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Short-Term Effect of Bezafibrate on the Expression of Adiponectin mRNA in the Adipose Tissues

A Study in Spontaneously Type 2 Diabetic Rats with Visceral Obesity

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The effect of short-term bezafibrate (BF) administration over time on the expression of adiponectin mRNA in the tissues was examined in Otsuka Long Evans Tokushima Fatty (OLETF) rats. Eight-week-old rats were divided into the high-dose (100 mg/kg) BF group (*n* = 15), the low-dose (10 mg/kg) BF group (*n* = 15), or the control group (*n* = 15) and followed up for 14 d. Triglyceride and free fatty acid levels significantly decreased in a dose-dependent manner in the high-dose BF group. The insulin levels increased with time, although they were significantly lower in the high-dose BF group on d 3 and 7 than the control group. Adiponectin levels significantly increased in the high-dose BF group. On d 14 of BF administration, the levels of VLDL and chylomicron were significantly lower in BF groups, and adiponectin mRNA expression in the white adipose tissue was significantly higher in the high-dose BF group. Findings from this study suggest that in type 2 diabetes with insulin resistance, hypertriglyceridemia is closely linked to adiponectin.

Key Words: Hypertriglyceridemia; adiponectin; bezafibrate; OLETF rats.

Introduction

Adiponectin, an adipose-specific hormone factor, has been confirmed to possess insulin sensitivity-enhancing activity and anti-atherogenic activity. Hypoadiponectinemia is reported to be related to the development of diseases such as type 2 diabetes and arteriosclerosis, which form part of the metabolic syndrome *(1–3)*.

It has been reported that the gene transcription factor peroxisome proliferators-activated receptor (PPAR) plays an important role in glucose and lipid metabolism *(4)*. PPAR-α, an isoform of PPAR, is a receptor of clofibrate, which is primarily expressed in liver, and its activation by derivatives of clofibrate including bezafibrate has been clinically used to improve lipid metabolism especially by reducing triglyceride (TG) levels *(5)*. A ligand of PPAR-α, bezafibrate, has recently been shown to improve insulin sensitivity in rats with diet-induced insulin resistance *(6)*, to improve glucose tolerance in IGT patients with hypertriglyceridemia *(7)*, and to reduce fasting plasma glucose levels and HbA1c levels in patients with type 2 diabetes *(8)*. Furthermore, a recently released report shows that bezafibrate suppressed and delayed the onset of type 2 diabetes in patients with impaired fasting glycemia (fasting blood glucose value of 110–125 mg/dL) during a 6.2-year follow-up period *(9)*.

This study examined the effects over time of short-term bezafibrate (BF) administration on the expression of adiponectin mRNA in the adipose tissues and on the blood adiponectin levels in spontaneously type 2 diabetic OLETF rats with visceral obesity, to investigate the relationship between hypertriglyceridemia and adiponectin.

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Results

Changes in Body Weight and Feed Intake

No significant differences in body weight and feed intake were seen among the various groups throughout the study period (Fig. 1).

Changes in Plasma Glucose, Free Fatty Acid, Insulin, and Adiponectin Levels

Throughout the entire course, the plasma glucose levels had unchanged in the all groups. Triglyceride levels significantly decreased in both low- and high-dose BF groups in a dose-dependent manner throughout the study. Likewise, free fatty acid levels decreased in these groups in a dose-dependent manner, with statistical significance reached in the high-dose BF group on d 3, 7, and 14 of administration (Fig. 1). The insulin levels increased with time in all

