

Bezafibrate-Induced Changes over Time in the Expression of Uncoupling Protein (UCP) mRNA in the 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 UCP mRNA in the tissues was examined in Otsuka Long Evans Tokushima Fatty (OLETF) rats. Eight-week-old rats were divided into a high-dose (100 mg/kg) BF group ($n = 15$), a low-dose (10 mg/kg) BF group ($n = 15$) and a control group ($n = 15$), and followed for 14 days. Feed intake by the high-dose BF group increased significantly between days 10 and 14 of administration. Triglyceride, free fatty acid, and T_4 levels decreased significantly in a dose-dependent manner in the high-dose BF group. Leptin and insulin levels significantly decreased on days 3 and 7. Throughout the study period, liver UCP2 mRNA increased in the high-dose BF group. On day 3 of BF administration, the levels of UCP2 mRNA expression in the skeletal muscles as well as UCP3 mRNA expression in the WAT were significantly increased in the high-dose BF group. PPAR- α mRNA significantly increased in the liver on day 3 of BF administration. We thus conclude that the PPAR- α -mediated effects of BF on the expression of liver UCP2 may be one of the factors that helped to decrease insulin levels. *J Atheroscler Thromb*, 2004; 11: 224–231.

Key words: Fibrate, UCP-2, PPAR- α , Thyroid hormone

Introduction

Findings have emerged that fibrates, which are in wide use as a treatment of hyperlipidemia, enhance the expression of uncoupling protein 2 (UCP2) mRNA in the liver and UCP3 mRNA in the skeletal muscles through activation of peroxisome proliferator-activated receptor (PPAR)- α (1–3), suggesting an effect of these drugs on energy homeostasis. In our previous report (4), we have

shown that long-term bezafibrate (BF) treatment not only arrests the development of obesity but also improves insulin resistance in obese, spontaneously type 2 diabetic Otsuka Long Evans Tokushima Fatty (OLETF) rats (5). However, the food intake was increased in the rats given BF compared to that in the control group so, we speculated that BF prevents obesity from developing by increasing energy expenditure. Cabrelo *et al.* reported that UCP3 may be involved in the improvement of insulin sensitivity caused by BF (3), and an upregulation of UCP2 has been observed in the liver during high-fat feeding and obesity (6). Our present study, therefore, was aimed at investigating the effect of short-term BF administration over time on the level of UCPs that regulate energy homeostasis.

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Materials and Methods

Forty-eight male OLETF rats (5) were provided at 4 weeks 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 ± 2°C) and relative humidity (55 ± 15%), and a 12-h light/12-h dark cycle. They were supplied with rat chow (CE-2; Clea Japan, Inc, Tokyo, Japan) and tap water ad libitum until 8 weeks of age. When the rats were 8 weeks old, they were randomly allocated to a control group or BF groups (Kissei Pharmaceutical, Nagano, Japan) including one administered 200 ppm BF mixed in to the food (which is 10 mg/kg B.W. in terms of normal food consumption amount; BF 10 mg/kg group hereafter) and an other administered 2,000 ppm BF (100 mg/kg B.W. in terms of normal food consumption amount; BF 100 mg/kg group). While they were individually reared in plastic cages, food consumption was determined by 3 day's average food intake from 0, 4, and 11 days after the start of administration. On days 3, 7 and 14 days after administration, non-fasting body weight and rectal temperature were 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, total cholesterol, triglyceride, free fatty acid, thyroid hormone (T₃, T₄), leptin, and insulin levels. Assay kits for plasma glucose, TG, FFA and TC were obtained from Wako Pure Chemical Industries (Osaka, Japan). IRI was measured by an insulin ELISA kit from Morinaga (Tokyo, Japan), IRL was measured by a leptin ELISA kit from Morinaga, and plasma thyroid hormones (T₃, T₄) were measured by T₃ and T₄ RIA Bead (Dainabot, Tokyo, Japan). The animals were then killed under nonfasting conditions, after which their liver, skeletal muscle (gastrocnemius), interscapular brown adipose tissue (BAT), and retroperitoneal white adipose tissue (WAT) were removed and frozen under liquid nitrogen, and then preserved at -80°C. Total RNA was separated from the frozen tissues (liver, gastrocnemius, interscapular BAT, and retroperitoneal WAT) using TRIzol RNA extraction liquid (Gibco-BRL), and the level of relative mRNA expression was measured by quantitative real-time polymerase chain reaction (PCR) via a TaqMan analysis that employed specific primers and probes. The oligonucleotide sequences of gene-specific primers and probes for the TaqMan analysis of rat UCP1, UCP2, UCP3, PPAR α , δ , γ and β -actin mRNAs were as follows: UCP1; forward (sense) 5'-CGGCA G-CCTTTTCAAAGG-3', reverse (antisense) 5'-CCTTT CTCCAAGTCGCCTATGT-3' fluorogenic probe FAM-5'-TTGCGCCTTCT TTTCTGCG ACTCG- 3'-TAMRA, UCP2; forward (sense) 5'-CCCACTGCTTCAGTTT TTCTCC-3', reverse (antisense)

