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ORIGINAL ARTICLE

Effect of the combination of metformin and fenofibrate on glucose homeostasis in diabetic Goto-Kakizaki rats

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Metformin has been reported to increase the expression of the glucagon-like peptide-1 (GLP-1) receptor in pancreatic beta cells in a peroxisome proliferator-activated receptor (PPAR)-α-dependent manner. We investigated whether a PPARα agonist, fenofibrate, exhibits an additive or synergistic effect on glucose metabolism, independent of its lipid-lowering effect, when added to metformin. Non-obese diabetic Goto-Kakizaki (GK) rats were divided into four groups and treated for 28 days with metformin, fenofibrate, metformin plus fenofibrate or vehicle. The random blood glucose levels, body weights, food intake and serum lipid profiles were not significantly different among the groups. After 4 weeks, metformin, but not fenofibrate, markedly reduced the blood glucose levels during oral glucose tolerance tests, and this effect was attenuated by adding fenofibrate. Metformin increased the expression of the GLP-1 receptor in pancreatic islets, whereas fenofibrate did not. During the intraperitoneal glucose tolerance tests with the injection of a GLP-1 analog, metformin and/or fenofibrate did not alter the insulin secretory responses. In conclusion, fenofibrate did not confer any beneficial effect on glucose homeostasis but reduced metformin's glucose-lowering activity in GK rats, thus discouraging the addition of fenofibrate to metformin to improve glycemic control.

INTRODUCTION

Relative insulin deficiency in the presence of prevailing insulin resistance is the main pathophysiology of type 2 diabetes mellitus.[1] The mechanisms of insulin secretion in response to a mixed meal are complex.[2] Among these mechanisms, the incretin effect contributes 50–70% of the insulin secretion in subjects with normal glucose tolerance.[3,4] The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are secreted from intestinal endocrine L- and K-cells, respectively, following the ingestion of nutrients and enhance insulin secretion from pancreatic beta cells in a glucose-dependent manner.[5–7] The effects of GLP-1 and GIP terminate shortly after secretion due to the enzymatic activity of dipeptidyl peptidase-4.[6] Intriguingly, the incretin effect is markedly attenuated by as much as 10–40% in patients with type 2 diabetes mellitus,[8–10] which can be explained not only by decreased beta cell function/mass *per se* but also by decreased incretin sensitivity due to the downregulation of

the relevant receptors.[11–13] Therefore, it may be possible to control the glucose level in type 2 diabetes mellitus patients by increasing the incretin sensitivity by upregulating the expression of incretin receptors in pancreatic beta cells.[14]

Incretin therapies such as GLP-1 analogs and dipeptidyl peptidase-4 inhibitors are currently available in clinical practice.[15,16] This type of new treatment has the advantages of low risks of hypoglycemia and weight gain relative to current anti-diabetes treatments based on sulfonylureas or insulin.[17] It is conceivable that the glucose-lowering effects of such incretin therapies would be augmented if they were combined with medications that effectively increase incretin sensitivity through the upregulation of incretin receptor expression. In addition to increased insulin secretion, we may expect an enhancement of other beneficial effects of incretin hormones, such as the preservation of or an increase in beta cell mass or function, delayed gastric emptying and decreased appetite.[6,17]

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The addition of GLP-1 analogs or dipeptidyl peptidase-4 inhibitors to metformin provides further glucose-lowering effects without causing hypoglycemia or weight gain.[18–20] The glucose-lowering effect of metformin is based on the suppression of hepatic glucose production and the enhancement of peripheral glucose utilization.[14,21] GLP-1 increases insulin secretion from pancreatic beta cells and decreases glucagon secretion from pancreatic alpha cells, which in turn leads to decreased hepatic glucose output.[22] Therefore, both metformin and incretin therapy converge to regulate hepatic glucose output. In addition, Maida et al.[23] recently demonstrated that metformin increases GLP-1 secretion from intestinal endocrine L cells and also increases the expression of the GLP-1 receptor in pancreatic islets via a peroxisome proliferator-activated receptor (PPAR)-α-dependent pathway. Furthermore, in an obese type 2 diabetes animal model, fenofibrate (a PPARα agonist) reduced the decline in beta cell mass and prevented the development of diabetes.[24] Based on these results, we hypothesized that a PPARα agonist, fenofibrate, may enhance the effect of GLP-1 when used either as a monotherapy or in combination with metformin.[14] To explore this possibility, we examined the effect of fenofibrate alone or in combination with metformin on glucose homeostasis and GLP-1 sensitivity in non-obese Goto-Kakizaki (GK) rats.