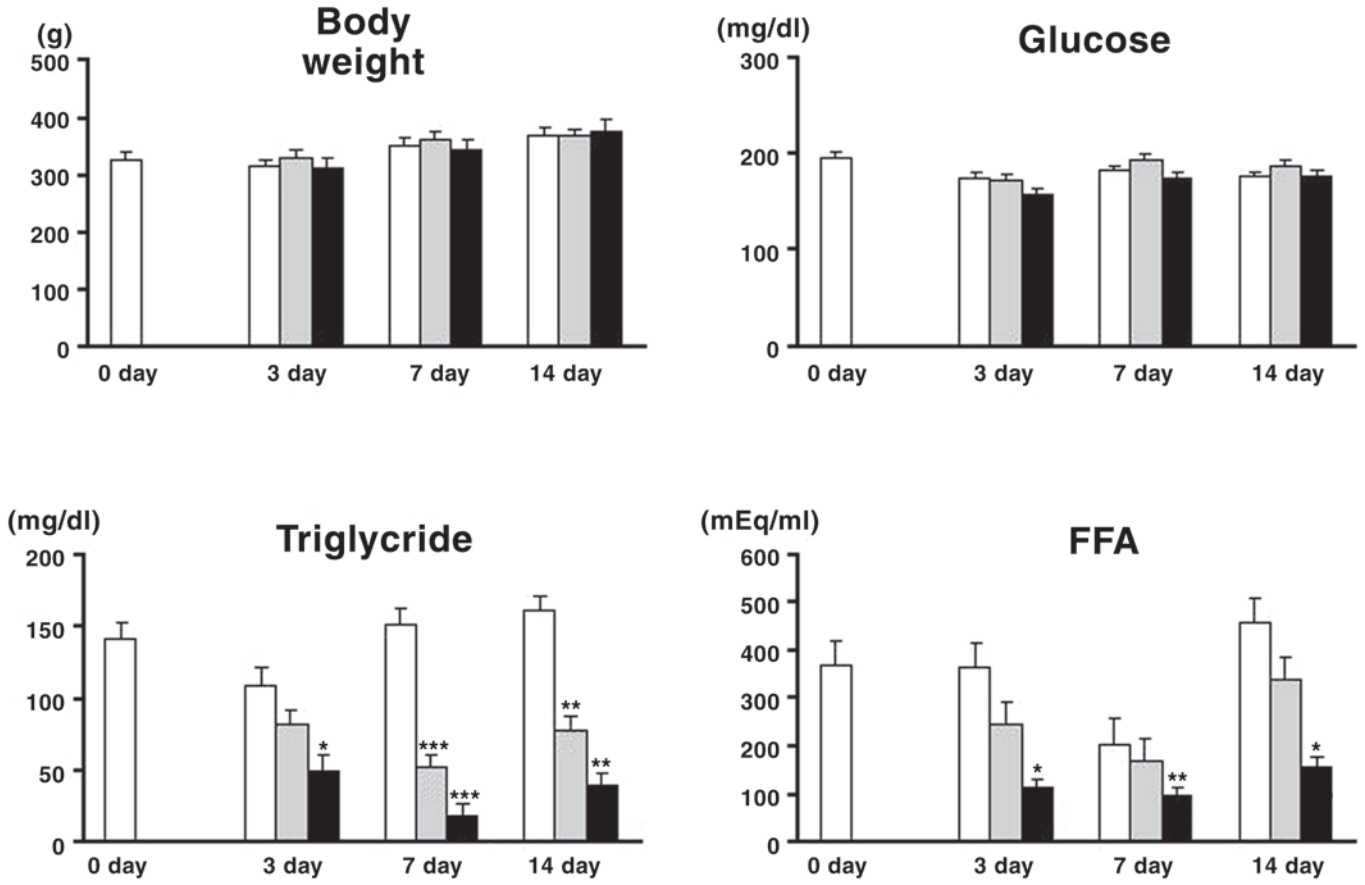

Fig. 1. Changes in body weight, plasma glucose, triglyceride, and free fatty acid levels in OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs control group.

