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Effects of Long-term Bezafibrate Treatment on Body Fat Accumulation and Tissue Triglyceride Content in Otsuka Long-Evans Tokushima Fatty Rats : Its Relation to the Expression of Uncoupling Protein 3 mRNA

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ABSTRACT

Fibrates stimulate β-oxidation of fatty acids in the liver and increase levels of uncoupling protein (UCP)-3 mRNA in white adipose tissue and skeletal muscle. We studied the effects of long-term bezafibrate treatment on body fat accumulation, tissue triglyceride content, and UCP-3 mRNA expression. Male Otsuka Long-Evans Tokushima Fatty rats were fed diets containing 2, 000 ppm (100 mg/kg), 200 ppm (10 mg/kg), or 0 ppm (control) bezafibrate and followed up for 16 weeks. Although food consumption increased in rats fed bezafibrate, increases in body weight were inhibited dose-dependently. Plasma levels of insulin, leptin, free fatty acids, and triglyceride decreased, although not significantly, in rats fed 10 mg/kg bezafibrate and decreased significantly in rats fed 100 mg/kg bezafibrate. Rats treated with bezafibrate showed significant decreases in body fat weight and triglyceride levels in liver, pancreas, and skeletal muscle. Expression of UCP-2 increased significantly in brown adipose tissue in the 10 mg/kg and 100 mg/kg groups and that of UCP-3 increased significantly in skeletal muscle in the 10 mg/kg group and in white adipose tissue in the 100 mg/kg group. We found that long-term bezafibrate treatment inhibited the progression of obesity in male Otsuka Long-Evans Tokushima Fatty rats. Our results suggest that a decrease in the triglyceride content of skeletal muscle is involved in the mechanism by which bezafibrate decreases hyperinsulinemia and that increased expression of UCP-3 mRNA in skeletal muscle promotes energy expenditure and decreases accumulated triglyceride, thereby decreasing hyperinsulinemia. (Jikeikai Med J 2003 ; 50 : 75-83)

Key words : bezafibrate, obesity, hyperinsulinemia, triglyceride content, uncoupling protein 3 expression

Introduction

Uncoupling protein (UCP)-2[1,2] and UCP-3[3,4] have recently been identified as new members of the UCP family. These molecules are present in white and brown adipose tissue and skeletal muscle. UCP-

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3 is a major UCP in skeletal muscle, where UCP-3 mRNA expression is decreased in patients with type 2 diabetes[5]. Expression of UCP-3 mRNA is positively correlated with the glucose utilization rate, as determined with the glucose clamp method[5]. In addition, UCP-3 mRNA expression in skeletal muscle is positively correlated with the resting metabolic rate in Pima Indians[6].

These findings suggest that both UCP-1 and UCP-3 play important roles in the energy-expenditure adjustment system. Moreover, transgenic mice overexpressing human UCP-3 in skeletal muscle are hyperphagic but weigh less than their wild-type littermates[7]. Fibrate drugs extensively used as antihyperlipidemia agents increase UCP-3 mRNA expression in white adipose tissue and skeletal muscle through activation of peroxisome proliferator-activated receptor alpha (PPARα)[8,9]. We investigated the effects of long-term administration of bezafibrate on body fat accumulation and tissue triglyceride content in relation to UCP-3 mRNA expression in Otsuka Long-Evans Tokushima Fatty (OLETF) rats[10,11], which are a spontaneously obese animal model of type 2 diabetes.

