

*Full Paper***Antidiabetic and Hypolipidemic Effects of a Novel Dual Peroxisome Proliferator-Activated Receptor (PPAR)  $\alpha/\gamma$  Agonist, E3030, in db/db Mice and Beagle Dogs**

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**Abstract.** We investigated the antidiabetic effects of E3030, which is a potent dual activator of peroxisome proliferator-activated receptor (PPAR)  $\alpha$  and PPAR $\gamma$ , in an animal model of diabetes, C57BL/KsJ-db/db mice (db/db mice), and the lipidemic effects of E3030 in beagle dogs, whose PPAR $\alpha$  and PPAR $\gamma$  transactivation responses to E3030 were similar to those of humans. E3030 activated human PPAR $\alpha$ , mouse PPAR $\alpha$ , dog PPAR $\alpha$ , human PPAR $\gamma$ , mouse PPAR $\gamma$ , and dog PPAR $\gamma$  with EC<sub>50</sub> values of 65, 920, 87, 34, 73, and 34 nM, respectively, in the chimeric GAL4-PPAR receptor transactivation reporter assay. In db/db mice orally administered E3030 decreased blood glucose, triglyceride (TG), non-esterified fatty acids (NEFA), and insulin levels and increased blood adiponectin levels during a 14-day experimental period. Significant effects on blood glucose and adiponectin levels were observed at a dose of 3 mg/kg or greater. Furthermore, significant effects on blood TG, NEFA, and insulin levels were observed at doses of 1 mg/kg or more. An oral glucose tolerance test (OGTT) performed on Day 15 showed that E3030 at 3 mg/kg improved glucose tolerance in this model. Fourteen days of oral treatment with E3030 at a dose of 0.03 mg/kg or greater showed remarkable TG- and non high-density lipoprotein (non-HDL) cholesterol-lowering effects in beagle dogs. These results were similar to those observed for the PPAR $\alpha$  agonist fenofibrate. E3030 also reduced apo C-III levels on Days 7 and 14, and elevated lipoprotein lipase (LPL) levels on Day 15. These results indicate that the TG- and non-HDL cholesterol-lowering actions of E3030 involve combined effects on reduction of apo C-III and elevation of LPL, resulting in increased lipolysis. The experimental results in animals suggest that E3030 has potential for use in the treatment of various aspects of metabolic dysfunction in type 2 diabetes, including dyslipidemia, hyperglycemia, hyperinsulinemia, and impaired glucose disposal.

**Keywords:** E3030, peroxisome proliferator-activated receptor (PPAR)  $\alpha$ , PPAR $\gamma$ , PPAR $\alpha/\gamma$  agonist

**Introduction**

Type 2 diabetes mellitus is a disease frequently associated with abnormal lipid metabolism. Abnormal lipid levels may be present even when glycemic control is adequate and nephropathy is absent. Elevated

triglyceride (TG) levels, reduced high-density lipoprotein (HDL) cholesterol, and a preponderance of small, dense low-density lipoprotein (LDL) particles are the key abnormalities that constitute diabetic dyslipidemia (1). It is reported that mortality due to heart disease in diabetic patients is two to three times higher than that in non-diabetics (2). Therefore, in addition to improving insulin resistance and glycemic control in patients with type 2 diabetes, improving the lipid profile in these patients would be very important.

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Peroxisome proliferator-activated receptors (PPARs) play a central role in regulating the storage and catabolism of glucose and lipids. Three classes of PPARs have been identified, PPAR $\alpha$ , PPAR $\beta$  ( $\delta$ ), and PPAR $\gamma$ . In humans, PPAR $\gamma$  agonists have demonstrated clinical utility for increasing insulin sensitivity and improving glycemic control in type 2 diabetes by enhancing peripheral insulin-mediated glucose disposal (3, 4), but these drugs have no or only a slight significant effect on TG, HDL cholesterol, and LDL cholesterol levels (5). On the other hand, PPAR $\alpha$  agonists have displayed clinical usefulness for lowering plasma TG and increasing HDL cholesterol levels in type 2 diabetes by enhancing lipid and lipoprotein metabolism. These drugs also reduce LDL cholesterol, particularly small dense LDL cholesterol, which is associated with increased risk of atherosclerosis (6, 7). Based on these findings, a dual PPAR $\alpha$ / $\gamma$  agonist might be expected to become a useful antidiabetic agent, improving both blood glucose levels and also lipid profile.

In a transactivation assay it has been shown that there are species differences in response to several synthetic compounds, KRP-297, GW9578, GI262570, L-796449, for PPAR $\alpha$  between human and mouse (8). Therefore, the results of in vivo studies of PPAR $\alpha$  agonists using rodents should be carefully reviewed in order to predict their biological actions in humans. Nagasawa (9) reported that dog PPAR $\alpha$  has 94.7% amino acid sequence identity to human PPAR $\alpha$ ; and importantly, in the ligand-binding domain, it is 97.0% identical. Overall, from this they concluded that the dog may be a useful model for investigating the in vivo biological actions of human PPAR $\alpha$  agonists.