MATERIALS AND METHODS

Animals and treatment
The animal studies were performed after receiving approval from the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (IACUC approval no. 11−0038). Twenty-four male GK rats (aged 5 weeks) were obtained from Japan SLC (Hamamatsu, Japan). They were provided with standard chow (Purina rat and mouse chow, Purina Korea, Seoul, Korea) and water ad libitum at the Seoul National University Hospital Biomedical Research Institute. After a 1-week adaptation period, the GK rats were divided into four groups ($n = 6$ in each group): the vehicle-treated control (Ctrl) group, the metformin alone (MA) group, the fenofibrate alone (FA) group and metformin plus fenofibrate (MF) group. The vehicle for both metformin and fenofibrate was 1% carboxymethyl cellulose (Sigma-Aldrich, St Louis, MO, USA). Each medication was administered by gastric gavage every day for 28 days. Metformin (Glucophage; Merck Serono, Geneva, Switzerland) was administered at a dose of 150 mg kg^{-1} for 14 days, and then the dose was increased to 300 mg kg^{-1} during the last 14 days. The dose of fenofibrate (Recipharm Fontaine, Dijon, France) was fixed at 150 mg kg^{-1} for 28 days.

Body weights, food intake, random blood glucose levels and lipid profiles
The body weights and random blood glucose levels were monitored every day, and food intake over 24 h was measured every week. The body weights and food intake were measured using an electrical scale (A&D, Tokyo, Japan), and the blood glucose levels were measured using a glucometer (OneTouch Ultra; LifeScan, Milpitas, CA, USA) and tail vein blood samples. The total blood cholesterol and triglyceride levels were measured enzymatically with an autoanalyzer (Hitachi 7070; Hitachi, Tokyo, Japan).

Oral glucose tolerance test
Oral glucose tolerance tests (OGTTs) were performed after 12-h fasts following 4 weeks of treatment. The baseline blood glucose levels were measured, and each medication was administered by gastric gavage 1 h before oral glucose loading. The OGTT consisted of 2.0 g kg^{-1} of glucose in a 20% solution delivered by gastric gavage. The glucose levels were monitored at 0, 15, 30, 60, 120 and 180 min using a glucometer (OneTouch Ultra) and tail vein blood samples.

Intraperitoneal glucose tolerance tests (IPGTTs) with exendin-4 injections
To evaluate the insulin secretory capacity in response to exendin-4, a GLP-1 analog, IPGTTs with exendin-4 injections were performed after 12-h fasts 2 days after the OGTTs. The baseline blood glucose levels were measured 1 h before glucose loading, and then exendin-4 (Sigma-Aldrich) at a dose of 25 nmol kg^{-1} was injected via the intraperitoneal (i.p.) route. An hour after the i.p. exendin-4 injection, a 2.0 g kg^{-1} load of glucose as a 20% solution was injected in the same way. The study medications were not administered on the day of the IPGTT to avoid the effect of the medications. Blood samples (150 μl) for glucose measurements and plasma samples for insulin measurements were obtained at 0, 30, 60, 120 and 180 min from the tail vein. The insulin levels were measured using a commercial ELISA kit (Alpco Diagnostics, Windham, NH, USA).

Histology
Following completion of the OGTTs and IPGTTs, the GK rats were killed by an i.p. injection of Zoletil 50 (zolazepam plus tiletamine; Virbac Laboratories, Carros, France) and xylazine (Bayer Korea, Seoul, Korea). The pancreas of each animal was fixed with 4% formalin solution and embedded in paraffin. The samples were exposed to an anti-insulin antibody (1:1000, mouse monoclonal antibody, I-2018, Sigma-Aldrich) followed by 5% normal goat serum (S-1000, Vector Laboratories, Burlingame, CA, USA). The sections were incubated with a secondary mouse antibody (1:1000, biotinylated anti-mouse IgG(H + L), BA-9200, Vector Laboratories) for 1 h. Then, the samples were reacted with an avidin–biotin complex (Vectastain Elite ABC kit, PK-6100, Vector Laboratories) for 30 min and exposed to 3,3′-diaminobenzidine (SK-4100, Vector Laboratories) for 20 s. The entire slide was scanned using an Aperio ScanScope (Aperio Technologies, Vista, CA, USA), and the beta cell area was quantified by counting the number of pixels with insulin staining using Aperio ImageScope. To measure the GLP-1 receptor expression in pancreatic islets, we performed immunofluorescent staining for the GLP-1 receptor and insulin. The samples were exposed to a primary GLP-1 receptor antibody (1:100, rabbit polyclonal GLP1R, ab39072, Abcam, Cambridge, UK) and an insulin antibody (1:1000, mouse monoclonal antibody, I-2018) for 12 h. Next, the samples were treated with Chromeo 488 (1:500, goat polyclonal secondary antibody to rabbit IgG(H + L), ab60314, Abcam) to visualize the bound GLP-1 receptor antibody and Alexa 594 (1:500, Alexa Fluor 594 goat anti-mouse IgG(H + L), A-11005, Invitrogen) to visualize the bound insulin antibody. Images were captured by a BX-50 Olympus microscope (Olympus, Tokyo, Japan) and quantified using Aperio ImageScope (Aperio Technologies). The level of GLP-1 receptor expression was expressed as the area of GLP-1 receptor staining/the area of insulin staining.