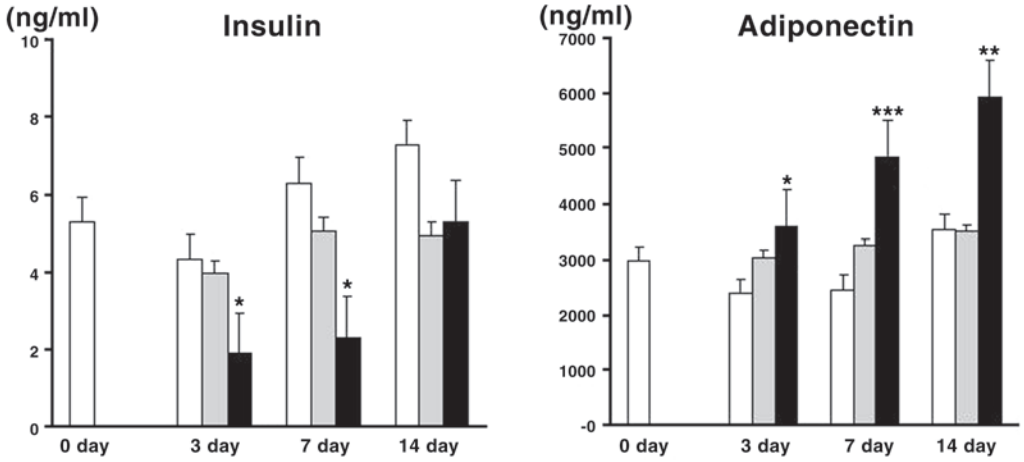

Fig. 2. Changes in insulin and adiponectin levels in OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs control group.

the groups, although they were significantly lower in the high-dose BF group on d 3 and 7 of administration than the control group. Adiponectin levels significantly increased on d 3, 7, and 14 of BF administration in the high-dose BF group (Fig. 2). Very-low-density lipoprotein (VLDL)–TG levels were significantly lower in both low- and high-dose BF groups in a dose-dependent manner, and chylomicron–TG levels were significantly lower in high-dose BF groups on d 14 of administration (Fig. 3).

Levels of Adiponectin mRNA Expression

The expression of adiponectin mRNA tended to increase after 3 d of high-dose BF administration and significantly increased 14 d after high-dose BF administration (Fig. 4).

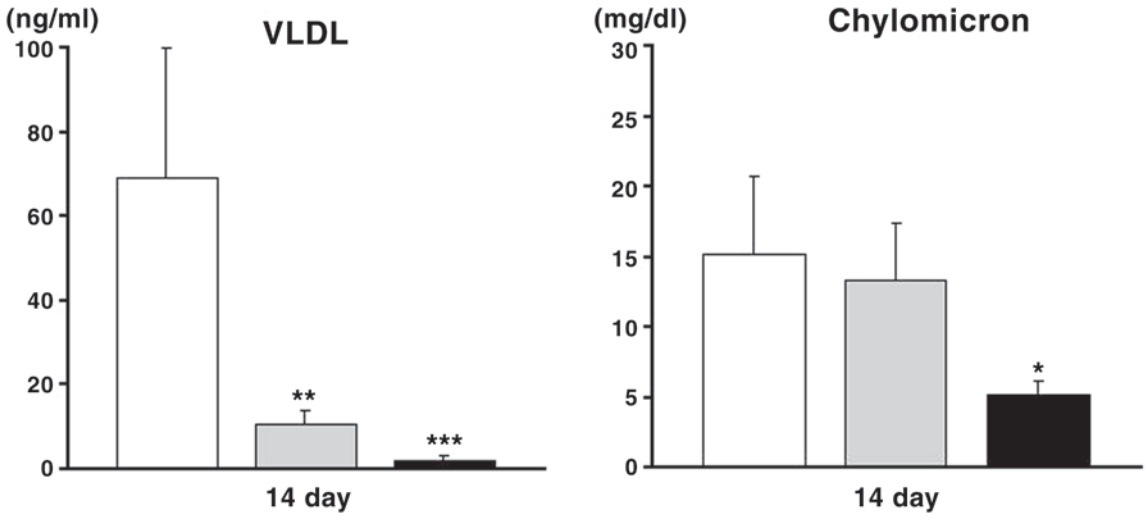

Fig. 3. Changes in VLDL–TG and chylomicron–TG levels in OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).*$p < 0.05$ and **$p < 0.01$ vs control group.