We report here the antidiabetic effects of E3030 monocalcium bis((2*S*)-3-{3-[(2*S*)-3-(4-chloro-2-cyanophenoxy)-2-fluoropropoxy]phenyl}-2-isopropoxypropionate) trihydrate (Fig. 1), a novel agent that activates human, mouse, and dog PPAR $\alpha$  and PPAR $\gamma$ , in C57BL/KsJ-*db/db* mice (*db/db* mice), which are characterized by hyperglycemia and hyperinsulinemia, and have been used as a type 2 diabetic model with insulin resistance. We also report hypolipidemic effects of E3030 in beagle dogs, whose PPAR $\alpha$  and PPAR $\gamma$  transactivation responses to E3030 were similar to those of humans.

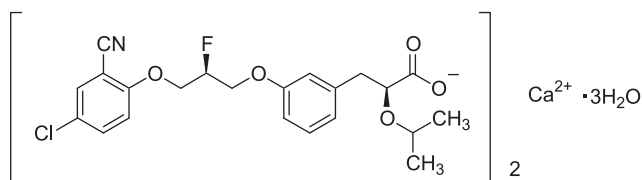


Fig. 1. Chemical structure of E3030.

## Materials and Methods

### Reagents

E3030 and pioglitazone HCl were synthesized in our laboratories. Fenofibrate was purchased from Sigma (St. Louis, MO, USA).

### Animals and cells

Male *db/db* mice and beagle dogs were purchased from CLEA Japan, Inc. (Tokyo) and NARC, Co. (Chiba), respectively. Animals were housed in a ventilated (10–15 times/h), temperature-controlled ( $23 \pm 3^\circ\text{C}$ ) room with constant humidity ( $55 \pm 15\%$ ) under a 12-h light/dark (7:00/19:00) cycle, and were fed with normal diet. All procedures were conducted according to Eisai Animal Care committee's guideline. Monkey kidney fibroblast CV-1 cells were purchased from Dainippon Pharmaceutical Co., Ltd. (Osaka) and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin.

### PPAR transactivation assay

The chimeric receptor expression plasmids pGAL4-hPPAR $\alpha$ , pGAL4-mPPAR $\alpha$ , pGAL4-dPPAR $\alpha$ , pGAL4-hPPAR $\gamma$ , pGAL4-mPPAR $\gamma$ , and pGAL4-dPPAR $\gamma$  were prepared by inserting the yeast GAL4 transcription factor DNA binding domain adjacent to the ligand binding domain of human PPAR $\alpha$ , mouse PPAR $\alpha$ , dog PPAR $\alpha$ , human PPAR $\gamma$ , mouse PPAR $\gamma$ , and dog PPAR $\gamma$ , respectively. The reporter plasmid, p(UAS)<sub>5</sub>-*tk* placental alkaline phosphatase (PLAP), was generated by inserting the five copies of the GAL4 response element upstream of the herpesvirus minimal thymidine kinase promoter (*tk* promoter) and the PLAP gene. CV-1 cells were transfected with chimeric receptor expression plasmid and reporter plasmid. The transfected cells were incubated for approx. 45 h with increasing concentrations of E3030, and then the PLAP activity was measured by mixing 10  $\mu\text{l}$  of culture supernatant, 50  $\mu\text{l}$  of PLAP buffer (16 mM sodium bicarbonate, 12 mM sodium carbonate, and 0.8 mM magnesium sulfate), and 50  $\mu\text{l}$  of CDP-Star Ready to Use with Sapphire II<sup>TM</sup> (Tropix, Bedford, MA, USA). After the reaction mixture had been incubated at room temperature for 60–120 min, the steady-state luminescence intensity was measured with a microplate luminometer (ARVO1420 MULTILABEL COUNTER; Wallac EG&G, Turku, Finland).

### Antidiabetic effects in *db/db* mice

Since it has already been clarified that both pioglitazone (10) and fenofibrate (11) show hypoglycemic and hypolipidemic effects in *db/db* mice, we did not

evaluate the effects of group with control in db/db mice. We chose the dosages at which E3030 showed dose-dependency in hypoglycemic effects.

E3030 (1, 3, 10, 30 mg/kg) or vehicle (0.5% methyl cellulose, 5 mL/kg) was orally administered to 12-week-old db/db mice once a day for 15 days ( $n=6$ ). After 4, 7, and 14 days of treatment, blood samples were collected from the tail vein. Blood glucose, TG, and NEFA levels were measured using commercial enzymatic assay kits (blood glucose: Glucose CII-test WAKO, Wako Pure Chemical Industries, Ltd., Osaka; TG: Determiner<sup>®</sup> L TGII, Kyowa Medex Co., Ltd., Tokyo; NEFA: NEFA C-test WAKO, Wako Pure Chemical Industries, Ltd.). Blood insulin and adiponectin levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (insulin: insulin ELISA kit, Morinaga Institute of Biological Science, Kanagawa; adiponectin: mouse/rat adiponectin ELISA kit, Otsuka Pharmaceutical Co., Ltd., Tokyo). Oral glucose tolerance test (OGTT) was performed after 15 days of treatment. Mice were fasted overnight after final administration and orally administered glucose (2 g/kg) as a 40% (w/v) solution. Blood samples were collected from the tail vein before and 30, 60, 120, and 180 min after glucose challenge, and then blood glucose and insulin levels were measured.