Statistical analysis

The data analysis was performed using Prism 5.0 (GraphPad, San Diego, CA, USA) and SPSS (Statistical Package for the Social Sciences) version 17.0 software (SPSS, Chicago, IL, USA). The initial blood glucose levels and body weights and the food intake were analyzed by nonparametric analysis using Kruskal–Wallis tests followed by Games–Howell *post hoc* tests. Two-way repeated measures analysis of variance with the Bonferroni *post hoc* test was used to analyze time-course differences in the glucose levels and body weights. The AUC of glucose and the incremental AUC (iAUC) of insulin were calculated using the trapezoidal rule. The AUC of glucose, the iAUC of insulin, the insulin-positive area, and the GLP-1 receptor-positive area were analyzed by the nonparametric Kruskal–Wallis test followed by the Games–Howell *post hoc* test. P-values < 0.05 were considered significant.

RESULTS

Body weights, food intake, random blood glucose levels and lipid profiles

The baseline body weights were not significantly different among the four groups. The changes in body weight over time also did not differ among the four groups ($P = 0.128$), but the Bonferroni *post hoc* test revealed differences between the Ctrl and MF groups from day 21 (Figure 1a). Food intake was comparable among the groups (Figure 1b). The random blood glucose levels at baseline and during the 4-week treatment period were similar among the groups (Figure 1c). The total cholesterol and triglyceride levels were comparable among the groups (Figure 1d), which indicated that fenofibrate had no effect on the plasma levels of total cholesterol and triglycerides in non-obese diabetic GK rats.

Oral glucose tolerance test

After 4 weeks of treatment, OGTTs were performed with the administration of the study medications (Figure 2a). The blood glucose levels were lower at 15, 30, 60 and 120 min in the MA group than in the Ctrl group. At 120 min, the glucose levels were higher in the FA group than in the Ctrl group. The MF group had lower blood glucose levels at 15 and 30 min than the Ctrl group. However, the MF group had higher blood glucose levels at 60, 120 and 180 min than the MA group. The FA group had higher blood glucose levels than the MA group from 30 min to the end of the test. The AUC of the glucose excursions over a 240-min period (from -60 min to $+180$ min) was significantly lower in the MA group than in the Ctrl group, whereas it was higher in the FA group than in the MA group (Figure 2b).

IPGTTs with exendin-4 injections

To examine the GLP-1 sensitivity, we performed IPGTTs with i.p. exendin-4. The glucose levels during the IPGTTs with i.p. exendin-4 injections did not differ between the Ctrl and MA groups but were higher in the FA and MF groups at 120 and 180 min. The blood glucose levels at 120 and 180 min were also higher in the FA and MF groups than in the MA group (Figure 3a). The AUCs for blood glucose during the IPGTTs were higher for the FA and MF groups than for the Ctrl group, and these values were higher in the MF group than in the MA group (Figure 3b).