METHODS

Twenty-four male OLETF rats provided by Tokushima Laboratories, Otsuka Pharmaceutical (Tokushima, Japan), were used. National Research Council Guidelines for the use and care of these animals were strictly adhered to. At the age of 8 weeks, 8 rats were randomly assigned to each of 3 groups: the 100 mg/kg group received 2,000 ppm bezafibrate (Kissei Pharmaceutical, Matsumoto, Japan) mixed in food (which is 100 mg/kg body weight based on average food consumption); the 10 mg/kg group received 200 ppm bezafibrate; and the control group received 0 ppm bezafibrate. Rats received the respective treatments for 16 weeks while individually housed in plastic cages. Food consumption was determined every 4 weeks, and body weight was determined every 2 weeks. Blood was collected from the retro-orbital venous plexus of nonfasted animals at baseline and at 8 and 16 weeks after the start of treatment to determine plasma levels of glucose, insulin, leptin, triglyceride, free fatty acid (FFA), and total cholesterol (TC). Sixteen weeks after the start of treatment, levels of plasma thyroid hormones (triiodothyronine [T3] and thyroxine [T4]) and corticosterone were determined. Plasma levels of insulin, leptin, thyroid hormones, and corticosterone were determined with radioimmunoassay; plasma levels of glucose, triglyceride, FFA, and TC were determined with the enzyme method.

After 16 weeks nonfasted animals were killed, and tissue weights were determined from left retroperitoneal fat, mesenteric fat, left epididymal fat, left abdominal subcutaneous fat, and liver. The range of abdominal subcutaneous fat resection was determined with the method of Krotkiewski and Björntorp[12]. The liver, pancreas, and skeletal muscle (paravertebral muscle) were homogenized, and the lipids were extracted according to method of Folch et al.[13] to determine tissue triglyceride content (mg/g tissue weight).

Total RNA was extracted[14] from liver, skeletal muscle (gastrocnemius), white adipose tissue (retroperitoneal fat), and brown adipose tissue in 5 rats of each group, and the samples were processed with Northern blotting according to the method of Lehrach et al.[15]. The cDNAs of UCP-1, UCP-2, UCP-3, PPARα, PPARγ, and PPARδ were prepared with the polymerase chain reaction method, and hybridization was performed with cDNA probes labeled with ^{32}P-cytidine-5′-triphosphate. The level of each mRNA was corrected with the intensity of the 28S rRNA band as an internal standard.

All values are expressed as means±SD. Differences between the 3 groups were examined with one-way variance analysis, and those between 2 groups were examined with nonpaired comparison. Differences with $p<0.05$ were considered significant.

RESULTS

Increases in body weight during the treatment period were inhibited dose-dependently in rats receiving bezafibrate. Body weight was significantly lower in rats receiving 100 mg/kg bezafibrate than in con-

trol rats after the 10th week of treatment (Fig. 1). Food consumption was significantly higher in rats receiving bezafibrate than in control rats after 14 weeks of treatment (Fig. 2). Glucose and TC levels did not differ between the groups. However, plasma levels of insulin, leptin, FFA, and triglyceride were slightly but not significantly lower than control in rats receiving 10 mg/kg bezafibrate and were significantly lower than control in rats receiving 100 mg/kg bezafibrate (Fig. 3). Although plasma T3 levels did not differ significantly between the groups, plasma T4 levels in both bezafibrate groups and corticosterone levels in the 100 mg/kg group were significantly lower than those in control rats (Table 1).

