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FK-614, a selective peroxisome proliferator–activated receptor γ agonist, improves peripheral glucose utilization while decreasing hepatic insulin extraction in alloxan-induced diabetic dogs

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Abstract

This is the first report of the effects of a nonthiazolidinedione activator of peroxisome proliferator–activated receptor (PPAR) γ, that is, FK-614 (a benzimidazole derivative), on glucose metabolism in vivo. To investigate the effect of FK-614 on peripheral and hepatic insulin action, we performed hyperinsulinemic-hyperglycemic clamp studies combined with the triple-catheter technique and a double-tracer approach in alloxan-diabetic dogs with (n = 5) or without (n = 6) treatment with FK-614 (0.32 mg/kg per day orally for 10 days). Throughout the experiment, insulin was infused intraportally at 18 pmol/kg per minute and hyperglycemia (~11 mmol/L) was maintained by a peripheral glucose infusion. After a 45-minute basal period (period I), a portal infusion of glucose labeled with [U-^{14}C]-glucose, was administered for 120 minutes (period II) to measure hepatic glucose uptake. This was followed by 90-minute recovery (period III). FK-614 marginally improved peripheral insulin sensitivity, did not affect hepatic glucose uptake, and surprisingly increased tracer-determined hepatic glucose production (19.0 ± 5.0 vs 10.6 ± 1.7 μmol/kg per minute, $P < .001$). Hepatic insulin extraction was decreased by FK-614 (47.8% ± 1.6% vs 55.9% ± 3.4%, $P < .01$), which led to greater peripheral insulin levels and glucose utilization. FK-614 treatment also decreased the daily insulin requirements (regular insulin, 0.18 ± 0.01 vs 0.32 ± 0.01 U/kg per day; and NPH insulin, 0.53 ± 0.02 vs 0.89 ± 0.04 U/kg per day; $P < .001$) to maintain fasting plasma glucose at approximately 10 mmol/L for 7 days before the experiments. We conclude that FK-614 treatment, at the dose used, improves peripheral glucose utilization because of an improvement in peripheral insulin sensitivity and a decrease in insulin clearance, but impairs hepatic insulin action in alloxan-induced diabetic dogs. The reason for the effects of FK-614 on hepatic glucose and insulin metabolism is unclear but they are both consistent with reports of hepatic steatosis by PPARγ activation when unopposed by concomitant activation of PPARα.

1. Introduction

The peroxisome proliferator–activated receptor (PPAR) γ has been implicated in the regulation of systemic insulin sensitivity. It is also the functional receptor for a class of insulin-sensitizing drugs, the thiazolidinediones (TZDs), now widely used in the treatment of type 2 diabetes mellitus [1,2]. Most of the ligands to PPARγ (pioglitazone, rosiglitazone, JTT-501 [3], MCC-555 [4], KRP-297 [5], NC-2100 [6]) share a similar chemical structure, being either TZD or TZD-like. This common structure seems to be important for their insulin-sensitizing action at both peripheral and hepatic sites. For example, troglitazone increased peripheral glucose uptake and suppressed hepatic glucose production during hyperinsulinemic-euglycemic clamps in patients with type 2 diabetes mellitus [7]. Furthermore, our recent studies showed that JTT-501 increased both peripheral glucose uptake and net hepatic glucose balance (NHGB) during hyperinsulinemic clamps with portal glucose infusion [8].

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FK-614 is a PPARγ agonist with a structure (benzimidazole) that is significantly different from that of TZD. Unlike high doses of TZD, FK-614 has no PPARα-activating effects [9]. In the present study, we evaluated the effects of FK-614 on insulin-mediated glucose flux in the liver and in peripheral tissues. Dogs were used for this study because in dogs it is possible to catheterize the carotid artery, the portal vein, and a hepatic vein to perform arteriovenous balance studies to evaluate hepatic glucose flux and insulin extraction. Experiments were performed in the alloxan-diabetic dog, which is the same model as we had previously used to evaluate the effects of pioglitazone [10] and JTT-501 [8], 2 insulin sensitizers. Thus, we could interpret our results in comparison to those obtained in the previous studies. Although alloxan-diabetic dogs are primarily insulin-deficient, they are also insulin-resistant [11], their insulin resistance being presumably secondary to imperfect control. Furthermore, they have impairment in hepatic glucose uptake (HGU) [10]. The present study is the first report of the effects of FK-614 on glucose metabolism.