5'-TGAGATC-TGCAATACAGGCTGC-3', fluorogenic probe FAM-5'-TTGGTTTCAAGGCCA CCGATGT-GC-3'-TAMRA, UCP3; forward (sense) 5'-GGCGCTTTTACTCAAGGAT-3', reverse (antisense) 5'-ACAATCAAAGTCCTCAGCCACA-3', fluorogenic probe FAM-5'-TGAAGGCG AC CGCAGTTGGTTG-3'-TAMRA. PPAR α ; forward (sense) 5'-GAAGCCATT CTGC GACATCA-3', reverse (antisense) 5'-GCCGATCTCCACAGCAAATT-3', PPAR δ ; forward (sense) 5'-GCTTCGTCACCCATGAGTTCTT-3', reverse (antisense) 5'-GC AATGAAAAG GGCCAGA TC -3', PPAR γ ; forward (sense) 5'-CTGTCGG TTTCAGA AGTGCCTT-3', reverse (antisense) 5'-AGCTGGTTCGATATCACTGG AGATC -3', and β -actin; forward (sense) 5'-GGCGCTTTTACTC AAGGAT-3', reverse (antisense) 5'-ACAATCAAAGTC-CTCAGCCACA-3', fluorogenic probe FAM-5'-TGAAGGC GACCGCAGTTGGTTG-3'-TAMRA. The PCR condition were 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 UCP copy number vs β actin.

The results of statistical analysis were represented as mean ± SEM. Statistical analysis was performed 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 ($p < 0.05$).

Results

Changes in body weight, rectal temperature, and feed intake

No significant difference in body weight or rectal temperature was seen among the various groups throughout the study period. The feed intake by the high-dose BF group was significantly higher than that in the control group between days 10 and 14 of BF administration (Fig. 1).

Changes in plasma glucose, free fatty acid, total cholesterol, thyroid hormone, leptin, and insulin levels

Throughout the entire course, the plasma glucose levels tended toward decline in the high-dose BF group, while failing to reach statistical significance. Triglyceride levels significantly decreased in both the 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 was reached in the high-dose BF group on days 7 and 14 of administration. There was a trend toward a decrease in total cholesterol levels in the high-dose BF group, but this difference failed to achieve significance (Fig. 2). T₄ levels dropped significantly in a dose-dependent manner throughout the course in both the low- and high-dose BF groups, while T₃ levels were