#### *Hypolipidemic effects in beagle dogs*

Since we had little information about hypolipidemic effects of PPAR $\alpha$  and PPAR $\gamma$  agonists in beagle dogs, fenofibrate and pioglitazone were used as reference agonists. We chose the dosages at which fenofibrate and pioglitazone showed clear changes in PPAR $\alpha$  and PPAR $\gamma$  biomarkers.

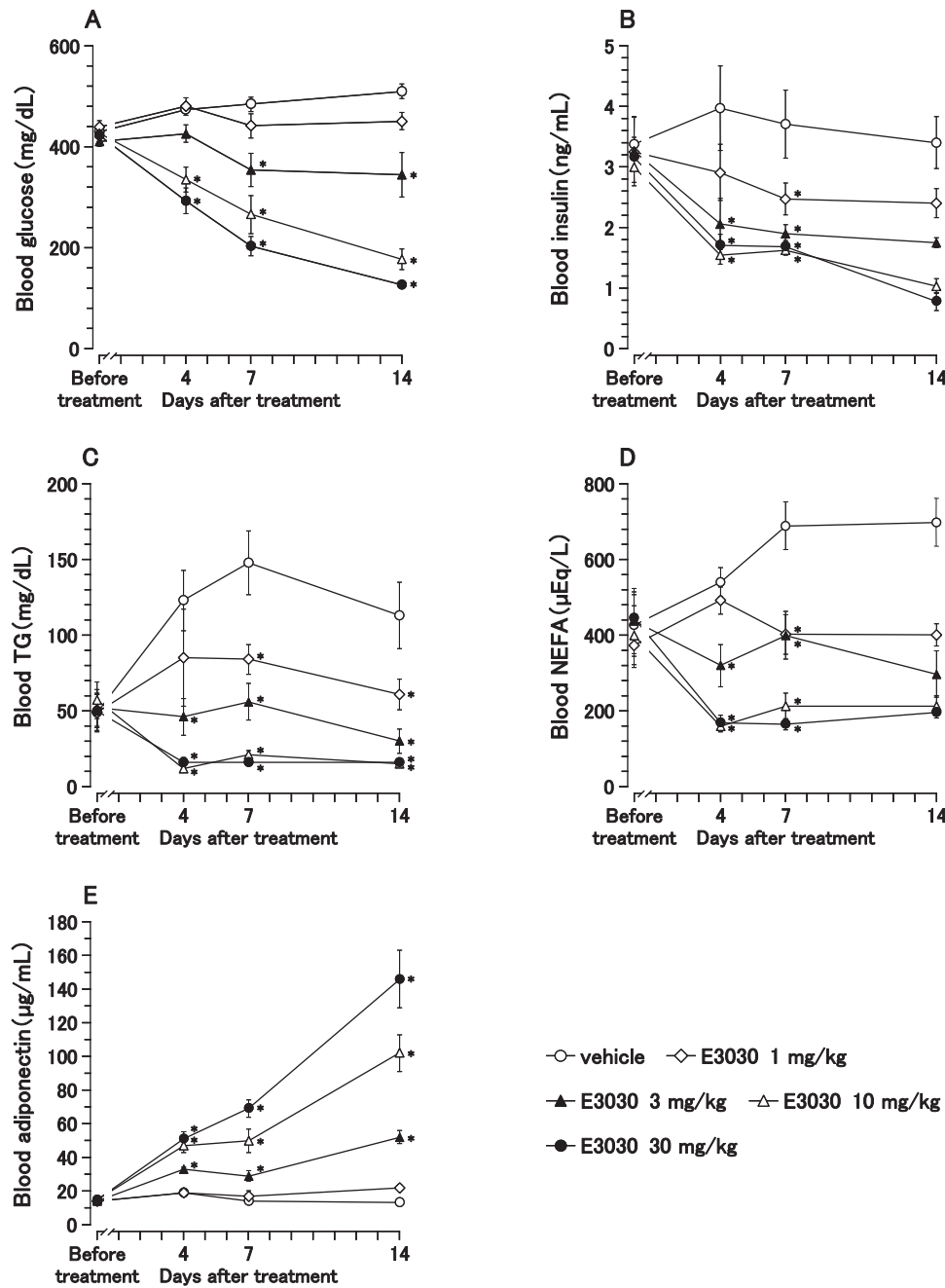
E3030 (0.03, 0.1, 0.3, 1 mg/kg), fenofibrate (100 mg/kg), pioglitazone HCl (1 mg/kg), or vehicle (0.5% methyl cellulose, 2 mL/kg) was orally administered to 6- to 16-month-old beagle dogs once a day for 15 days ( $n=6$ ). After 7 and 14 days of treatment, blood samples were collected from a cephalic vein and plasma glucose, TG, total cholesterol, non-HDL cholesterol, HDL cholesterol, apo C-III, and adiponectin were determined. LPL is located on the luminal surface of extrahepatic capillaries where it hydrolyses TG predominantly in two lipoprotein particles, chylomicrons and very low density lipoproteins (VLDLs). Intravenous injection of heparin can detach LPL from the luminal surface of extrahepatic capillaries. After 15 days of treatment, post-heparin plasma was drawn 10 min after intravenous injection of heparin (30 units/kg). LPL mass concentration in post-heparin plasma was measured using a commercial LPL ELISA kit (LPL ELISA "DAIICHI"; Daiichi Pure Chemicals Co., Ltd., Tokyo). Concentrations of plasma