2. Methods

2.1. Animals

Experiments were carried out in 6 male mongrel dogs (25-30 kg). The dogs were injected with alloxan 65 mg/kg (Aldrich, Milwaukee, Wis) dissolved at pH 4.4 in 0.1 mol/L acetate buffer to induce diabetes [11]. With this regimen of alloxan administration, borderline elevations of serum alanine aminotransferase can be found in the first few days but disappear 5 days after alloxan administration, whereas plasma creatinine levels are not affected. This was confirmed in our dogs. Approximately 7 days after induction of diabetes, cannulation surgery was carried out. The dogs were fed standard dog chow diet 400 to 450 g (25% protein, 9% fat, 38% carbohydrate; Ralston Purina, Mississauga, Ontario, Canada) and 350g beef (Romar Pet Supply, Toronto, Ontario, Canada) once daily. Subcutaneous injections of a combination of intermediate- (NPH) and short-acting (regular) porcine insulin (Lilly, Indianapolis, Ind) were given once daily at feedings to maintain fasting glucose levels to less than 11 mmol/L. The treatment protocol was a control period of 10 days, to be initiated after approximately 7 days of postsurgery recovery, a treatment period of 10 days and a washout period of 2 weeks. We had previously found that a 2-week washout period was sufficient for JTT-501, a drug with similar pharmacokinetics to FK-614 [8]. After oral administration in dogs, the half-life of FK-614 and its metabolites in plasma is less than 24 hours (S Takakura, Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan, unpublished data). The dogs were randomly assigned to 2 groups, one of which was to receive the control experiment before the treatment with FK-614, whereas the other group was to receive the control experiment after the washout period. Two dogs received the control experiment before the treatment experiment, whereas 3 received the control experiment after the washout period. Our statistical analysis confirmed that the order of experiments had no significant effect on any of the results. One additional dog received only 1 control experiment because of catheter failure before the FK-614 treatment experiment. Therefore, there were n = 5 in the FK-614 treatment group and n = 6 in the control group. FK-614 treatment was given orally with the morning feeding at a dose of 0.32 mg/kg per day. A comparable dose given for 14 days decreased blood glucose levels in *db*/*db* mice and improved oral glucose tolerance in *ob*/*ob* mice [9].

2.2. Surgical procedures

All procedures were in accordance with the Canadian Council on Animal Care Standards and were approved by the Animal Care Committee of the University of Toronto. Laparotomy and vessel cannulation were performed under general anesthesia, induced with thiamylal sodium and maintained with nitrous oxide, isoflurane, and assisted ventilation. Silastic catheters (Dow Corning, Midland, Mich) were inserted into the carotid artery, portal vein, and left common hepatic vein for sampling and the jugular, jejunal, and splenic veins for infusion [12]. The tips of the jejunal and splenic vein catheters were placed at 2 cm proximal to the point of the first vessel bifurcation. The tip of the portal vein catheter was placed at 2 cm distal to the point at which the vessel enters the liver, and the tip of the hepatic vein catheter was placed at 1 cm inside the left common hepatic vein. The gastrointestinal vein was ligated, and Doppler flow probes (Transonic System, Ithaca, NY) were positioned around the portal vein and the common hepatic artery. After insertion, the catheters were filled with heparin solution (100 IU/mL Hepalean; Organon Canada, Toronto, Ontario, Canada), and their free ends were knotted. The free ends of the catheters and the leads to the flow probes were then placed in subcutaneous pockets at the back of the neck through a subcutaneous tunnel to allow complete closure of the incisions.

2.3. Experimental protocol

On the day before the experiment, the free ends of the catheter and flow probe leads were exteriorized from the subcutaneous pockets through small incisions made under local anesthesia (1% lidocaine). The contents of each catheter were aspirated, and the catheters were flushed with saline and filled with heparin solution (1000 U/mL). The dogs were fasted 24 hours before the experiments. The last dose of FK-614 (0.32 mg/kg per day) was given orally 2 hours before the experiments without any food. A hyperinsulinemic-hyperglycemic clamp combined with a double-tracer technique [13] and an intraportal glucose load was used to determine peripheral glucose uptake, HGU, and NHGB in the conscious alloxan-diabetic dogs. Before insulin infusion, blood samples were taken to determine fasting parameters. Arterial blood pressure was determined by a pressure transducer connected to the sampling catheter of the carotid artery and recorded on a physiograph.