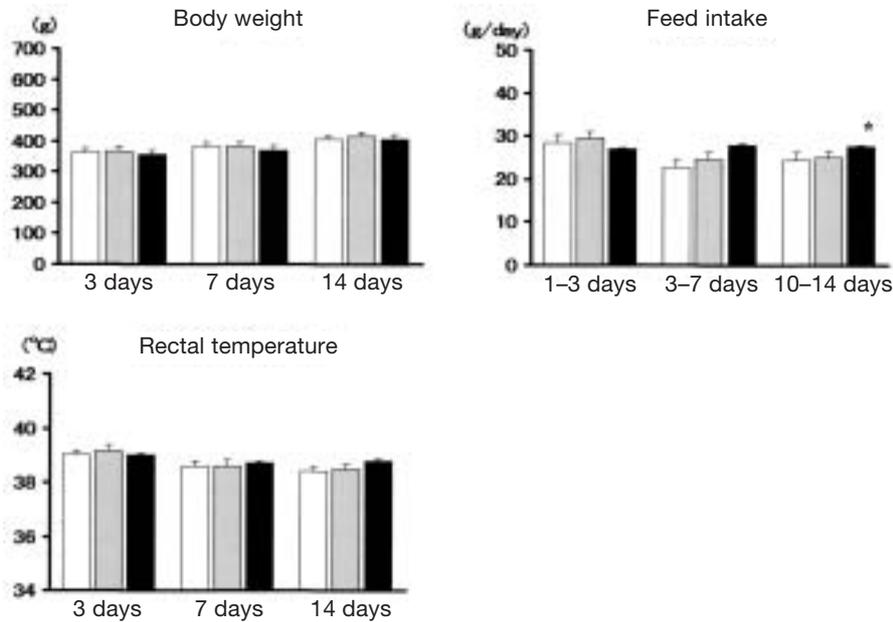


Fig. 1. Changes over time in body weight, feed intake, and rectal temperature in OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).
* $p < 0.05$ vs control group

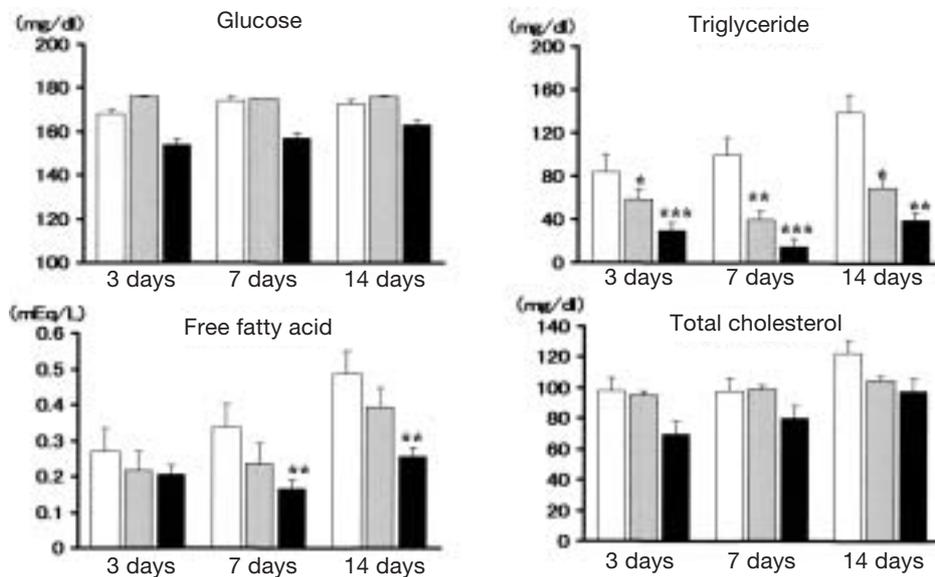


Fig. 2. Changes over time in plasma glucose, triglyceride, free fatty acid and total cholesterol levels in OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).
* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs control group

significantly decreased only in the high-dose BF group on day 3 of administration. Leptin and insulin levels were significantly decreased on days 3 and 7 of BF administration in the high-dose BF group, while no significant difference was seen on day 14 (Fig. 3).