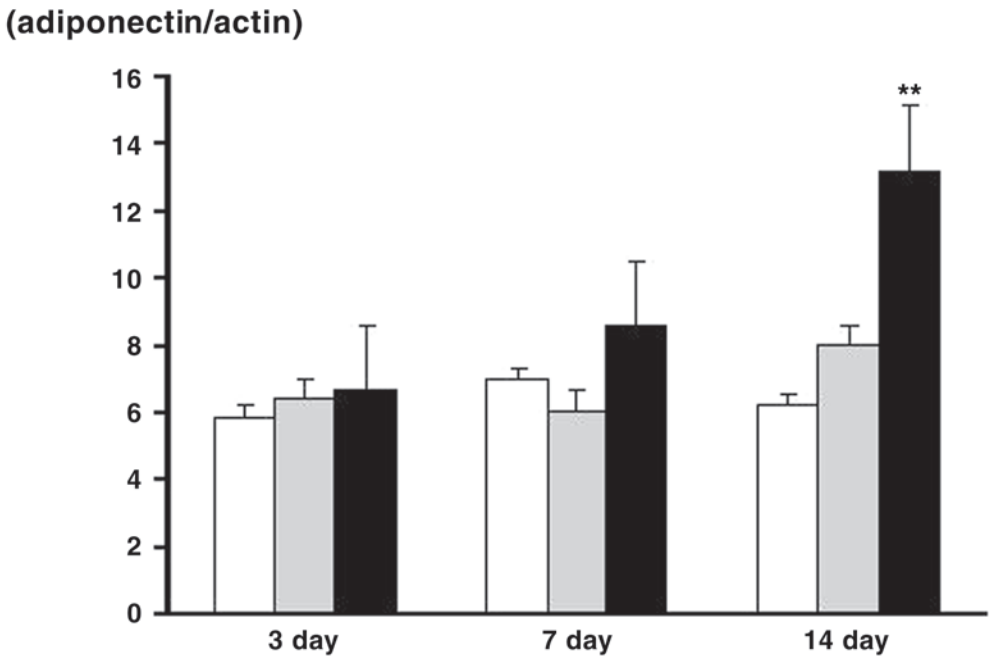

Fig. 4. Changes in the levels of adiponectin mRNA expressed in the white adipose tissues of OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).*$p < 0.05$, and **$p < 0.01$ vs control group.

Discussion

This study used spontaneously type 2 diabetic OLETF rats *(10)* that are reported to have reduced LPL mRNA expression in the subcutaneous adipose tissues and the skeletal muscles because of insulin resistance, as well as delayed triglyceride metabolism. The rats were given bezafibrate *(11)*, which is reported to increase LPL by way of PPAR-α, for 4 wk. As a result, a significant decrease was observed in plasma triglyceride, free fatty acid, chylomicron, VLDL, and insulin levels. In conjunction with this, a significant increase was noted in the expression of adiponectin mRNA in the adipose tissues as well as in blood adiponectin levels.

A peroxisome proliferator-activated receptor γ (PPAR-γ) agonist is reported to elevate the concentration of adiponectin in the blood *(12)*. With regard to the potential mechanisms of action involved, Iwaki et al. report that (1) a PPAR-responsive element (PPRE) exists in adiponectin promoter region, and that located close to it is LRH-RE, a binding element of another orphan nuclear hormone receptor, the liver receptor homolog-1 (LRH-1) and that (2) the heterodimers of the retinoid X receptor (RXR) and PPAR-γ work in a collaborative fashion with LRH-1 to maximally elevate adiponectin's transcription activity *(13)*. This suggests that in our study also, bezafibrate may have acted directly

on the adipose tissues via its PPAR-γ activity *(14)*, affecting adiponectin as a result.

In their study in healthy volunteers, Möhlig et al. reported that, in steady states achieved with a euglycemic hyperinsulinemic clamp, adiponectin significantly decreases from baseline, and that hyperinsulinemia lasting about 2 h induces hypoadiponectinemia *(15)*. However, the relationship between acute effects of insulin on circulating adiponectin levels is unclear and to clarify the relation of bezafibrate-induced change in insulin levels for the early times of treatment to the adiponectin expression in the adipose tissues or circulating adiponectin levels in our study, further investigations are necessary.

In our study, a inverse relationship between the changes in triglyceride or free fatty acid levels and the changes in adiponection levels was observed. There were several reports concerning the relationship between hypertriglyceridemia and blood TNF-α levels or TNF-α production *(16,17)*. Therefore, while TNF-α production was not investigated in our study, there is a possibility that the bezafibrate-induced reduction in VLDL may have led to decreased TNF-α production, and diminished the ability of TNF-α to suppress adiponectin production, thus elevating the level of adiponectin.