Each experiment consisted of a 120-minute tracer equilibration period (from −165 to −45 minutes), a 45-minute basal sampling period (from −45 to 0 minute, period I), a 120-minute portal glucose infusion period (0-120 minutes, period II), and a 90-minute recovery period (120-210 minutes, period III). Throughout the experiments, insulin was infused portally at a constant rate (18 pmol/kg per minute) via the jejunal and splenic veins, whereas exogenous glucose (25% dextrose) was administered peripherally via the jugular vein to maintain moderate hyperglycemia (~10 mmol/L). Potassium chloride (20 μEq/mL) was mixed with peripheral glucose infusate to prevent insulin-induced hypokalemia. To determine the glucose turnover rate [3-^{3}H]-glucose (Du Pont–New England Nuclear, Lachine, Quebec, Canada) was given as a primed constant infusion ($148 \times 10^{10} + 0.629 \times 10^{10}$ μBq/min) throughout the experiment. [3-^{3}H]-glucose (1.48×10^{10} μBq/mL) was also added to the peripheral glucose infusate to minimize the changes in plasma glucose specific activity induced by variations of the glucose infusion rate. After a 45-minute basal period, portal glucose infusion (25 % dextrose, 22.2 μmol/kg per minute), which contained 370×10^{10} μBq (1.258×10^{10} μBq/kg per minute) of [U-^{14}C]-glucose (Du Pont–New England Nuclear) was administered for 120 minutes. During portal glucose infusion, a fraction of the portal glucose load that is not extracted by the liver enters the systemic circulation and reduces peripheral glucose infusion requirements to maintain the peripheral hyperglycemic clamp. *p*-Aminohippuric acid (PAH) was given intraportally with glucose to estimate glucose mixing with portal blood [14]. During period III, peripheral glucose infusion alone was given to maintain the hyperglycemic clamp. Arterial glucose levels were determined at 5-minute intervals, and the peripheral glucose infusion rate was adjusted accordingly to maintain steady arterial hyperglycemia throughout the experiments [15]. Blood samples were drawn at 15-minute intervals from the carotid artery to determine glucose turnover and first-pass HGU, and from the portal vein and hepatic vein cannulas to determine NHGB. During the last 30 minutes of all periods, samples were taken from all sampling lines for hepatic extraction of substrates and insulin. The arterial and portal vein blood samples were collected simultaneously, and hepatic vein samples were taken with a 30-second delay to compensate for transit time through the liver [14]. The amount of blood withdrawn in each experiment was approximately 120 mL. Portal and hepatic artery blood flow was determined every 15 minutes using Doppler flow meters [14].

2.4. Laboratory methods

Plasma glucose concentrations were measured by glucose oxidase method using a Glucose Analyzer II (Beckman, Fullerton, Calif). Insulin and glucagon were analyzed by radioimmunoassays (coefficient of variation, 12% and 15%, respectively). Plasma free fatty acids (FFAs) were determined using a colorimetric kit (Wako Chemicals, Neuss, Germany). Plasma PAH levels were determined spectrophotometrically at 465 nm [14]. To measure the radioactivity of [3-^{3}H]- and [U-^{14}C]-glucose, samples were deproteinized with zinc sulfate and barium hydroxide. The supernatant was evaporated at 50°C, redissolved in water, and counted in Ready Safe scintillation fluid (Beckman) by a liquid scintillation counter. Aliquots of the infused [3-^{3}H]- and [U-^{14}C]-glucose tracer were run along with the plasma samples for the precise determination of the infusion rate of both tracers. Results were expressed as disintegrations per minute per milliliter of plasma after correction for counting efficiency and for spillover of ^{14}C counts into the ^{3}H channel.

2.5. Calculations

To assess the completeness of glucose mixing in the portal blood, PAH (Sigma, St Louis, Mo) was mixed with intraportal glucose infusate and the recovery of PAH in both portal and hepatic vein was determined according to the method of Myers et al [12]. Experiments were consisted valid only when the recovery PAH fell within the acceptable range (100% ± 40%).

The rates of glucose appearance (Ra) and disappearance were calculated with [3-^{3}H]-glucose as tracer using a modified 1-compartment model [16] after data smoothing by the Optimal Segments method [17]. The rate of glucose disappearance corresponded to glucose utilization (GU) because the plasma glucose levels were less than the renal glucose threshold in dogs [18]. In periods I and III, endogenous glucose production (EGP) was calculated as the difference between total Ra and exogenous glucose infusion. In period II, the [U-^{14}C]-glucose data were used to calculate the rate of appearance of portal glucose (RaP). The contribution to the plasma glucose concentration made by the glucose infused portally was estimated by dividing the measured plasma [^{14}C]glucose counts by the ^{14}C-specific

Table 1
Daily requirement of regular and NPH insulin during 7 days before experiments; fasting metabolic variables and arterial pressure on the day of experiment

	Control (n = 6)	FK-614 (n = 5)
Regular insulin (U/kg per d)	0.32 ± 0.01	0.18 ± 0.01*
NPH insulin (U/kg per d)	0.89 ± 0.04	0.53 ± 0.02*
Fasting plasma glucose (mmol/L)	10.6 ± 0.5	10.8 ± 0.4
Insulin (pmol/L)	32 ± 7	26 ± 4
Glucagon (pg/mL)	98 ± 9	85 ± 12
FFAs (μEq/L)	1179 ± 108	980 ± 81
Lactate (μmol/L)	1054 ± 118	1193 ± 163
Glycerol (μmol/L)	55 ± 24	47 ± 15
Triglycerides (mmol/L)	0.39 ± 0.04	0.41 ± 0.03
sBP (mm Hg)	135 ± 9	133 ± 6
dBP (mm Hg)	101 ± 6	98 ± 5
mBP (mm Hg)	112 ± 7	110 ± 6

Values are expressed as mean ± SEM. sBP indicates systolic blood pressure; dBP, diastolic blood pressure; mBP, mean blood pressure.