glucose, TG, and total cholesterol were determined using commercial enzymatic assay kits (plasma glucose: Glucose CII-test WAKO, TG: L-Type WAKO-TG-H, total cholesterol: L-Type WAKO-CHO-H). Lipoproteins were separated by ultracentrifugation according to the method of Bronzert and Brewer (12), and cholesterol in the bottom fraction (HDL) determined enzymatically. Concentrations of non-HDL cholesterol were calculated by subtracting those of HDL cholesterol from those of total cholesterol. Concentration of plasma apo C-III was determined by ELISA. Briefly, each plasma sample diluted with phosphate-buffered saline containing 0.05% Tween 20 (PBST) was applied to a 96-well EIA plate (Corning Inc., NY, USA) and incubated overnight at 4°C. The plate was then incubated with rabbit anti-dog apo C-III polyclonal antibody, followed by incubation with horseradish peroxidase-labeled anti-rabbit IgG. Each well was reacted with *o*-phenylenediamine dihydrochloride and terminated with 3 M HCl, and then the absorbance at 490 nm was measured. Concentration of plasma adiponectin was also determined by ELISA. Briefly, each plasma sample diluted with PBST was applied to a 96-well EIA plate coated with rabbit polyclonal anti-dog adiponectin antibody and incubated at room temperature for 1 h. The plate was then incubated with biotinylated anti-dog adiponectin polyclonal antibody, followed by incubation with horseradish peroxidase-labeled streptavidin. Each well was reacted with *o*-phenylenediamine dihydrochloride and terminated with 3 M HCl, and then the absorbance at 490 nm was measured.

#### *Statistical analysis*

Db/db mouse experiments: Data are expressed as the mean  $\pm$  S.E.M. Differences in blood glucose, TG, NEFA, insulin, and adiponectin levels between the vehicle-treated control group and E3030-treated groups were analyzed by repeated measures analysis of covariance (ANCOVA), followed by the Dunnett type multiple comparison test using baseline values as covariates. To determine the integrated glucose and insulin response to oral glucose challenge, areas under the curves (AUCs) of blood glucose and insulin were calculated by the trapezoidal rule. Differences in AUCs of glucose and insulin during OGTT between vehicle-treated control group and E3030-treated groups were determined by one-way analysis of variance (ANOVA), followed by the Dunnett type multiple comparison test.

Beagle dog experiments: Data are expressed as the mean  $\pm$  S.E.M. For plasma glucose, TG, total cholesterol, non-HDL cholesterol, HDL cholesterol, non-HDL cholesterol/HDL cholesterol ratio, apo C-III, and adiponectin, differences between the vehicle-treated

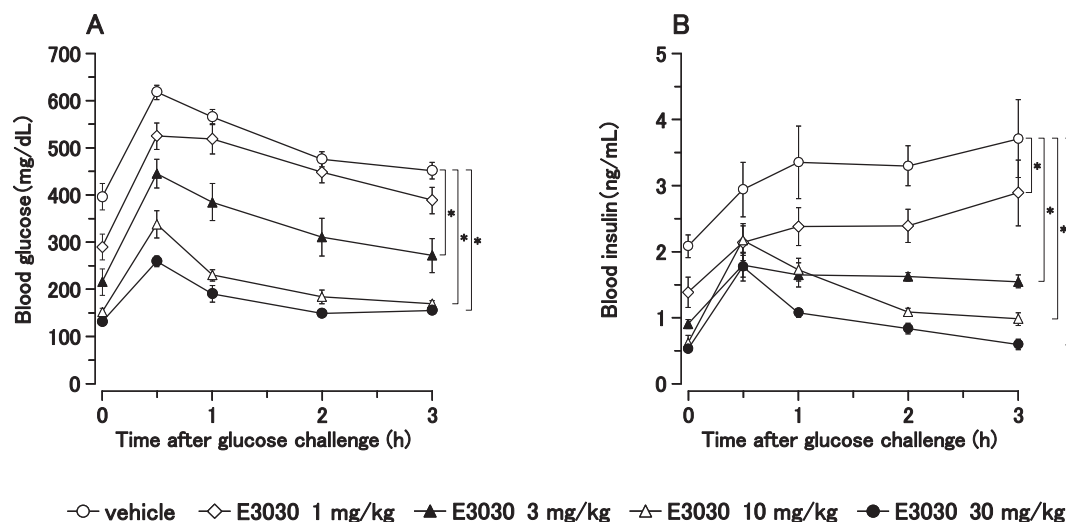


**Fig. 2.** Effects of E3030 on blood glucose (A), insulin (B), TG (C), NEFA (D), and adiponectin (E) levels in db/db mice. E3030 was orally administered once a day for 14 days. Results are each expressed as the mean  $\pm$  S.E.M. of six animals. \* $P < 0.05$  vs vehicle control [repeated measures analysis of covariance (ANCOVA) followed by Dunnett type multiple comparison test using baseline values as covariates].