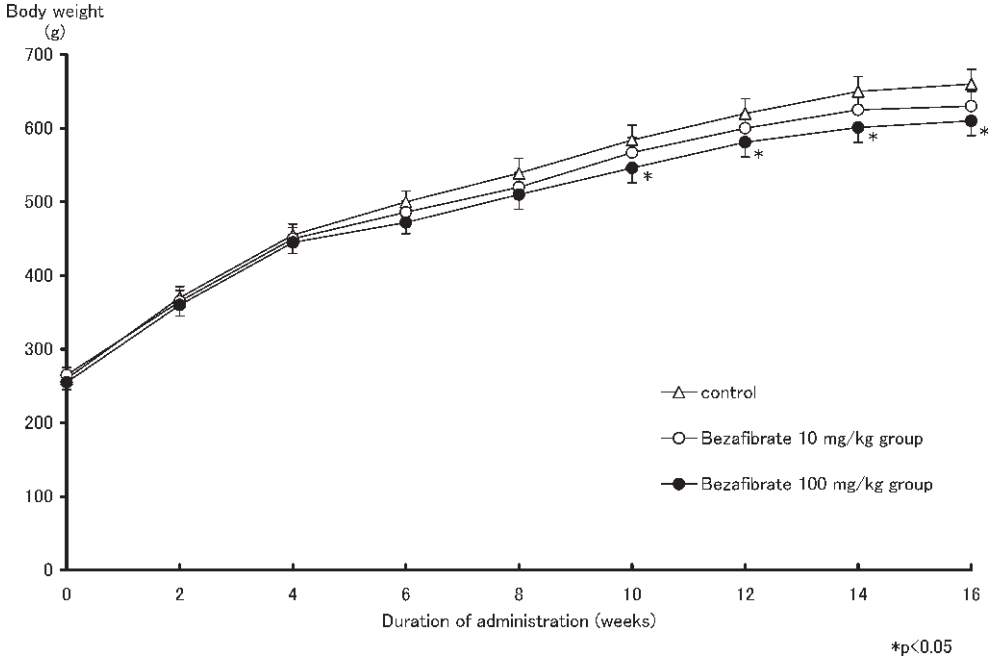

Fig. 1. Changes in body weight of OLETF rats treated with bezafibrate.

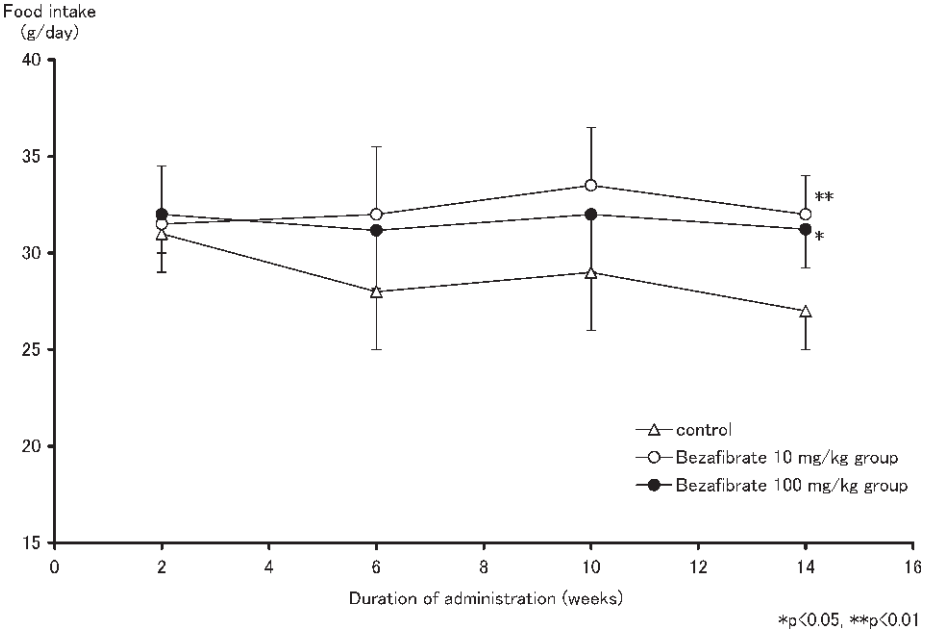

Fig. 2. Changes in food intake by OLETF rats treated with bezafibrate.

The retroperitoneal fat weight at the end of treatment was significantly lower ($p<0.01$) in rats receiving bezafibrate than in control rats, but weights of other fat tissues did not differ significantly between the groups (Table 2). Liver weight in rats receiving 10 mg/kg ($p<0.05$) or 100 mg/kg ($p<0.01$) bezafibrate was significantly higher than control (Table 2).

Tissue triglyceride content was significantly lower than control in the liver of rats receiving 100 mg/kg bezafibrate and in the pancreas and paravertebral muscle of rats receiving 10 mg/kg or 100 mg/kg bezafibrate (Fig. 4).