* $P < .001$ vs control.

activity in the portal infusate [13]. These calculated plasma glucose concentrations represent the glucose profile that would exist if the only source of glucose entry into the body were from the portally infused glucose load. Using these calculated (ie, from the [^{14}C]glucose data) plasma glucose concentration and the measured plasma [3-^{3}H]-glucose counts, the rate of peripheral appearance of portally delivered glucose (RaP) was computed as described previously for total Ra. Tracer-determined first-pass HGU in period II was portal infusion rate minus RaP. Endogenous glucose production in period II was calculated as total Ra minus the exogenous peripheral glucose infusion minus RaP.

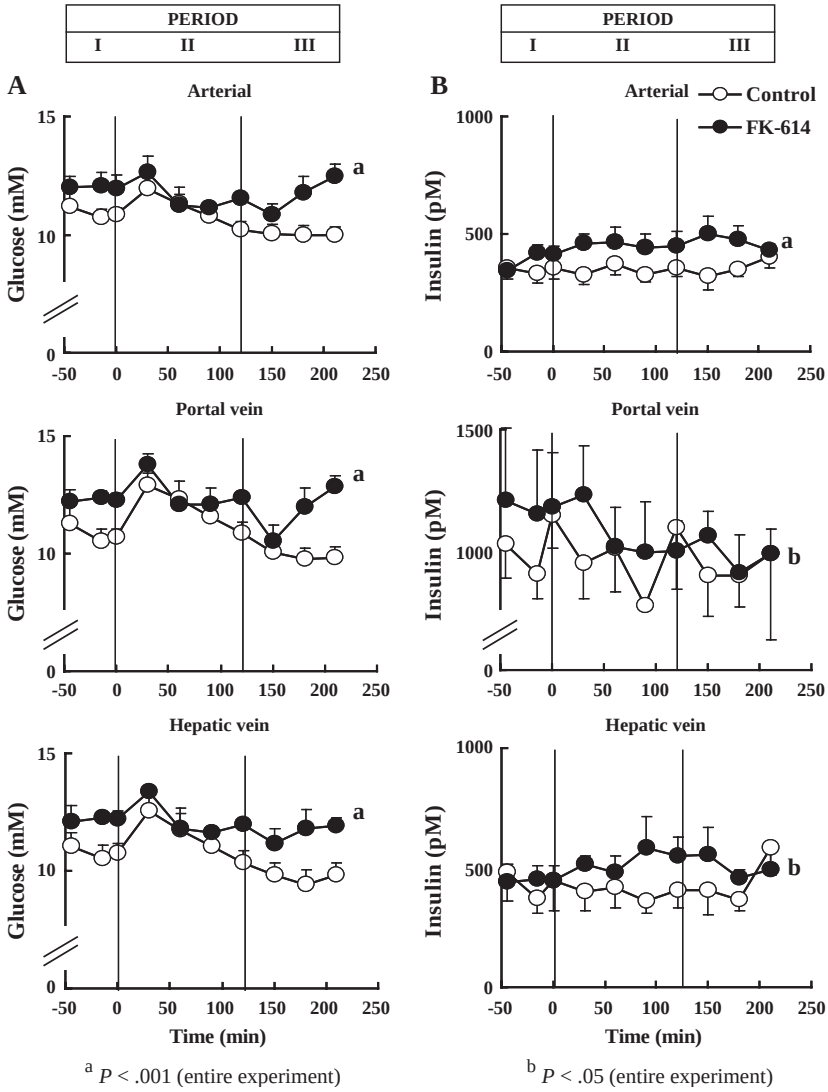
The arterial-venous (A-V) difference method was applied to calculate the NHGB. Hepatic balances of gluconeogenic precursors and of insulin were also determined in these dogs. The load of substrate reaching the liver (Influx) was calculated using the formula

$$\text{Influx} = [A] \times F_A + [P] \times F_P,$$

where $[A]$ and $[P]$ are the arterial and portal vein plasma concentration of the substrate/hormone, and F_A and F_P are the plasma flow of hepatic artery and hepatic vein, respectively. Plasma flow was determined from blood flow (measured by flow probes) multiplied by (1 − hematocrit). The amount of substrate/hormone leaving the liver (efflux) was calculated by the formula

$$\text{Efflux} = [H] \times \text{HPF},$$

where $[H]$ is hepatic vein plasma concentration of substrate hormone. Hepatic plasma flow (HPF) is $F_A + F_P$. The



[a] $P < .001$ (entire experiment)

[b] $P < .05$ (entire experiment)

Fig. 1. Glucose (A) and insulin (B) levels in the arterial (upper panel), portal vein (middle panel), and hepatic vein (lower panel) plasma of control dogs (n = 6) and dogs treated with FK-614 (n = 5) (0.32 mg/kg per day) for 10 days. Throughout the experiments, insulin was infused intraportally. After a basal period from −45 to 0 minutes (period I), a portal infusion of glucose labeled with [U-^{14}C]-glucose was administered from 0 to 120 minutes (period II) to measure hepatic glucose uptake. This was followed by a recovery period from 120 to 210 minutes (period III). Data are expressed as mean ± SEM.

balances across the liver (net hepatic balance [NHB]) and hepatic extraction ratio (HER, %) of glucose, gluconeogenic precursors, and insulin were determined by the following formulas:

$$\mathrm{NHB} = \mathrm{Influx} - \mathrm{Efflux}$$

$$\mathrm{HER} = \frac{\mathrm{NHB}}{\mathrm{Influx}} \times 100,$$

where positive balance indicates uptake.