control group and E3030-, fenofibrate-, or pioglitazone-treated groups were analyzed by repeated measures ANCOVA, followed by the Dunnett type multiple comparison test using baseline values as covariates. For LPL, difference between the vehicle-treated control group and fenofibrate- or pioglitazone-treated group was analyzed by the unpaired *t* test. Differences between

the vehicle-treated control group and E3030-treated groups were analyzed by one-way ANOVA, followed by the Dunnett type multiple comparison test.

A value of  $P < 0.05$  (2-sided) was considered statistically significant. Statistical analysis was conducted using the software package SAS 8.1 (SAS Institute Japan, Ltd., Tokyo).



**Fig. 3.** Effects of E3030 on changes in blood glucose (A) and insulin (B) levels during OGTT in db/db mice. E3030 was orally administered once a day for 15 days. One day after the final drug treatment, mice orally received 2 g/kg glucose (5 mL/kg). Results are each expressed as the mean  $\pm$  S.E.M. of six animals. Statistical analysis was performed on AUCs of glucose and insulin. \* $P < 0.05$  vs vehicle control [one-way analysis of variance (ANOVA) followed by the Dunnett type multiple comparison test].

## Results

### PPAR transactivation activity of E3030

To validate our chimeric GAL4-PPAR receptor transactivation reporter assay, we measured the PPAR transactivation activity of some PPAR agonists in our assay.  $EC_{50}$  values of pioglitazone for human and mouse PPAR $\gamma$  were 329 and 382 nM, respectively; and those of fenofibric acid for human, mouse, and dog PPAR $\alpha$  were 17.1, 21.2, and 17.0  $\mu$ M, respectively. These values in our assay were similar to those of previous reports (8, 13).

E3030 activated human PPAR $\alpha$ , mouse PPAR $\alpha$ , dog PPAR $\alpha$ , human PPAR $\gamma$ , mouse PPAR $\gamma$ , and dog PPAR $\gamma$  with  $EC_{50}$  values (and the 95% confidence interval) of 65 (60 to 70), 920 (780 to 1100), 87 (75 to 100), 34 (23 to 50), 73 (61 to 88), and 34 (31 to 37) nM, respectively, in the chimeric GAL4-PPAR receptor transactivation reporter assay. These results suggest that E3030 is a dual PPAR $\alpha/\gamma$  agonist.

### Antidiabetic effects of E3030 in db/db mice

The effects of E3030 on blood glucose, insulin, TG, NEFA, and adiponectin levels in db/db mice are summarized in Fig. 2. During the experimental period, E3030 decreased blood glucose, TG, NEFA, and insulin levels and increased blood adiponectin levels in a dose-dependent manner. Significant effects on blood glucose and adiponectin levels were observed at 3 mg/kg or greater. Furthermore, significant effects on blood TG, NEFA, and insulin levels were observed at 1 mg/kg or