Expression of UCP-1 was found only in brown adipose tissue and was not increased by bezafibrate. Expression of UCP-2 was found in skeletal muscle, liver, and brown and white adipose tissue but was increased by bezafibrate only in brown adipose tissue. Expression of UCP-3 was observed in skeletal muscle

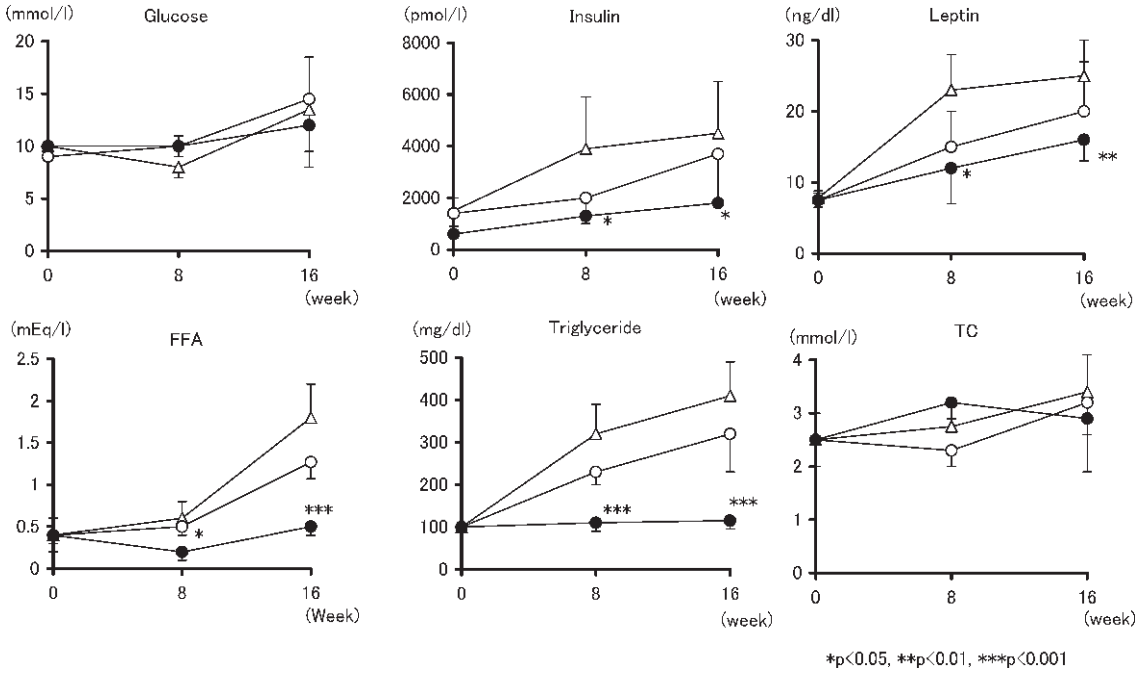

Fig. 3. Changes in plasma levels of glucose, insulin, leptin, FFA, triglyceride, and TC in OLETF rats treated with bezafibrate. -△- control, -○- bezafibrate 10 mg/kg group, -●- bezafibrate 100 mg/kg group

Table 1. Plasma triiodothyronine, thyroxine, and corticosterone levels in OLETF rats treated with bezafibrate

	T3 (ng/ml)	T4 (μg/dl)	Corticosterone (ng/mL)
Control	1.16±0.09	5.36±0.36	255.4±67.0
Bezafibrate 10 mg/kg	1.09±0.10	4.74±0.48*	201.6±43.2
Bezafibrate 100 mg/kg	1.18±0.10	3.80±0.35**	148.4±57.9*

*$p<0.05$, **$p<0.01$, vs. control.

