkins TW, Umpierrez GE, Kitabchi AE. Management of type 2 diabetes: evolving strategies for the treatment of patients with type 2 diabetes. Metabolism 2011;60:1–23
6. Ye JM, Dzamko N, Cleasby ME, et al. Direct demonstration of lipid sequestration as a mechanism by which rosiglitazone prevents fatty-acid-induced insulin resistance in the rat: comparison with metformin. Diabetologia 2004;47:1306–1313
7. Semple RK, Chatterjee VK, O'Rahilly S. PPAR gamma and human metabolic disease. J Clin Invest 2006;116:581–589
8. Gupta D, Kono T, Evans-Molina C. The role of peroxisome proliferator-activated receptor γ in pancreatic β cell function and survival: therapeutic implications for the treatment of type 2 diabetes mellitus. Diabetes Obes Metab 2010;12:1036–1047
9. Lamontagne J, Pepin E, Peyot ML, et al. Pioglitazone acutely reduces insulin secretion and causes metabolic deceleration of the pancreatic beta-cell at submaximal glucose concentrations. Endocrinology 2009;150:3465–3474
10. Brunmair B, Staniek K, Gras F, et al. Thiazolidinediones, like metformin, inhibit respiratory complex I: a common mechanism contributing to their antidiabetic actions? Diabetes 2004;53:1052–1059
11. Andrikopoulos S. Obesity and type 2 diabetes: slow down!—Can metabolic deceleration protect the islet beta cell from excess nutrient-induced damage? Mol Cell Endocrinol 2010;316:140–146
12. Aston-Mourney K, Proietto J, Morahan G, Andrikopoulos S. Too much of a good thing: why it is bad to stimulate the beta cell to secrete insulin. Diabetologia 2008;51:540–545
13. Hagman DK, Latour MG, Chakrabarti SK, et al. Cyclical and alternating infusions of glucose and intralipid in rats inhibit insulin gene expression and Pdx-1 binding in islets. Diabetes 2008;57:424–431
14. Dutil J, Deng AY. Further chromosomal mapping of a blood pressure QTL in Dahl rats on chromosome 2 using congenic strains. Physiol Genomics 2001;6:3–9
15. Goh TT, Mason TM, Gupta N, et al. Lipid-induced beta-cell dysfunction in vivo in models of progressive beta-cell failure. Am J Physiol Endocrinol Metab 2007;292:E549–E560
16. Makimura H, Mizuno TM, Yang XJ, Silverstein J, Beasley J, Mobbs CV. Cerulenin mimics effects of leptin on metabolic rate, food intake, and body weight independent of the melanocortin system, but unlike leptin, cerulenin fails to block neuroendocrine effects of fasting. Diabetes 2001;50:733–739
17. Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB. Isolation of INS-1-derived cell lines with robust ATP-sensitive K+ channel-dependent and -independent glucose-stimulated insulin secretion. Diabetes 2000;49:424–430
18. Gotoh M, Maki T, Satomi S, et al. Reproducible high yield of rat islets by stationary in vitro digestion following pancreatic ductal or portal venous collagenase injection. Transplantation 1987;43:725–730
19. Uwaifo GI, Parikh SJ, Keil M, Elberg J, Chin J, Yanovski JA. Comparison of insulin sensitivity, clearance, and secretion estimates using euglycemic and hyperglycemic clamps in children. J Clin Endocrinol Metab 2002;87:2899–2905
20. Campbell IW. Long-term glycaemic control with pioglitazone in patients with type 2 diabetes. Int J Clin Pract 2004;58:192–200
21. Henke BR, Blanchard SG, Brackeen MF, et al. N-(2-Benzoylphenyl)-L-tyrosine PPARgamma agonists. 1. Discovery of a novel series of potent antihyperglycemic and antihyperlipidemic agents. J Med Chem 1998;41:5020–5036
22. Lin CH, Peng YH, Coumar MS, et al. Design and structural analysis of novel pharmacophores for potent and selective peroxisome proliferator-activated receptor gamma agonists. J Med Chem 2009;52:2618–2622
23. Campbell IW, Mariz S. Beta-cell preservation with thiazolidinediones. Diabetes Res Clin Pract 2007;76:163–176
24. Patanè G, Piro S, Rabuazzo AM, Anello M, Vigneri R, Purrello F. Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. Diabetes 2000;49:735–740
25. Kim SH, Abbasi F, Chu JW, et al. Rosiglitazone reduces glucose-stimulated insulin secretion rate and increases insulin clearance in nondiabetic, insulin-resistant individuals. Diabetes 2005;54:2447–2452
26. Farret A, Chevassus H, Roux B, Petit P, Galtier F. Direct rosiglitazone-induced modifications in insulin secretion, action and clearance: a single-dose hyperglycaemic clamp study. Diabetologia 2007;50:1384–1387
27. Osei K, Gaillard T, Schuster D. Thiazolidinediones increase hepatic insulin extraction in African Americans with impaired glucose tolerance and type 2 diabetes mellitus. A pilot study of rosiglitazone. Metabolism 2007;56:24–29
28. LeBrasseur NK, Kelly M, Tsao TS, et al. Thiazolidinediones can rapidly activate AMP-activated protein kinase in mammalian tissues. Am J Physiol Endocrinol Metab 2006;291:E175–E181
29. Blalock EM, Phelps JT, Pancani T, et al. Effects of long-term pioglitazone treatment on peripheral and central markers of aging. PLoS ONE 2010;5:e10405
30. Syversen U, Stunes AK, Gustafsson BI, et al. Different skeletal effects of the peroxisome proliferator activated receptor (PPAR)alpha agonist fenofibrate and the PPARgamma agonist pioglitazone. BMC Endocr Disord 2009;9:10
31. Calixto LA, de Oliveira AR, Jabor VA, Bonato PS. In vitro characterization of rosiglitazone metabolites and determination of the kinetic parameters employing rat liver microsomal fraction. Eur J Drug Metab Pharmacokinet 2011;36:159–166
32. Stephenne X, Foretz M, Taleux N, et al. Metformin activates AMP-activated protein kinase in primary human hepatocytes by decreasing cellular energy status. Diabetologia 2011;54:3101–3110
33. Foretz M, Hébrard S, Leclerc J, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. J Clin Invest 2010;120:2355–2369
34. Ruderman NB, Park H, Kaushik VK, et al. AMPK as a metabolic switch in rat muscle, liver and adipose tissue after exercise. Acta Physiol Scand 2003;178:435–442
35. Wei S, Kulp SK, Chen CS. Energy restriction as an antitumor target of thiazolidinediones. J Biol Chem 2010;285:9780–9791
36. Gohil VM, Sheth SA, Nilsson R, et al. Nutrient-sensitized screening for drugs that shift energy metabolism from mitochondrial respiration to glycolysis. Nat Biotechnol 2010;28:249–255
37. Dhahbi JM, Mote PL, Fahy GM, Spindler SR. Identification of potential caloric restriction mimetics by microarray profiling. Physiol Genomics 2005;23:343–350
38. Nolan CJ, Prentki M. The islet beta-cell: fuel responsive and vulnerable. Trends Endocrinol Metab 2008;19:285–291