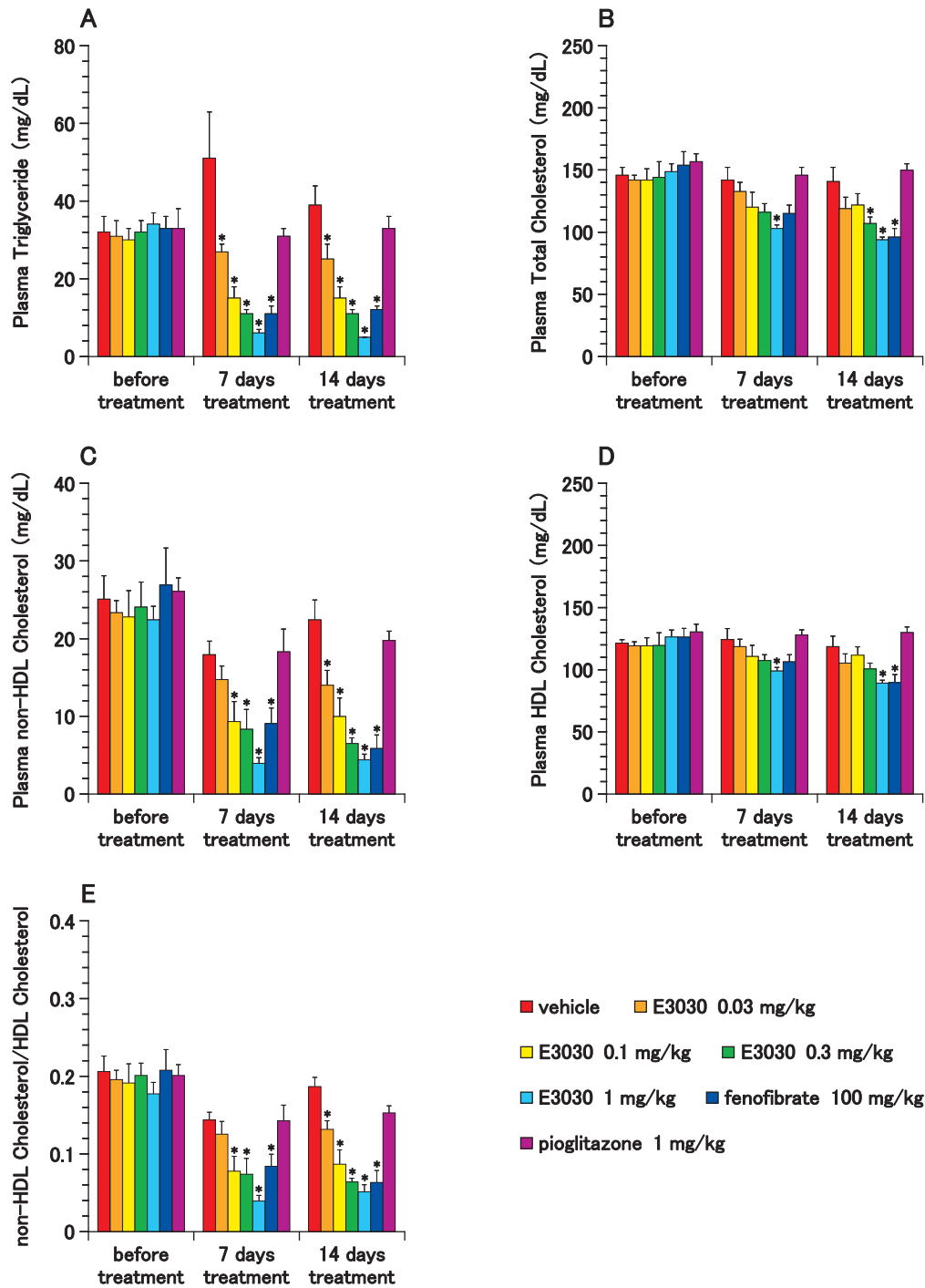
greater. The effect of E3030 on changes in blood glucose and insulin levels during OGTT in db/db mice is shown in Fig. 3. Before and after glucose challenge, blood glucose and insulin levels in E3030-treated groups showed low values compared with the vehicle-treated group, and AUCs of blood glucose and insulin during OGTT were significantly decreased at 3 and 1 mg/kg, respectively.

Loss in body weight was not observed in E3030-treated db/db mice at all doses (data not shown).

### Hypolipidemic effects of E3030 in beagle dogs

The hypolipidemic effects of E3030 were examined in comparison with those of fenofibrate and pioglitazone in beagle dogs.

Changes in plasma TG and total cholesterol levels after 2 weeks of treatment with E3030, fenofibrate, and pioglitazone are shown in Fig. 4. After 2 weeks of treatment with E3030 at doses of 0.03, 0.1, 0.3, and 1 mg/kg, plasma concentrations of TG were significantly reduced by 36%, 62%, 72%, and 87%, respectively, compared with the vehicle-treated control. E3030 reduced not only plasma TG but also total cholesterol. Indeed, animals exhibited dose-dependent reductions in total cholesterol of 16%, 13%, 24%, and 33%, respectively, with the latter two being significant. Two weeks of treatment with fenofibrate, which is clinically used as a TG- and cholesterol-lowering drug, significantly reduced both TG and total cholesterol at a dose of 100 mg/kg. On the other hand, pioglitazone, which is clinically used as an antidiabetic drug, did not signifi-

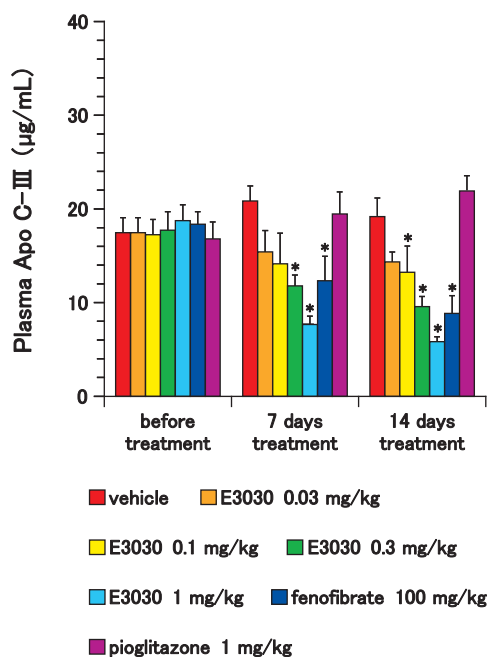


**Fig. 4.** Effects of E3030, fenofibrate, and pioglitazone on plasma TG (A), total cholesterol (B), non-HDL cholesterol (C), HDL cholesterol (D) levels, and non-HDL cholesterol / HDL cholesterol ratio (E) in beagle dogs. Drugs were orally administered once a day for 14 days. Results are each expressed as the mean  $\pm$  S.E.M. of six animals. \* $P$  < 0.05 vs vehicle control (repeated measures ANCOVA followed by the Dunnett type multiple comparison test using baseline values as covariates).

cantly reduce either TG or total cholesterol at 1 mg/kg.