Levels of UCP 1, 2, and 3 mRNA expression

The expression of UCP1 mRNA was seen only in the BAT and WAT. However, BF did not enhance the levels of UCP1 mRNA expressed (Fig. 4 and Fig. 5). In contrast, the expression of UCP2 mRNA was seen in the BAT and WAT, the liver, and the skeletal muscles, with the UCP2 mRNA expression enhanced, especially in the liver, in the high-dose BF group. This increased expression of liver UCP2 mRNA was continually observed after day 3 of BF administration (Fig. 6). Furthermore, on day 3, the expression of UCP2 mRNA increased in the skeletal muscles (Fig. 7). The expression of UCP3 mRNA was seen in the BAT, WAT and skeletal muscles, with a significant increase seen especially in the WAT on day 3 in the high-dose BF group (Fig. 5). However, BF did not increase the expression of UCP3 mRNA in the skeletal muscles (Fig. 7).

Changes in the level of PPAR mRNA expression

PPAR- α and PPAR- δ mRNA were expressed in the BAT, liver, and skeletal muscles; PPAR- γ mRNA was expressed

in the BAT and WAT. The level of PPAR- α mRNA expression was significantly increased in the high-dose BF group compared to the control group in liver (Fig. 6).

Discussion

Fibrate drugs such as BF (7–11) are widely used clinically to induce the expression of lipoprotein lipase genes (12), as well as a variety of other genes such as Apo A-I and A-II, through activation of PPAR- α , thereby improving lipid metabolism. According to the recently published results of a study with PPAR- α knockout mice (13), these genes appear to increase plasma lipid levels and body weight. The relationship between PPAR- α and obesity is becoming of considerable clinical interest.

In our study of BF, while UCP1 mRNA was seen to be expressed only in the BAT and WAT, BF administration led to no enhanced expression of UCP1 mRNA, which was in agreement with the results reported by Cabrero *et al.* (3), thus ruling out the possibility that UCP1 may be involved in the BF-induced accelerated energy expenditure, or in the prevention of disease progression to obesity (4).

In contrast, the expression of UCP2 mRNA was noted in the BAT, WAT, liver, and skeletal muscles, and was especially enhanced in the liver, a finding that coincides with the results of a study by Cabrero *et al.* (3). However,

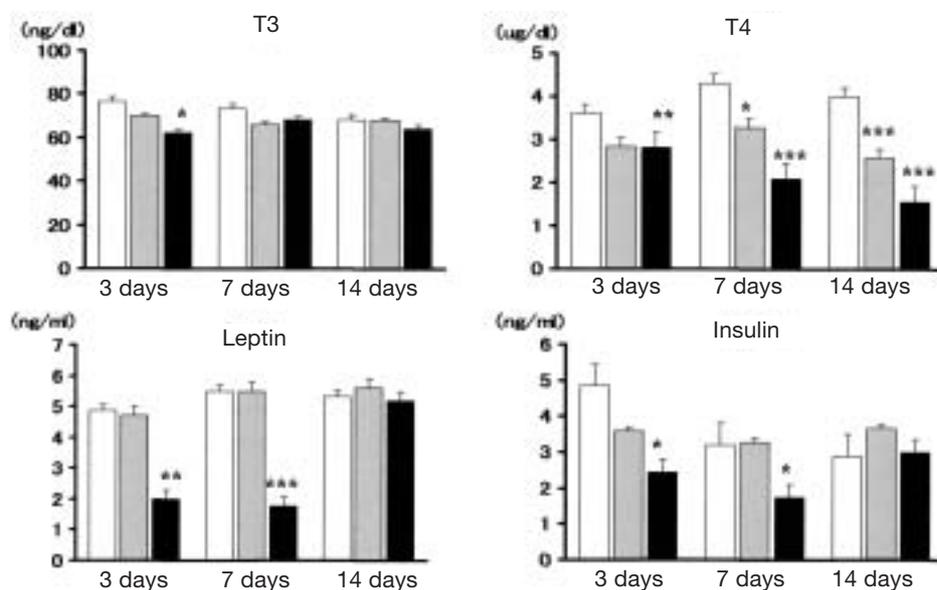


Fig. 3. Changes over time in thyroid hormone, leptin and insulin levels in OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs control group