Total hepatic glucose uptake (THGU) was calculated, in periods I and III, as

$$\mathrm{THGU} = \mathrm{EGP} + \mathrm{NHGB}.$$

In period II, THGU was calculated as EGP + RaP + NHGB.

2.6. Statistical analysis

The data are expressed as the mean ± SEM and represent the average values for the periods. Two-way analysis of variance for repeated measurements was used for comparisons between treatments and periods.

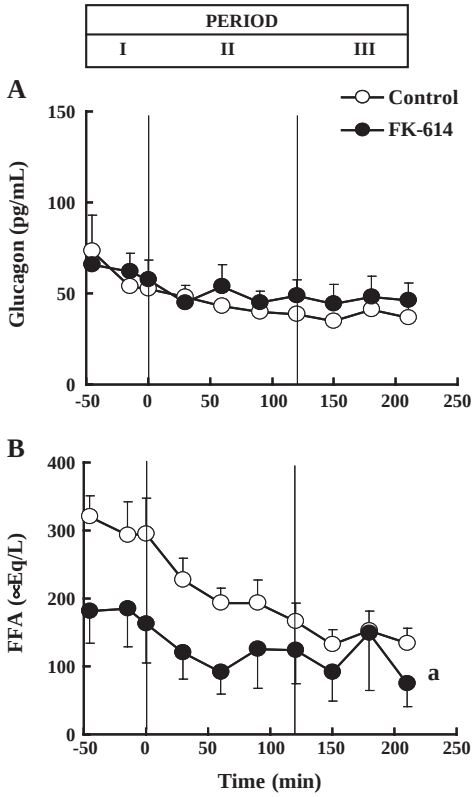
3. Results

3.1. Arterial levels of glucose, insulin, glucagon, and other substrates, and arterial blood pressure in the postabsorptive state; insulin requirements of regular and NPH insulin during 7 days before experiments

FK-614 treatment significantly decreased the daily requirements of both short- (regular) and intermediate-acting (NPH) insulin to maintain similar fasting plasma glucose levels for 7 days before the experiments (Table 1). FK-614 treatment did not significantly affect plasma insulin, glucagon, FFA, lactate, glycerol, and triglyceride levels in the postabsorptive state (Table 1). The systolic, diastolic, and mean arterial blood pressures were not affected by FK-614 (Table 1).

3.2. Plasma glucose, insulin, glucagon, and FFA levels during the hyperinsulinemic-hyperglycemic clamp

Despite our effort to maintain plasma glucose at the basal levels of approximately 11 mmol/L, arterial, portal, and hepatic plasma glucose levels were slightly but significantly higher ($P < .001$) in the FK-614 than in the control treatment group (Fig. 1A) during the hyperinsulinemic-hyperglycemic clamp. Despite the same rate of portal insulin infusion, arterial ($P < .001$), portal, and hepatic (both $P < .05$) insulin levels were also higher with FK-614 treatment than with the control (Fig. 1B). Accordingly, hepatic insulin extraction was lower with FK-614 (47.8% ± 1.6% vs 55.9% ± 3.4%, $P < .01$). In both groups, arterial glucagon and FFA levels were low (ie, suppressed by insulin). Glucagon levels were stable and similar between FK-614 treatment and control (Fig. 2A). However, plasma FFA levels was significantly lower in the FK-614 treatment group ($P < .001$) (Fig. 2B).



[a] $P < .001$ (entire experiment)

Fig. 2. Plasma glucagon (A) and FFA levels (B) during the hyperinsulinemic-hyperglycemic clamp studies. The experimental protocol is described in the legend of Fig. 1. Data are expressed as mean ± SEM.

3.3. Peripheral glucose infusion rate, GU, and EGP

Peripheral glucose infusion rate was significantly higher in the FK-614 vs the control group ($P < .001$) (Fig. 3A). Plasma specific activity of [3-^{3}H]-glucose (not shown) was stable (±20%) throughout the experiments. Tracer-derived GU (Fig. 3B) was also significantly higher with FK-614 vs control ($P < .001$). However, when normalized by the glucose and insulin levels, GU remained higher in the FK-614 group only in period I (Fig. 3C), suggesting that the FK-614–induced improvement in peripheral insulin sensitivity was marginal. Both peripheral glucose infusion rate and GU were higher ($P < .001$) during period III than during period II or I. Peripheral glucose infusion rate was also higher ($P < .001$) in period II than in I. Endogenous glucose production was not fully suppressed in any group during any period (Fig. 3D). Unexpectedly, EGP was higher in the FK-614 than in the control group ($P < .001$). As expected because of the portal glucose infusion, EGP was lower in period II than in period I ($P < .001$) or period III ($P < .05$).