The plasma insulin levels during the IPGTTs were lower between 30 and 180 min in the MF group and at 180 min for both the MA and FA groups than in the Ctrl group
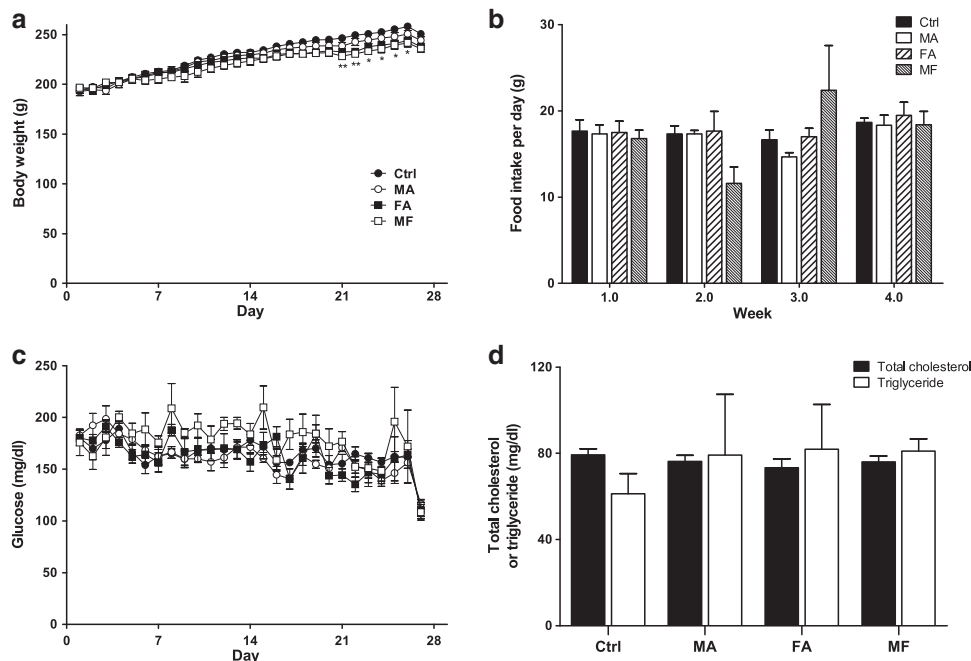

Figure 1 Tracking data for body weights (**a**), food intake (**b**), random blood glucose concentrations (**c**) and plasma levels of total cholesterol and triglycerides (**d**) after 4 weeks of treatment. Filled circles and black bars: Ctrl, control group; open circles and white bars: MA, metformin alone group; filled squares and upward dashed bars: FA, fenofibrate alone group; open squares and downward dashed bars: MF, metformin plus fenofibrate group (**a–c**). Black bars: total cholesterol; white bars: triglycerides (**d**). $^{*}P < 0.05$, $^{**}P < 0.01$ vs Ctrl.
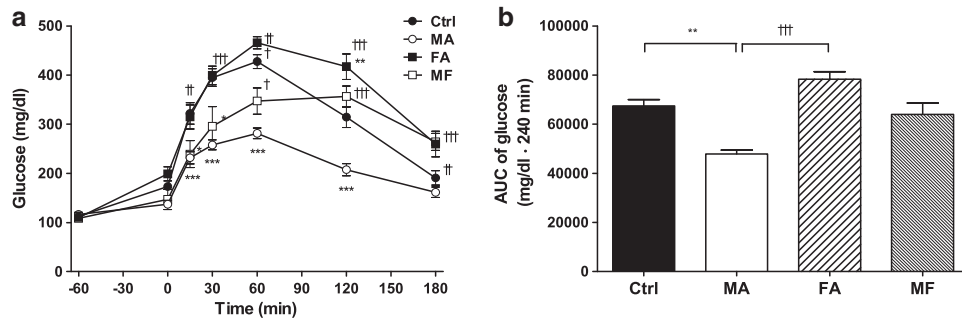
Figure 2 The oral glucose tolerance test results. The blood glucose levels (**a**) and AUCs (**b**). Filled circles and black bars: Ctrl, control group; open circles and white bars: MA, metformin alone group; filled squares and upward dashed bars: FA, fenofibrate alone group; open squares and downward dashed bars: MF, metformin plus fenofibrate group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs Ctrl, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs MA.
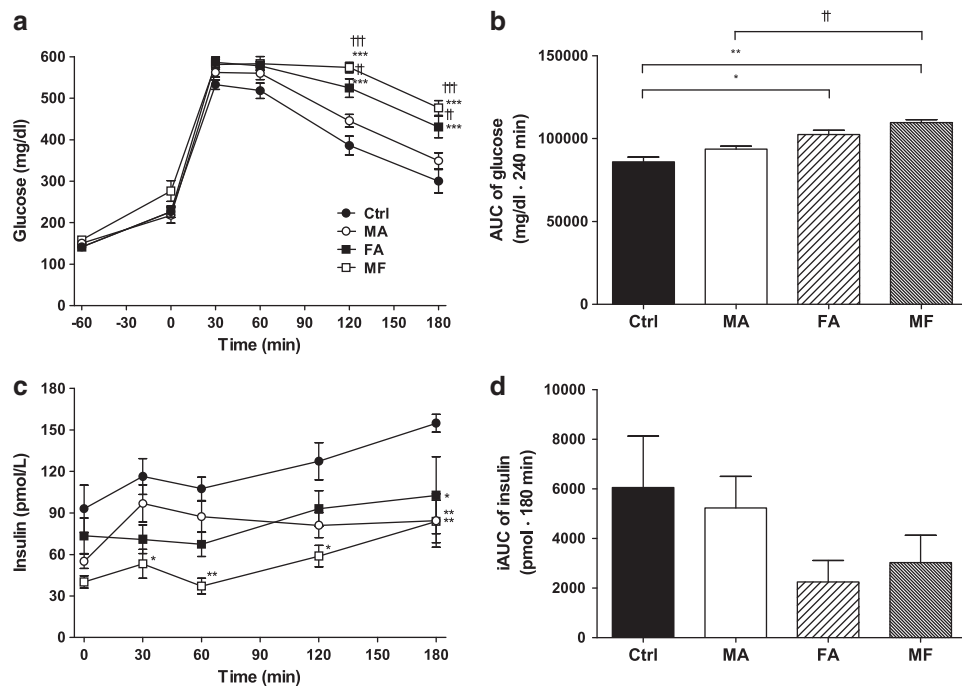