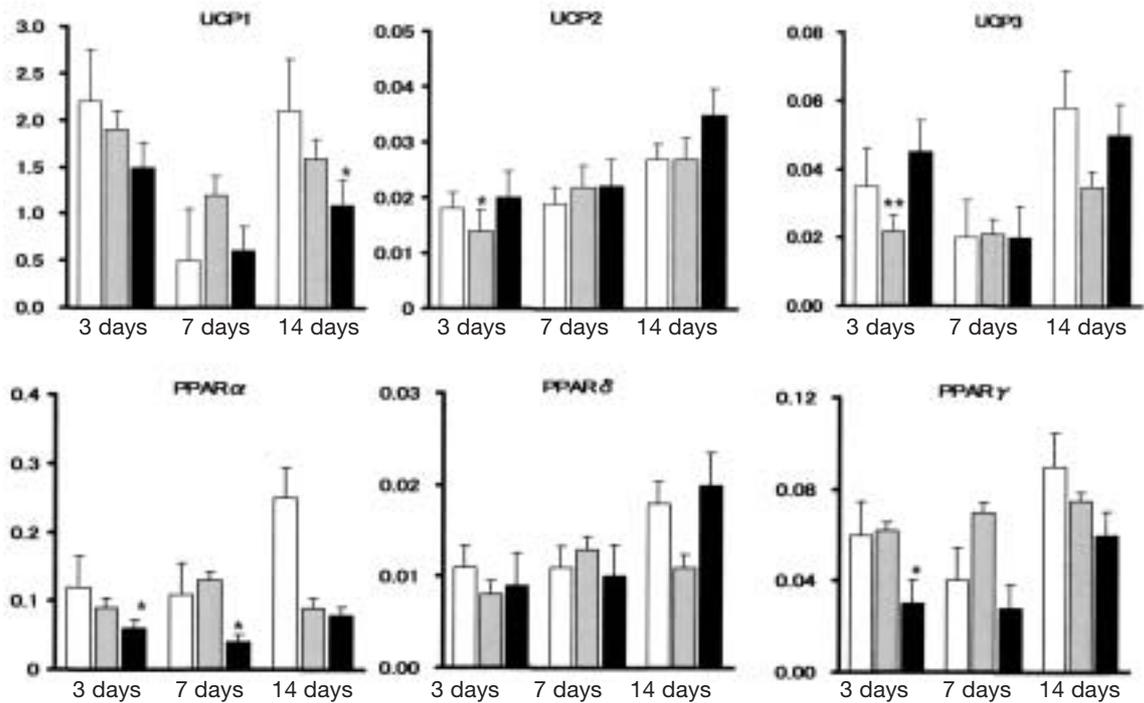


Fig. 4. Changes over time in the levels of UCP mRNA and PPAR mRNA expressed in the brown adipose tissues of OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).