Table 2. Tissue weight in OLETF rats treated with bezafibrate (g/100 g body weight)

	Retroperitoneal fat	Mesenteric fat	Epididymal fat	Subcutaneous fat	Liver
Control	2.69±0.22	2.06±0.16	1.23±0.13	1.71±0.29	4.46±0.52
Bezafibrate 10 mg/kg	2.15±0.21**	2.12±0.17	1.22±0.08	1.50±0.18	5.44±0.58*
Bezafibrate 100 mg/kg	2.10±0.12**	2.05±0.24	1.25±0.22	1.57±0.16	6.34±0.55**

*$p<0.05$, **$p<0.01$, vs. control.

and brown and white adipose tissue. The increase in UCP-3 expression by bezafibrate was especially noted in the skeletal muscle and white adipose tissue (Fig. 5). When expression was quantified with an imaging analyzer, significant increases in UCP-2 expression were noted in brown adipose tissue in both bezafibrate

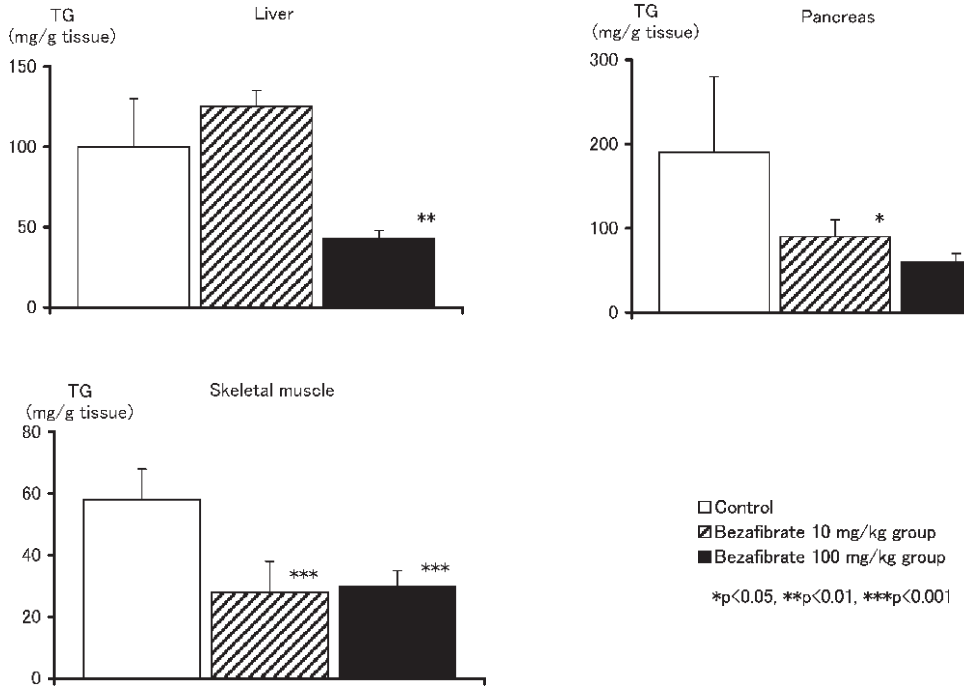

Fig. 4. Tissue triglyceride content in OLETF rats treated with bezafibrate.