Figure 3 The sensitivity to an exogenous GLP-1 analog. The blood glucose levels (**a**) and AUCs (**b**), and the blood insulin levels (**c**) and iAUCs (**d**). Filled circles and black bars: Ctrl, control group; open circles and white bars: MA, metformin alone group; filled squares and upward dashed bars: FA, fenofibrate alone group; open squares and downward dashed bars: MF, metformin plus fenofibrate group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs Ctrl, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs MA.

(Figure 3c). There were no significant differences in the iAUCs for the plasma insulin levels among the groups (Figure 3d). However, compared with the Ctrl or MA group, the FA and MF groups had lower iAUCs for the plasma insulin levels, but these differences did not reach statistical significance (Figure 3d).

Beta cell area and GLP-1 receptor area
Representative images of pancreatic islets stained for insulin after 4 weeks of treatment are shown in Figure 4a. The morphology of the islets of GK rats were typically distorted, as reported elsewhere.[25] The average insulin-positive area corrected by the total pancreatic area was $1.3 \pm 0.2\%$ in the Ctrl group, $1.1 \pm 0.2\%$ in the MA group, $1.1 \pm 0.2\%$ in the FA

group and $0.7 \pm 0.1\%$ in the MF group; thus, the values for the different groups were comparable. According to the *post hoc* analysis, the average percentage of the total pancreas area positive for insulin tended to be lower in the MF group than in the Ctrl group ($P = 0.108$) (Figure 4b).