* $p < 0.05$, and ** $p < 0.01$ vs control group

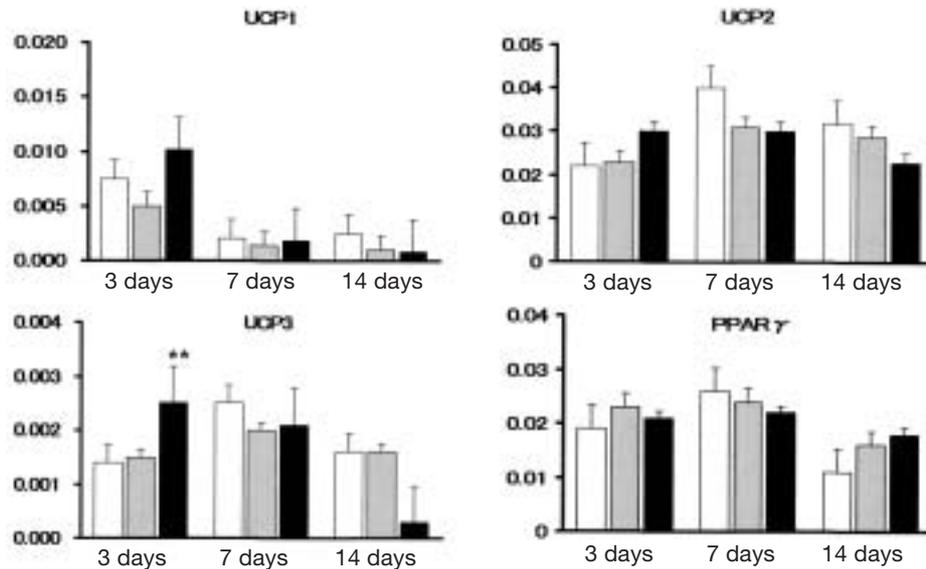


Fig. 5. Changes over time in the levels of UCP mRNA and PPAR mRNA expressed in the white adipose tissues of OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).

** $p < 0.01$ vs control group

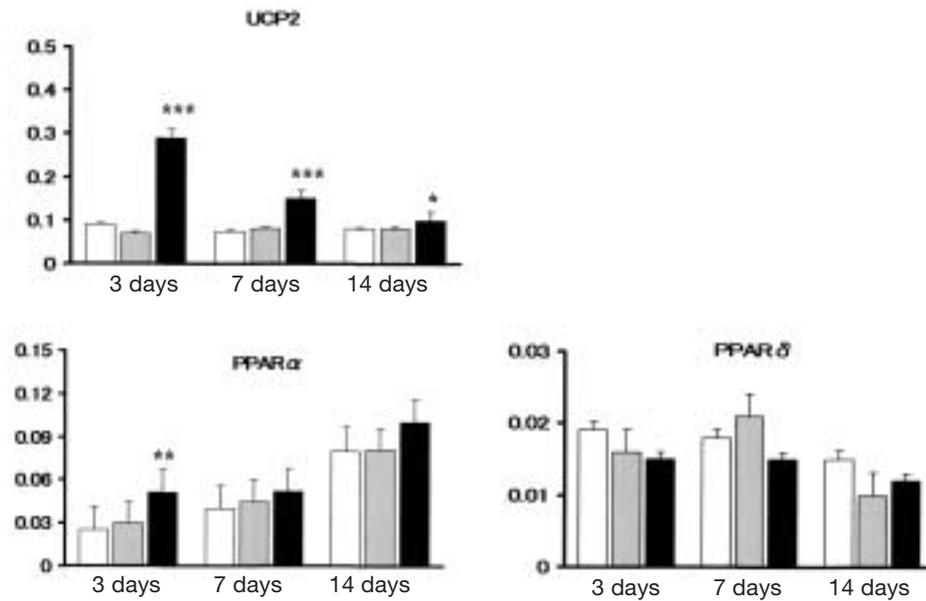


Fig. 6. Changes over time in the levels of UCP mRNA and PPAR mRNA expressed in the liver of OLETF rats given bezafibrate. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs control group