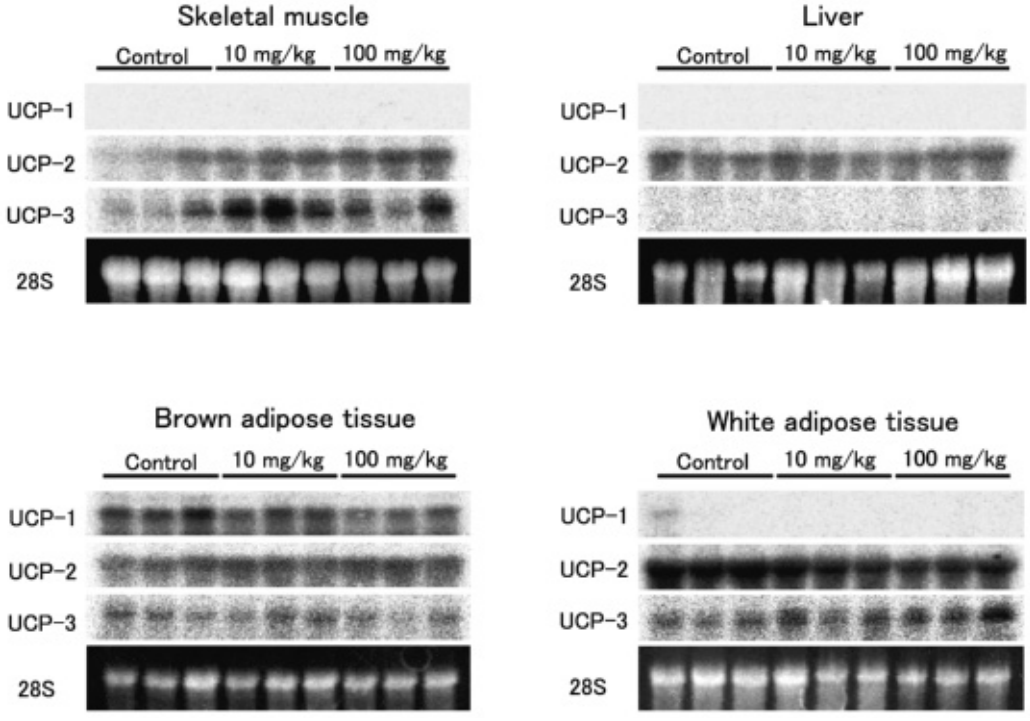

Fig. 5. Representative UCP-1, UCP-2, and UCP-3 mRNA expression in skeletal muscle, liver and brown and white adipose tissue of OLETF rats treated with bezafibrate.

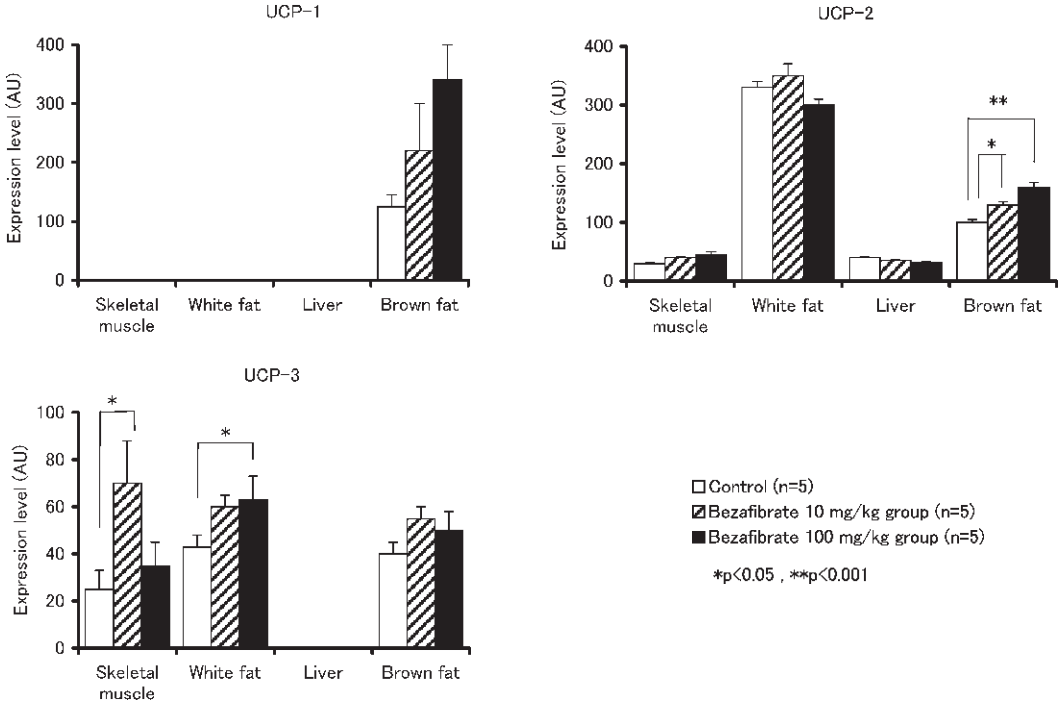

Fig. 6. Comparison of UCP-1, UCP-2, and UCP-3 mRNA expression in skeletal muscle, white adipose tissue, liver, and brown adipose tissue of OLETF rats treated with bezafibrate.