Representative images of pancreatic islets immunostained for insulin (red fluorescence) and the GLP-1 receptor (green fluorescence) are shown in Figure 4c. Most GLP-1 receptor immunoreactivity overlapped with the insulin immunostaining. The average GLP-1 receptor-positive area per insulin-positive area was significantly higher in the MA group than in the Ctrl group ($45.4 \pm 3.3\%$ vs $26.1 \pm 2.1\%$, $P < 0.001$). The average percentage of GLP-1 receptor-positive area per insulin-positive area in the FA group was lower than that in
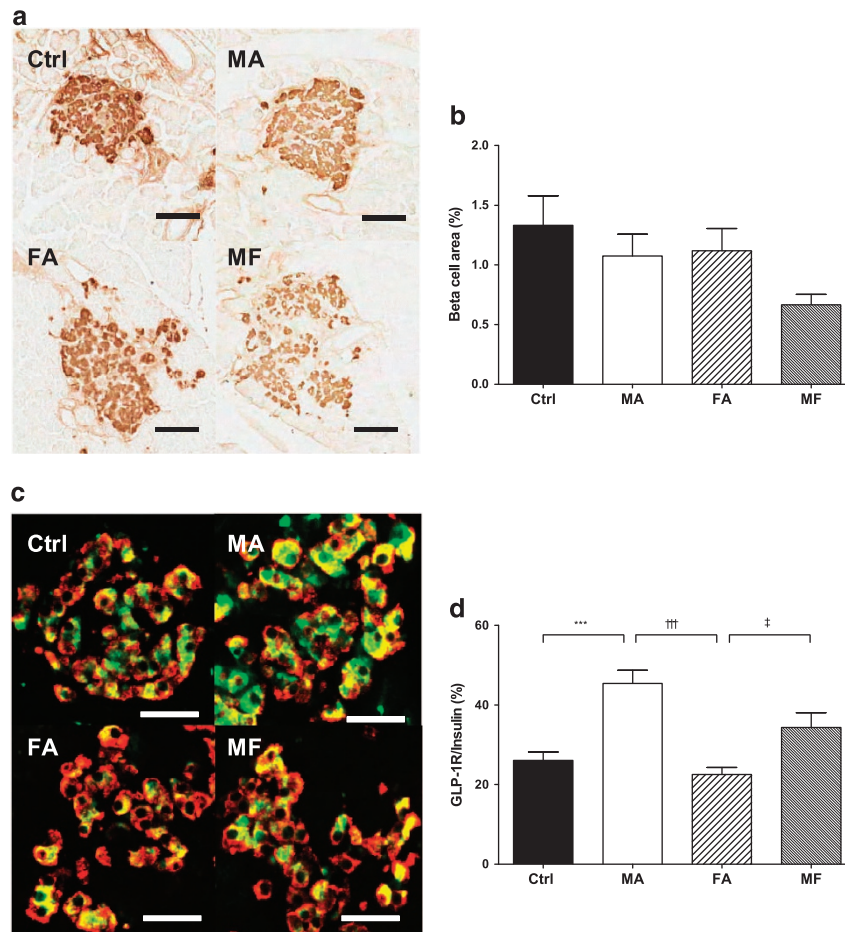
Figure 4 Immunohistochemical staining for insulin (**a**), percent beta cell area in the pancreas (**b**), immunofluorescence staining for the GLP-1 receptor (green) and insulin (red) (**c**), and ratio of the GLP-1 receptor-positive area to the insulin-positive area (**d**). Scale bars, 50 μm. Black bars: Ctrl, control group; white bars: MA, metformin alone group; upward dashed bars: FA, fenofibrate alone group; downward dashed bars: MF, metformin plus fenofibrate group. ***$P < 0.001$ vs Ctrl, †††$P < 0.001$ vs MA, ‡$P < 0.05$ vs FA.

the MA group ($22.6 \pm 1.7\%$ vs $45.4 \pm 3.3\%$, $P < 0.001$), and that in the MF group was higher than that in the FA group ($34.3 \pm 3.7\%$ vs $22.6 \pm 1.7\%$, $P = 0.029$) (Figure 4d).

DISCUSSION

In this study, we examined whether a PPARα agonist, fenofibrate, with or without metformin can improve glucose homeostasis through enhancing GLP-1 sensitivity by increasing the expression of the GLP-1 receptor in the pancreatic beta cells of non-obese diabetic GK rats. There were no differences in body weight and food intake among the groups during the study period. The total cholesterol and triglyceride levels were comparable among the four treatment groups, which allowed us to examine the effect of fenofibrate on glucose homeostasis independent of its lipid-lowering effects in non-obese diabetic GK rats.

Although metformin lowered the blood glucose levels during the OGTTs, fenofibrate did not exhibit glucose-lowering effects, but rather, caused a higher glucose level during the late postprandial period. Similarly, a recent study revealed that 6 weeks of treatment with bezafibrate, a PPARα agonist,

did not affect glucose homeostasis in db/db mice, an obese diabetic rodent model.[26] Furthermore, in our study, fenofibrate offset the glucose-lowering effect of metformin during the OGTTs, which indicates that fenofibrate has a negative effect on glucose homeostasis when added to metformin. However, in human studies, even though they were not designed to evaluate the glucose-lowering effect, fenofibrate had no adverse effect on glucose homeostasis when added to metformin in type 2 diabetic patients with mixed dyslipidemia[27] or in patients with metabolic syndrome.[28] In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, more than 40% of the study subjects enrolled in each treatment group (placebo vs fenofibrate) were taking metformin, but there was no meaningful change in the HbA1c levels from the baseline values in either group.[29] The mechanism responsible for the discrepancy between our experimental results and the results of previous clinical trials needs to be elucidated.