Changes in plasma non-HDL cholesterol and HDL cholesterol levels after 2 weeks of treatment with E3030, fenofibrate, and pioglitazone are also shown in Fig. 4. Both E3030 and fenofibrate remarkably reduced

non-HDL cholesterol levels. E3030 at doses of 0.03, 0.1, 0.3, and 1 mg/kg significantly decreased non-HDL cholesterol levels by 38%, 55%, 71%, and 80%, respectively, compared with the vehicle-treated control. E3030 also caused reductions in HDL cholesterol of

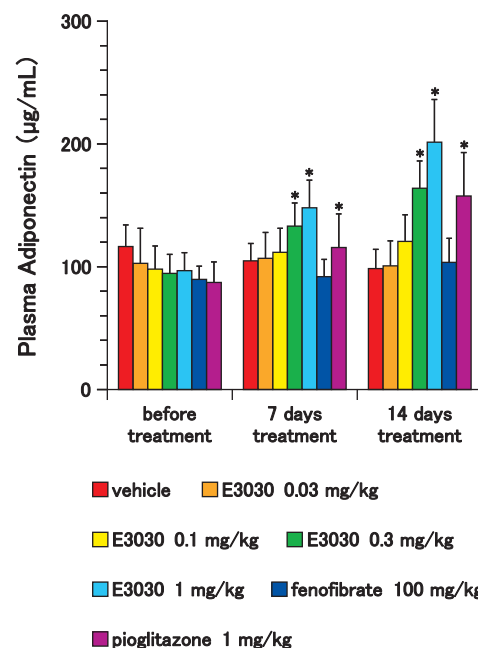


**Fig. 5.** Effects of E3030, fenofibrate, and pioglitazone on plasma apo C-III levels in beagle dogs. Drugs were orally administered once a day for 14 days. Results are each expressed as the mean  $\pm$  S.E.M. of six animals. \* $P$  < 0.05 vs vehicle control (repeated measures ANCOVA followed by the Dunnett type multiple comparison test using baseline values as covariates).

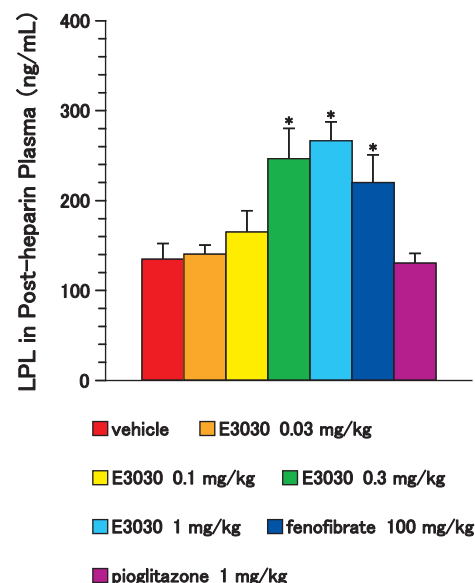
11%, 6%, 15%, and 25%, respectively, with the last being significant. Percent decreases in non-HDL cholesterol were more than those in HDL cholesterol. As a consequence of this more potent effect of E3030 on non-HDL cholesterol, a significant reduction in the atherogenic index (non-HDL cholesterol/HDL cholesterol ratio) was observed in E3030-treated beagle dogs. A similar result was obtained in fenofibrate-treated dogs, but not in pioglitazone-treated dogs.

We investigated the effects of E3030, fenofibrate, and pioglitazone on changes in plasma apo C-III and adiponectin levels in beagle dogs. Two weeks of treatment with E3030 significantly reduced plasma apo C-III levels at 0.1 mg/kg or greater and increased plasma adiponectin levels at 0.3 mg/kg or greater (Figs. 5 and 6). Fenofibrate significantly reduced plasma apo C-III, but did not significantly alter plasma adiponectin levels at a dose of 100 mg/kg. Pioglitazone did not significantly alter plasma apo C-III levels, but significantly increased plasma adiponectin levels at a dose of 1 mg/kg.

We also investigated the effects of E3030, fenofibrate, and pioglitazone on changes in LPL levels in post-heparin plasma in beagle dogs. In this study, the increase in LPL levels not only by fenofibrate but also by E3030 was observed (Fig. 7). On the other hand, pioglitazone caused no significant change.



**Fig. 6.** Effects of E3030, fenofibrate, and pioglitazone on plasma adiponectin levels in beagle dogs. Drugs were orally administered once a day for 14 days. Results are each expressed as the mean  $\pm$  S.E.M. of six animals. \* $P$  < 0.05 vs vehicle control (repeated measures ANCOVA followed by the Dunnett type multiple comparison test using baseline values as covariates).