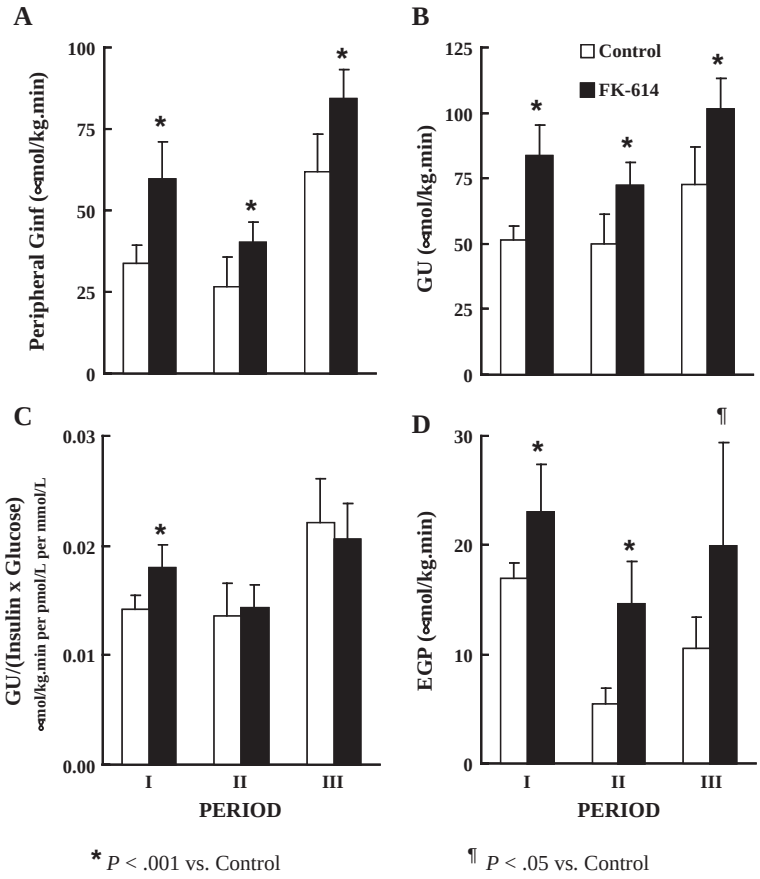


Fig. 3. Peripheral glucose infusion rate (A), GU (B), GU normalized by glucose and insulin (C), and EGP (D) during the hyperinsulinemic-hyperglycemic clamp studies. The experimental protocol is described in the legend of Fig. 1. Significance levels between periods are reported in the text. Data are expressed as mean ± SEM and represent the average values of each period. Ginf indicates glucose infusion rate; GU, glucose utilization; EGP, endogenous glucose production.

3.4. Net hepatic glucose balance (determined by A-V difference method), THGU, and tracer-determined first-pass HGU

There was no difference in hepatic blood flow between control and FK-614 groups (Table 2). Net hepatic glucose balance determined by the A-V difference method was positive in all periods, indicating net uptake. Despite a greater hepatic glucose load (Table 2) in the FK-614 group, NHGB was not different from the control group (Table 2). Total hepatic glucose uptake (Table 2) was greater in the FK-614 than in the control group ($P < .001$) because of the greater EGP. During period II, tracer-determined first-pass HGU, which is the amount of portally infused glucose taken up by the liver on a first-pass, was comparable between control and FK-614 groups (5.2 ± 1.6 vs 5.4 ± 1.2 μmol/kg per minute, respectively). The large difference between THGU and first-pass HGU indicates that most of the portally infused glucose was taken up by the liver after being recirculated from the periphery.

Table 2
Hepatic blood flow, hepatic glucose load, NHGB, and THGU

	Period 1	Period 2	Period 3
HBF (mL/kg per min)			
Control	24.2 ± 1.6	23.9 ± 1.6	24.8 ± 0.7
FK-614	21.8 ± 0.9	24.4 ± 1.7	22.7 ± 1.9
HGL			
Control	157 ± 9	163 ± 14	149 ± 6
FK-614	163 ± 8	181 ± 13*	170 ± 16*
NHGB			
Control	1.2 ± 0.8	3.2 ± 2.2	3.3 ± 1.0
FK-614	0.8 ± 1.8	3.5 ± 5.7	2.6 ± 0.9
THGU			
Control	18.2 ± 2.0	26.2 ± 5.1	12.7 ± 4.3
FK-614	25.6 ± 5.2**	33.2 ± 7.4**	25.8 ± 12.3**

Values are expressed as mean ± SEM and are in micromoles per kilogram per minute unless otherwise noted. HBF indicates hepatic blood flow; HGL, hepatic glucose load; NHGB, net hepatic glucose balance; THGU, total hepatic glucose uptake.