On the other hand, in this study, the bezafibrate-induced increase in insulin sensitivity or decrease in glucose intolerance, which have been previously reported *(6–8)*, was not apparent. However, we previously reported that long-term (from 8 to 24 wk of age) bezafibrate treatment inhibited the progression of obesity and decreased plasma insulin levels in the same OLETF rat models *(18)*. Therefore, the reasons why these effects were not apparent in this experiment are as follows: the ages from 8 to 12 wk in OLETF rats are the stage at which the fat accumulation rapidly progresses and a significant increase in insulin resistance that was estimated by euglycemic insulin clamp study *(19)* occurs, and the experiment duration is too short to clarify whether the bezafibrate treatment increase the insulin sensitivity or not.

We conclude that the results obtained in our study—that bezafibrate reduced plasma triglyceride and free fatty acid levels while increasing adiponectin levels—indicate that hypertriglyceridemia is closely related to adiponectin and the bezafibrate-induced increase in adiponectin levels may account at least in part for its insulin resistance ameliorating effect.

Materials and Methods

Fifty male OLETF rats *(20)* were provided at 4 wk of age by the Tokushima Research Institute, Otsuka Pharmaceutical Co (Tokushima, Japan). The animals were housed in plastic cages (320 × 270 × 175 mm) in an animal room with a controlled temperature (23°C ± 2°C) and relative humidity (55% ± 15%), and a 12-h light/12-h dark cycle (lights on at 0700 hour). They were supplied with rat chow (CE-2; Clea Japan, Inc.) and tap water *ad libitum* until 8 wk of age. When the rats were 8 wk old, five rats were sacrified and the rest of them ($n = 45$) were randomly allocated to three groups: one control group ($n = 15$) and two bezafibrate (Kissei Pharmaceutical, Matsumoto) groups including one ($n = 15$) administered 200 ppm mixed in food (which is 10 mg/kg bw in terms of normal food consumption amount; bezafibrate 10 mg/kg group hereafter) and the other group ($n = 15$) administered 2000 ppm (100 mg/kg bw in terms of normal food consumption amount; bezafibrate 100 mg/kg group hereafter). While they were individually reared in plastic cages, food consumption was determined by 3 days' average food intake from d 0, 4, and 11 after the start of administration. On d 3, 7, and 14 after administration, non-fasting body weight was measured. The guidelines for Laboratory Animal Facilities of the Jikei University School of Medicine were followed for the care and use of the animals in this study. Blood was subsequently drawn from these animals to measure plasma glucose, triglyceride, free fatty acid, insulin, and adiponectin levels. The assay kits for plasma glucose, TG, and FFA were obtained from Wako Pure Chemical Industries (Osaka, Japan). The plasma triglyceride levels in lipoprotein fractions were determined by agarose gel electrophoresis, followed by triglyceride-specific staining using the Chol/Trig Combo system (Helena Laboratories, Saitama, Japan). Insulin and adiponectin were measured by insulin ELISA kit from Morinaga (Tokyo, Japan) and adiponectin ELISA kit from Otsuka (Tokushima, Japan). The animals were then killed under non-fasting conditions, after which their retroperitoneal white adipose tissues (WAT) were removed and frozen under liquid nitrogen, and then preserved at –80°C. Total RNA was separated from frozen tissues by using TRIZOL Reagent (Invitrogen, California, USA), and the level of relative mRNA expression was measured by using quantitative real-time polymerase chain reaction (PCR) via a TaqMan analysis that employed specific primers and probes. Oligonucleotide sequences of gene-specific primers and probes for TaqMan analysis of rat adiponectin mRNAs were as follows: forward (sense) 5'- CCC TCC ACC CAA GGA AAC TT -3', reverse (sense) 5'- GGT ATC CCA TTG TGA CCA GGA -3', TaqMan probe 5'- AGG TTG GAT GGC AGG CAT CCC A -3'. PCR was set for 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 120 s. Relative expression levels are indicated as ratio of adiponectin copy number to β actin.

The results of statistical analysis were represented as mean ± SEM. Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Scheffe's method, as a post-hoc test to detect any significant differences among the groups ($p < 0.05$).

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