**Fig. 7.** Effects of E3030, fenofibrate, and pioglitazone on LPL levels in post-heparin plasma in beagle dogs. Drugs were orally administered once a day for 15 days. Results are each expressed as the mean  $\pm$  S.E.M. of six animals. \* $P$  < 0.05 vs vehicle control (difference between the vehicle control group and fenofibrate or pioglitazone group was analyzed by the unpaired  $t$ -test; differences between the vehicle control and E3030 treated groups were analyzed by one-way ANOVA followed by the Dunnett type multiple comparison test).



E3030, fenofibrate, and pioglitazone did not significantly affect normoglycemia and body weight in normal dogs at all doses (data not shown).

## Discussion

The cornerstone of therapy for type 2 diabetes mellitus remains exercise and weight reduction. However, this often fails and patients require a pharmacologic agent. Drug therapy includes insulin, sulfonamides, biguanides,  $\alpha$  glucosidase inhibitor, insulin secretagogues, and insulin sensitizers. Diabetes is a complex disorder that has implications for the direct or indirect progression of disease affecting a variety of target organs. One of these target organs is the vascular system. Diabetics are prone to the development of atherosclerosis. Seventy percent of patients with type 2 diabetes die of cardiovascular disease and diabetes is associated with a two to three fold excess risk of coronary heart disease (2). Type 2 diabetes mellitus often is characterized by elevated levels of fasting blood glucose, hemoglobin A<sub>1c</sub>, and insulin with insulin resistance. It is also characterized by increased levels of cholesterol and TG and decreased levels of HDL cholesterol. Clinically, patients tend to be overweight. The elevated glucose, insulin, cholesterol, and TG all contribute to an increased risk of comorbidities and death. Therefore, an agent that can control both blood glucose and lipid levels would be a significant advancement in the treatment of diabetes.

We investigated the antidiabetic effects of E3030, which is a potent dual activator of PPAR $\alpha$  and PPAR $\gamma$ , in an animal model of diabetes (db/db mice). E3030 improved insulin resistance, lowered blood glucose levels, and raised adiponectin levels in db/db mice (Figs. 2 and 3), similar to other PPAR $\gamma$  agonists (14–16). As adiponectin is known to improve glucose metabolism by increasing insulin sensitivity (17, 18), it can be suggested that E3030's effects on glucose metabolism are mediated through this rise.

We also investigated the hypolipidemic effects of E3030 in beagle dogs, whose PPAR $\alpha$  and PPAR $\gamma$  transactivation responses to E3030 were similar to those of humans. After 2 weeks of treatment, E3030 significantly reduced both plasma TG and non-HDL cholesterol levels at a dose of 0.03 mg/kg or greater (Fig. 4). Percent decreases in non-HDL cholesterol were more than those in HDL cholesterol. As a consequence of this more potent effect of E3030 on non-HDL cholesterol, a significant reduction in the non-HDL cholesterol/HDL cholesterol ratio was observed in E3030-treated dogs.

In this study, a reduction in apo C-III levels and increase in LPL levels not only by fenofibrate but also

by E3030 in beagle dogs was observed (Figs. 5 and 7). LPL is an enzyme involved in the hydrolysis of TG. On the other hand, apo C-III is an apolipoprotein that inhibits hydrolysis of TG by LPL. It is reported that fenofibrate decreases plasma TG and non-HDL cholesterol levels, at least in part, through the following mechanism (19–21): Fenofibrate increases LPL level and decreases apo C-III level through a PPAR $\alpha$  agonistic action and reduces TG content of very low density lipoprotein (VLDL), a TG-rich lipoprotein, thus converting VLDL first to intermediate density lipoprotein (IDL) and then to LDL. IDL and LDL are taken up by the liver via the LDL receptor and cholesterol is eliminated in bile as bile acids. These results indicate that the TG- and non-HDL cholesterol-lowering actions of E3030 involve combined effects on reduction of apo C-III and elevation of LPL, resulting in increased lipolysis.

Plasma apo C-III and adiponectin are biomarkers of PPAR $\alpha$  (20, 21) and PPAR $\gamma$  (15, 22) activation, respectively. Two weeks of treatment with E3030 significantly reduced plasma apo C-III levels at 0.1 mg/kg or greater (Fig. 5) and elevated plasma adiponectin levels at 0.3 mg/kg or greater (Fig. 6). The changes in plasma apo C-III and adiponectin indicated that E3030 could be expected to activate PPAR $\alpha$  and PPAR $\gamma$  at a similar dose in beagle dogs. From the changes in biomarkers of PPAR $\alpha$  and PPAR $\gamma$ , fenofibrate and pioglitazone were confirmed to activate PPAR $\alpha$  and PPAR $\gamma$  at 100 and 1 mg/kg, respectively, in beagle dogs. Although fenofibrate at a dose of 100 mg/kg exhibited hypolipidemic effects similar to those observed for E3030, pioglitazone at a dose of 1 mg/kg had little effect (Fig. 4). These results indicate that the hypolipidemic effects of E3030 in beagle dogs are mostly based on its PPAR $\alpha$  agonist activity.

In conclusion, the experimental results in animals suggest that E3030 has the potential for use in treatment of various aspects of metabolic dysfunction in type 2 diabetes, including dyslipidemia, hyperglycemia, hyperinsulinemia, and impaired glucose disposal.

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