* $P < .01$ vs control.

** $P < .001$ vs control.

3.5. Arterial levels and hepatic extraction of substrates

During the hyperinsulinemic clamps, arterial levels of lactate and glycine were higher ($P < .05$) with FK-614 than with control, whereas the arterial levels of other gluconeogenic precursors were not different (Table 3). However, there was no significant difference in the hepatic balance of any of the measured gluconeogenic precursors, including lactate (control: 1.18 ± 0.53 vs FK-614: 2.20 ±

Table 3
Arterial levels of lactate, glycerol, and gluconeogenic amino acids

	Period 1	Period 2	Period 3
Lactate			
Control	843 ± 89	749 ± 112	701 ± 102
FK-614	1185 ± 222*	916 ± 179*	798 ± 166
Glycerol			
Control	46 ± 7	53 ± 12	46 ± 11
FK-614	55 ± 10	65 ± 20	41 ± 9
Alanine			
Control	205 ± 19	156 ± 20	149 ± 23
FK-614	218 ± 35	154 ± 25	126 ± 13
Serine			
Control	53 ± 8	46 ± 4	49 ± 6
FK-614	64 ± 7	53 ± 6	48 ± 6
Glycine			
Control	74 ± 10	71 ± 5	76 ± 7
FK-614	96 ± 16	88 ± 12*	81 ± 9
Threonine			
Control	65 ± 7	59 ± 5	70 ± 1
FK-614	77 ± 16	64 ± 13	55 ± 9

Values are expressed as mean ± SEM and are in micromoles per liter.
* $P < .05$ vs control.

0.82 μmol/kg per minute) and glycine (control: 0.43 ± 0.12 vs FK-614: 0.34 ± 0.11 μmol/kg per minute).

4. Discussion

In this study, we demonstrated that a novel non–TZD-like derivative PPARγ agonist, FK-614, increases peripheral GU and unexpectedly also EGP, and decreases hepatic insulin extraction during a hyperinsulinemic clamp. In contrast, both pioglitazone (TZD) [10] and JTT-501 (isoxazolidinedione, ie, a TZD-like compound) [8] decreased EGP and had no effect on hepatic insulin extraction in the same animal model as the present study.

Consistent with our previous studies using pioglitazone and JTT-501, the levels of FFA in the FK-614 treatment groups were lower during the hyperinsulinemic clamp. Slightly lower levels of FFA might have contributed to increased GU; however, the main cause of the greater GU in the FK-614 group was the greater glucose and insulin levels because GU normalized by glucose and insulin was similar between control and FK-614 treatment in periods 2 and 3. Thus, FK-614 had no greater effect on peripheral insulin sensitivity than JTT-501 [8]. Pioglitazone did not increase peripheral insulin sensitivity in a slightly different experimental protocol using the same animal model [10]. In spite the lower level of FFA and higher levels of glucose and insulin, which would be expected to decrease EGP in the FK-614 group, there was an increase in EGP compared with that of controls. Although lactate levels were higher with FK-614 than with control treatment, perhaps reflecting increased peripheral GU, the difference in the hepatic uptake of lactate was not significant (and could theoretically account for only 10% of the observed difference in EGP) and there was no difference in the hepatic uptake of other gluconeogenic precursors. This suggests that the rate of gluconeogenesis may be similar between treatments, unless FK-614 altered hepatic gluconeogenic conversion of precursors. Thus, the increase in EGP may be mainly accounted for by an increase in glycogenolysis, likely due to hepatic insulin resistance. This unexpected increase in EGP was accompanied by a decrease in the hepatic extraction of insulin, indicating that FK-614 affected both insulin action and kinetics in the liver. As discussed previously, the slightly greater glucose levels in the FK-614 group, which likely contributed to the greater GU, would have been expected to decrease rather than increase EGP. It is unlikely that the slight difference in glucose levels affected the hepatic insulin extraction processes because it is still controversial whether even substantial hyperglycemia affects hepatic insulin extraction [19,20]. The reason for the effects of FK-614 on both hepatic insulin action and clearance is not clear, however reductions of both hepatic insulin action [21,22] and clearance [23,24] have been previously linked to increased hepatic fat content. It should be noted that PPARγ activation by TZD has been associated with hepatic steatosis [25-27] and with decreased hepatic insulin sensitivity [27] in some obese and diabetic animal models. To our knowledge, there are no reports about the effect of selective PPARγ activators on hepatic fat content. However, liver-specific knockout of PPARγ was found to decrease hepatic fat content [26,27], whereas liver overexpression of PPARγ increased hepatic fat content in wild type mice and induced frank hepatic steatosis in PPARα-null mice [28]. FK-614 does not activate PPARα unlike high doses of TZD [9]. Indeed, the treatment of lean and *ob*/*ob* mice with TZD increased the expression of hepatic fatty acid translocase (FAT/CD36) which is a gene up-regulated by PPARα [29]. Thus, activation of both PPARα and PPARγ by TZD treatment and activation of PPARγ alone by FK-614, resulting in increased hepatic fat content, may partly explain the difference in hepatic glucose metabolism between FK-614 and TZD or TZD-like PPARγ agonists. Unfortunately, we did not take biopsies to measure tissue fat content in our dogs. Species-specific difference in PPARα and PPARγ expression and activation should be considered with regard to the effect of PPARs in the liver, as TZD, which increased hepatic steatosis in obese diabetic animal models [25,26], improved nonalcoholic steatohepatitis in humans [30]. It is also possible that in our model, hyperglycemia and/or previous alloxanization influenced the hepatic response to selective PPARγ activation because very recent studies with FK-614 indicate that this drug does not decrease but rather increases hepatic insulin sensitivity in Zucker fatty rats [31]. In our dogs, liver enzymes were within the reference range at the onset of FK-614 treatment, but alanine aminotransferase was marginally elevated soon after alloxanization in 2 of 6 dogs. Whether even minimal liver damage could affect the hepatic response to selective PPARγ activation would be important to know, given the prevalence of nonalcoholic fatty liver disease in metabolic syndrome and type 2 diabetes mellitus [32].