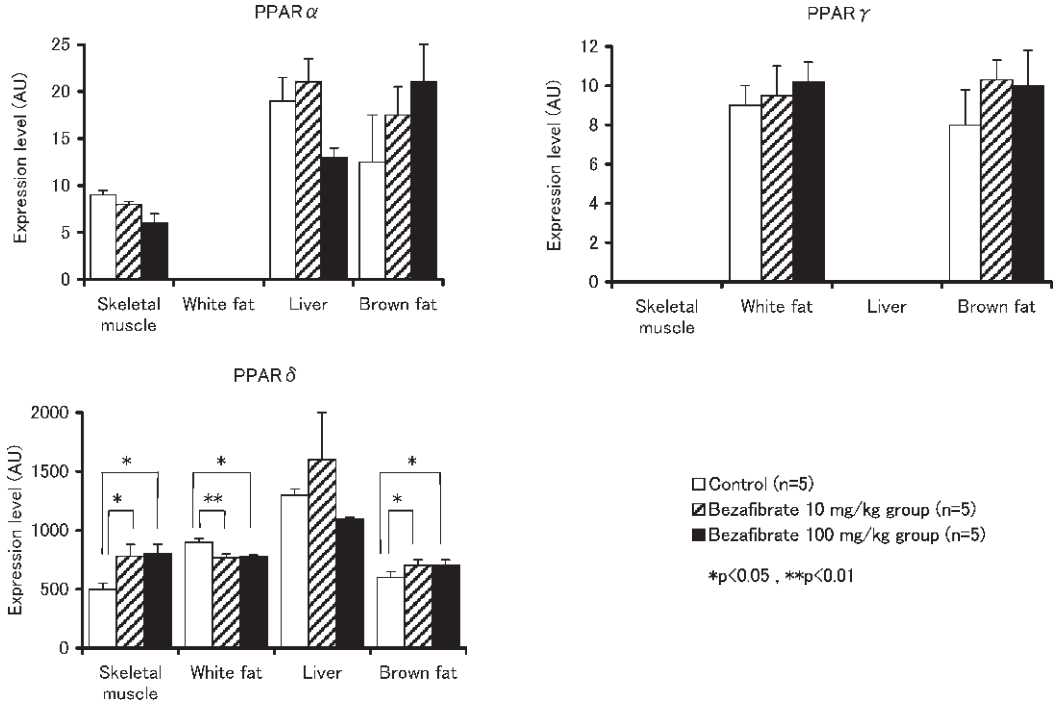

Fig. 7. Comparison of PPARα, PPARγ, and PPARδ mRNA expression in skeletal muscle and white and brown adipose tissue of OLETF rats treated with bezafibrate.

groups, in UCP-3 expression in the skeletal muscle of rats receiving 10 mg/kg bezafibrate, and in UCP-3 expression in white adipose tissue of rats receiving 100 mg/kg bezafibrate (Fig. 6).

Expression of PPARα was noted in skeletal muscle, liver, and brown adipose tissue, and expression of PPARγ was noted in white and brown adipose tissue; however, expression was not increased by bezafibrate. In contrast, expression of PPARδ was noted in skeletal muscle, white and brown adipose tissue, and liver. Bezafibrate significantly increased expression of PPARδ in skeletal muscle and brown adipose tissue and significantly decreased expression in white adipose tissue (Fig. 7).

Discussion

We found that long-term treatment with bezafibrate inhibited the progression of obesity and hyperinsulinemia in OLETF rats. Because the amount of food consumed by rats given bezafibrate tended to increase over that consumed by control rats throughout the treatment period, we believe that bezafibrate inhibits the progression of obesity by promoting energy expenditure.

In the present study, expression of UCP-1 mRNA was observed in only brown adipose tissue and was not increased by bezafibrate. This result agrees with that of Cabrero et al.[9], ruling out the involvement of UCP-1 in bezafibrate's promotion of energy expenditure and inhibition of the progression of obesity.