Maida et al.[23] reported that short-term (48 h) metformin treatment significantly increased GLP-1 receptor expression in the pancreatic islets of non-diabetic mice relative to

vehicle-treated mice. In this study, we found that long-term (4 weeks) treatment with metformin also increased the expression of the GLP-1 receptor in pancreatic islets in non-obese diabetic GK rats, which are characterized by prominent beta cell destruction.[30] Furthermore, the expression level of the GLP-1 receptor in the pancreatic islets of GK rats was higher when the rats were treated with metformin and fenofibrate than with fenofibrate alone. Taken together, these results indicate that metformin increases the expression of the GLP-1 receptor in pancreatic islets in both non-diabetic and diabetic animal models regardless of whether fenofibrate is used. However, the increased expression of the GLP-1 receptor was not translated into an increased insulin response to an intraperitoneally administered GLP-1 receptor agonist (exendin-4). Severely compromised beta cell function and/or mass in GK rats might compromise the ability of metformin to enhance insulin secretion in response to exendin-4.

It has been reported that metformin-treated mice exhibit increased expression of the GLP-1 receptor in pancreatic islets independent of the effect of AMP-activated protein kinase.[23] Instead, the increased expression of the GLP-1 receptor was attributed to the action of PPARα because no such increase was observed in PPARα-knockout mice in vivo and because metformin increased GLP-1 receptor expression in INS-1 beta cells in vitro in a PPARα-dependent fashion.[23] In contrast, in our study, fenofibrate, a potent PPARα agonist, did not have any additive or synergistic effect on the expression of the GLP-1 receptor when used in combination with metformin. The concept of the selective modulation of nuclear receptors by different ligands[31] may provide a plausible explanation for this apparent discrepancy.

For PPARγ, another PPAR family member, different ligands induce different receptor conformations and therefore different biological responses, as explained by the selective PPAR modulator (SPPARM) model.[32] The SPPARM model could be extended to the regulation of PPARα activity. Gemfibrozil, another PPARα agonist available in clinical practice for the treatment of dyslipidemia, behaves as a partial agonist, whereas fenofibrate behaves as a full agonist, recruiting coactivators to the human apoA-I DR-2 site more effectively than gemfibrozil.[33] Accordingly, we may be able to explain the divergent regulation of apoA-I in humans by gemfibrozil or fenofibrate.[33] In Maida et al.,[23] PPARα was modulated by unidentified endogenous ligands that were presumably induced by metformin treatment, whereas we used a synthetic full agonist of PPARα in this study. We speculate that different PPARα ligands might be responsible for the metformin response at the level of GLP-1 receptor expression in pancreatic islets.

In summary, fenofibrate did not confer any beneficial effect on glucose homeostasis but instead reduced metformin's glucose-lowering activity in GK rats. Because GK rats are non-obese and are characterized by decreased beta cell mass associated with extensive islet fibrosis,[30] animal models with obesity or mild beta cell dysfunction may have different responses to metformin and/or fenofibrate treatment, and therefore, further investigation is required.

1 DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 1992; **15**: 318–368.
2 Kieffer TJ, Habener JF. The glucagon-like peptides. *Endocr Rev* 1999; **20**: 876–913.
3 Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab* 1986; **63**: 492–498.
4 Oh TJ, Kim MY, Shin JY, Lee JC, Kim S, Park KS et al. The incretin effect in korean subjects with normal glucose tolerance or type 2 diabetes. *Clin Endocrinol (Oxf)*, (doi:10.1111/cen.12167).
5 Creutzfeldt W, Nauck M. Gut hormones and diabetes mellitus. *Diabetes Metab Rev* 1992; **8**: 149–177.
6 Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007; **132**: 2131–2157.
7 Cho YM, Kieffer TJ. K-cells and glucose-dependent insulinotropic polypeptide in health and disease. *Vitam Horm* 2010; **84**: 111–150.
8 Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1986; **29**: 46–52.
9 Stege H, Bagger J, Nielsen JP, Ersboll AK. Effect of breeding strategy and feeding system on the within-herd variation of lean meat percents in Danish slaughter pigs. *Prev Vet Med* 2011; **101**: 73–78.
10 Knop FK, Vilsboll T, Hojberg PV, Larsen S, Madsbad S, Volund A et al. Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? *Diabetes* 2007; **56**: 1951–1959.
11 Xu G, Kaneto H, Laybutt DR, Duvivier-Kali VF, Trivedi N, Suzuma K et al. Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. *Diabetes* 2007; **56**: 1551–1558.
12 Lynn FC, Pamir N, Ng EH, McIntosh CH, Kieffer TJ, Pederson RA. Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* 2001; **50**: 1004–1011.
13 Meier JJ, Nauck MA. Is the diminished incretin effect in type 2 diabetes just an epi-phenomenon of impaired beta-cell function? *Diabetes* 2010; **59**: 1117–1125.
14 Cho YM, Kieffer TJ. New aspects of an old drug: metformin as a glucagon-like peptide 1 (GLP-1) enhancer and sensitiser. *Diabetologia* 2011; **54**: 219–222.
15 Chia CW, Egan JM. Incretin-based therapies in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2008; **93**: 3703–3716.
16 Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R et al. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2009; **32**: 193–203.
17 Drucker DJ. The biology of incretin hormones. *Cell Metab* 2006; **3**: 153–165.
18 Zander M, Taskiran M, Toft-Nielsen MB, Madsbad S, Holst JJ. Additive glucose-lowering effects of glucagon-like peptide-1 and metformin in type 2 diabetes. *Diabetes Care* 2001; **24**: 720–725.
19 Cuthbertson J, Patterson S, O'Harte FP, Bell PM. Addition of metformin to exogenous glucagon-like peptide-1 results in increased serum glucagon-like peptide-1 concentrations and greater glucose lowering in type 2 diabetes mellitus. *Metabolism* 2011; **60**: 52–56.