With regard to the FK-614–induced decrease in hepatic insulin extraction, it was associated with hepatic but not peripheral insulin resistance in our model contrary to the decrease in hepatic insulin extraction observed in obesity, which is generally associated with both hepatic and peripheral insulin resistance [33]. This suggests that changes in muscle and hepatic fat content might have been discordant in our model, whereas concordant changes in fat content of both muscle and liver might explain the associations of peripheral insulin resistance with decreased hepatic insulin action and extraction in obesity. It is also possible that FK-614 affects hepatic insulin extraction because of a direct effect of the drug on the uptake and metabolism of insulin by the liver, independent of changes in hepatic fat content. Other oral hypoglycemic agents [34-36], including TZD [37], have been reported to affect hepatic insulin extraction, although their effect may not be direct [38,39].

One surprising feature of 10-day treatment with FK-614 in alloxan-induced diabetic dogs was that it significantly reduced the daily requirements of both regular and NPH insulin, unlike 10-day treatment with pioglitazone 10 or JTT-5018. The effectiveness of FK-614 in reducing the daily insulin requirements is in accordance with the effect of FK-614 to decrease hepatic insulin extraction.

FK-614 also had a small enhancing effect on peripheral insulin sensitivity in our model. This effect may be underestimated when normalizing GU by insulin and glucose, because GU may be near maximal at the insulin and glucose levels of the present study. Also, PPARγ agonists have greater effect on insulin sensitivity in models of obesity-associated type 2 diabetes mellitus [40,41], where insulin resistance is the primary defect, than in models of type 1 diabetes mellitus [42], where insulin resistance is secondary. The alloxan-diabetic dog is primarily a model of type 1 rather than type 2 diabetes mellitus, and thus may not be the ideal model to test a presumed insulin sensitizer. Unfortunately, there are no good models of type 2 diabetes mellitus in the dog, where arteriovenous balance studies across the liver are feasible. The mechanisms by which PPARγ agonists improve peripheral insulin resistance are not fully understood. Peroxisome proliferator–activated receptor γ activation is known to lower the plasma level of adipocytokines and FFA by increasing the capacity of fat storage in the adipose tissue, thus improving insulin resistance indirectly [43]. Recent evidence suggests that PPARγ agonists also improve insulin sensitivity through a direct effect on skeletal muscle, as in transgenic mice lacking adipose tissue where TZD enhanced muscle glucose uptake [44]. The antioxidant/anti-inflammatory [45] and AMPK-stimulating effects of PPARγ activators [46] could also contribute to their insulin-sensitizing effect. To date, there has been no study done to compare the extent of these peripheral insulin-sensitizing mechanisms with FK-614 vs TZD. The effects of FK-614 to instead impair insulin sensitivity in the liver and decrease hepatic insulin extraction, which were observed in the present study, may be dose-dependent. The dose of FK-614 used in the present study was based on preliminary results in rodent models (see Methods). A lower dose of FK-614 may result in more selective effects and specific peripheral insulin sensitization.

We conclude that FK-614 treatment at the dose used has marginal effect on peripheral insulin sensitivity, impairs hepatic glucose metabolism, and decreases hepatic insulin extraction in alloxan-induced diabetic dogs. The decrease in hepatic insulin clearance likely contributes to the reduction in daily insulin requirements. Because TZD [10] and TZD-like [8] compounds do not decrease hepatic insulin sensitivity and clearance in alloxan-diabetic dogs, these results suggest that at least in this model, some degree of hepatic PPARα activation may be required for high doses of PPARγ agonists to exert favorable effects on glucose metabolism in the liver.

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