Expression of UCP-2 mRNA was observed in skeletal muscle, liver, and white and brown adipose tissue. However, unlike Cabrero et al.[9], we observed an increase in expression by bezafibrate in only brown adipose tissue. The result of Cabrero et al.[9] might be attributed to their substantially different experimental conditions, that is, shorter treatment period and healthy animals. In contrast, fenofibrate[16] and Wy-14644[17], which are also fibrate-type drugs, have been reported to increase UCP-2 mRNA expression in the liver. These increases are probably because the intensity of PPARα, PPARγ, and PPARδ as ligands for the bezafibrate used in this experiment is somewhat different[18] from that for fenofibrate and Wy-14643.

We observed expression of UCP-3 mRNA in skeletal muscle and white and brown adipose tissue. The increase in expression by bezafibrate was especially significant in the skeletal muscle and white adipose tissue. This result agrees with that of Cabrero et al.[9]. However, we found that bezafibrate increased UCP-3 mRNA expression in skeletal muscle in rats receiving 10 mg/kg but not in rats receiving 100 mg/kg. Although the reason for this difference is unclear, Weigle et al.[19] have reported that UCP-3 expression in skeletal muscle is enhanced by injection of lipids into the blood. A positive correlation between the amount of UCP-3 mRNA in skeletal muscle and the plasma level of FFA has also been reported[20]; these findings suggest that the increased expression of UCP-3 in skeletal muscle is related to the use of lipids as energy[19,21]. In other words, FFA levels in the blood were decreased in rats that received 100 mg/kg bezafibrate, which also seems to have decreased UCP-3 mRNA expression in skeletal muscle. In a long-term experiment such as our present study, secondary changes could have occurred in UCP-3 mRNA expression in skeletal muscle after initial induction. Therefore, changes over time in UCP-3 mRNA expression should be studied. Although UCP-3 mRNA expression in skeletal muscle is reportedly influenced by thyroid hormones[22,23], leptin[22], and glucocorticoids[22], levels of these hormones decreased significantly in the present study. We assume that the observed decreases in thyroid hormones, leptin, and corticosterone with bezafibrate were secondary changes.

Expression of PPARα was observed in skeletal muscle, liver, and brown adipose tissue, whereas expression of PPARγ was observed in white and brown adipose tissue. However, expression of these PPAR subtypes did not differ between the groups. In contrast, the expression of PPARδ was commonly observed in skeletal muscle, liver, and white and brown adipose tissue and was significantly increased by bezafibrate in skeletal muscle and brown adipose tissue. We cannot assume that all changes in UCP mRNA expression we observed were mediated by PPARδ. Instead, as has been suggested, lipid metab-

olism and energy expenditure might be promoted by activation of PPARα, mainly in the liver, to adjust the proteins involved in lipid biosynthesis and lipoprotein metabolism. Alternatively, UCP-3 expression in white adipose tissue might be mediated by PPARδ, because PPARα was not expressed there. Results of a study using cultured skeletal muscle L6 cells suggest that PPARδ is involved in the increase of UCP-3 mRNA expression by fatty acids[24]. Accordingly, a direct action mediated by PPARδ cannot be ruled out. Because the complete function of PPARβ/δ/NUC1 remains unclear, the relation of PPAR and UCP should be investigated further.

Adipocytes not only store energy but also secrete various physiologically active substances (adipocytokines), including tumor necrosis factor alpha (TNF-α), to influence distant organs. Among these adipocytokines, TNF-α[25,26] is attracting attention as a humoral factor that induces insulin resistance. Hotamisligil et al.[25] have suggested that TNF-α secreted by adipocytes in skeletal muscle has a paracrinologic effect on skeletal muscle cells. Both animal experiments[11] and clinical experience[27-29] indicate that triglyceride accumulation in skeletal muscle is related to insulin resistance. A decrease in the triglyceride content of skeletal muscle might be involved in the mechanism by which bezafibrate decreases insulin resistance[30-32]. The increase in UCP-3 mRNA expression in skeletal muscle observed in the present study may have promoted energy expenditure and decreased triglyceride accumulation in skeletal muscle, thereby decreasing insulin resistance.

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