20 Ahren B. Novel combination treatment of type 2 diabetes DPP-4 inhibition + metformin. *Vasc Health Risk Manag* 2008; **4**: 383–394.

21 Bailey CJ, Turner RC. Metformin. *N Engl J Med* 1996; **334**: 574–579.

22 Cho YM, Merchant CE, Kieffer TJ. Targeting the glucagon receptor family for diabetes and obesity therapy. *Pharmacol Therapeut* 2012; **135**: 247–278.

23 Maida A, Lamont BJ, Cao X, Drucker DJ. Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor-alpha in mice. *Diabetologia* 2011; **54**: 339–349.

24 Koh EH, Kim MS, Park JY, Kim HS, Youn JY, Park HS *et al*. Peroxisome proliferator-activated receptor (PPAR)-alpha activation prevents diabetes in OLETF rats: comparison with PPAR-gamma activation. *Diabetes* 2003; **52**: 2331–2337.

25 Speck M, Cho YM, Asadi A, Rubino F, Kieffer TJ. Duodenal-jejunal bypass protects GK rats from {beta}-cell loss and aggravation of hyperglycemia and increases enteroendocrine cells coexpressing GIP and GLP-1. *Am J Physiol Endocrinol Metab* 2011; **300**: E923–E932.

26 Kang ZF, Deng Y, Zhou Y, Fan RR, Chan JC, Laybutt DR *et al*. Pharmacological reduction of NEFA restores the efficacy of incretin-based therapies through GLP-1 receptor signalling in the beta cell in mouse models of diabetes. *Diabetologia* 2013; **56**: 423–433.

27 Pruski M, Krysiak R, Okopien B. Pleiotropic action of short-term metformin and fenofibrate treatment, combined with lifestyle intervention, in type 2 diabetic patients with mixed dyslipidemia. *Diabetes Care* 2009; **32**: 1421–1424.

28 Nieuwdorp M, Stroes ES, Kastelein JJ. Normalization of metabolic syndrome using fenofibrate, metformin or their combination. *Diabetes Obes Metab* 2007; **9**: 869–878.

29 Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR *et al*. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005; **366**: 1849–1861.

30 Portha B, Lacraz G, Kergoat M, Homo-Delarche F, Giroix MH, Bailbe D *et al*. The GK rat beta-cell: a prototype for the diseased human beta-cell in type 2 diabetes? *Mol Cell Endocrinol* 2009; **297**: 73–85.

31 Clarke BL, Khosla S. New selective estrogen and androgen receptor modulators. *Curr Opin Rheumatol* 2009; **21**: 374–379.

32 Olefsky JM. Treatment of insulin resistance with peroxisome proliferator-activated receptor gamma agonists. *J Clin Invest* 2000; **106**: 467–472.

33 Duez H, Lefebvre B, Poulain P, Torra IP, Percevault F, Luc G *et al*. Regulation of human apoA-I by gemfibrozil and fenofibrate through selective peroxisome proliferator-activated receptor alpha modulation. *Arterioscler Thromb Vasc Biol* 2005; **25**: 585–591.