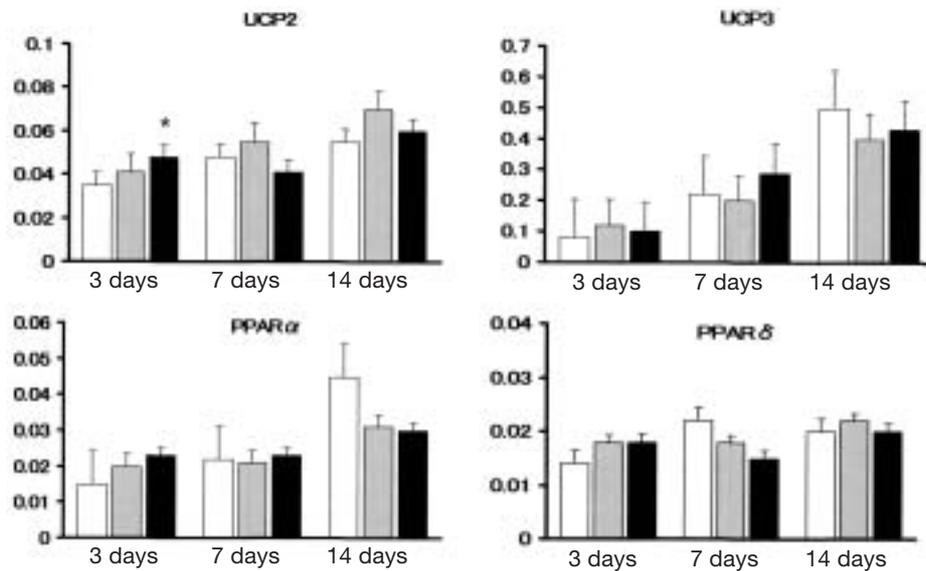


Fig. 7. Changes in the levels of UCP mRNA and PPAR mRNA expressed in the skeletal muscles of OLETF rats given BF. Open square, control group; shaded square, low-dose BF group (10 mg/kg); and closed square, high-dose BF group (100 mg/kg).

* $p < 0.05$ vs control group

unlike their findings, in our study, enhanced expression of liver UCP2 mRNA was noted even after day 3 of BF administration. This discrepancy may have been attributable to Cabrero *et al.*'s use of normal rats, which may have affected the results of the experiments. There are also reports of other fibrates, such as phenofibrate (1) and Wy-14643 (2), having enhanced the expression of UCP2 mRNA in the liver. Therefore, this effect on liver UCP2 mRNA expression is considered to be common to all fibrates.

The expression of UCP3 mRNA was noted in the BAT, WAT, and skeletal muscles. In agreement with the results of Cabrero *et al.* (3), a significant increase in the UCP3 mRNA expression level was seen, especially in the WAT on day 3 in the high-dose BF group. In the skeletal muscles, however, BF administration did not enhance expression of UCP3 mRNA, a finding that differed from those of Cabrero *et al.* Although the reason for this is unclear, it was speculated that, unlike UCP2 mRNA, which reportedly remains stable for a long period, UCP3, with its half-life of only several hours and rapid excretion (14), requires that changes in its expression level be studied within several hours of BF administration.

We found that PPAR- α and PPAR- σ mRNA were expressed in the BAT, liver, and skeletal muscles; however, PPAR- γ was expressed only in the BAT and WAT. The level of PPAR- α expressed on day 3 was significantly increased in the high-dose BF group. Therefore, we considered the increased expression of liver UCP2 mRNA to be a PPAR- α -mediated change.

Thyroid hormones are reported to enhance the expression of UCP2 mRNA in the BAT and WAT, skeletal muscles, and the heart, as well as the expression of UCP3 mRNA in the BAT and skeletal muscles (15–18). In our study, thyroid hormone levels decreased significantly in a dose-dependent manner with BF administration. However, the relationships between blood thyroid hormone levels and expression of UCP-2 mRNA in the liver and skeletal muscles and expression of UCP-3 mRNA in the WAT remain to be clarified. Because we did not measure the resting metabolic rate (RMR) through analysis of expired gas in this study, the observed reduction in the thyroid hormone (T_4) level suggests a trend toward reduction of RMR, representing the process with which the body responded to the BF-induced increase in local energy expenditure via UCP by slowing down its metabolism to maintain energy homeostasis. While leptin is also reported to enhance the expression of UCP2 mRNA in the WAT, and the expression of UCP3 mRNA in the BAT and skeletal muscles (19, 20), a significant decrease in plasma leptin levels was noted in the high-dose BF group on days 3 and 7 in our study.

We thus conclude that the PPAR- α -mediated effect of BF on the expression of liver UCP2 may have been one of the factors that helped decrease insulin levels.

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