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Original Article

Fenofibrate Inhibits Visceral Adiposity by Inhibiting UCPs in C57BL/6J Mice Fed on a High Fat Diet

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We investigated to verify whether the PPARα agonist fenofibrate regulates adipose tissue metabolism and to determine the molecular mechanism involved in this regulation. After male mice (C57BL/6J) received a high fat diet with or without fenofibrate for 6 weeks, the effects of fenofibrate on not only adipose tissue weight, visceral adipocyte size, serum lipid and glucose levels, but also the expression of uncoupling proteins (UCPs). Mice given a fenofibrate-supplemented high fat diet showed reduced both visceral and subcutaneous adipose tissue weights *versus* high fat diet-fed animals. The size of visceral adipocytes was significantly decreased by fenofibrate treatment. The administration of fenofibrate resulted in decreased serum levels of triglycerides, free fatty acids, and glucose. Moreover, fenofibrate up-regulated mRNA levels of visceral adipose tissue UCP2 and skeletal muscle UCP3. Therefore, our results suggest that the increases in the expression of UCPs by fenofibrate seem to suppress diet-induced visceral adiposity as well as severe hypertriglyceridemia and hyperglycemia in male mice.

Key Words: Fenofibrate, PPARα, Visceral adiposity, Visceral adipocyte hypertrophy, Hypertriglyceridemia, Hyperglycemia

INTRODUCTION

Fibrates are a class of hypolipidemic drugs used to treat dyslipidemic patients. At the molecular level, fibrates act as peroxisome proliferator-activated receptor α (PPARα) ligands that regulate the expression of a number of genes responsible for lipid and lipoprotein metabolism (Schoonjans et al., 1996; Steals et al., 1998; Kliewer et al., 1999). Fibrate-bound PPARα heterodimerizes with retinoid X receptor and then modulates the expression of target genes with a PPAR response element (PPRE) in their promoter regions (Dreyer et al., 1992).

Fibrates seem to regulate energy homeostasis. Excess energy intake increases the concentrations of plasma triglycerides and cholesterol, and lipids accumulated in the adipose tissue are believed largely to derive from circulating triglycerides (Bourgeois et al., 1983; Costet et al., 1998; Chaput et al., 2000). Thus, increased hepatic fatty acid oxidation and decreased hepatic triglycerides by fenofibrate may decrease body weight gain indicating that PPARα may be involved in the regulation of obesity. This is supported by a report that PPARα-deficient mice have abnormal triglyceride and cholesterol metabolism, and become obese with age (Costet et al., 1998). Other studies have produced evidence that fenofibrate can modulate the body weight of animal models, such as fatty Zucker rats, high fat-fed C57BL/6 mice and high fat-fed obese rats (Chaput et al., 2000; Guerre-Millo et al., 2000; Mancini et al., 2001; Yoon et al., 2002; Jeong et al., 2004a), although the reported effects of fibrates are contradictory.

Chemical uncoupling of mitochondrial membranes by uncoupling proteins (UCPs) has been found to reduce body weight gain and adiposity (Kopecky et al., 1995; Lentes et

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al., 1999; Li et al., 2000; Schrauwen et al., 2002; Hesselink et al., 2003; Liu et al., 2003). UCPs are members of the mitochondrial carrier protein family, which includes UCP1, UCP2 and UCP3. UCP1 is expressed exclusively in brown adipose tissue, while UCP2 is expressed in a variety of tissues such as white adipose tissue and the liver, and UCP3 is highly expressed in skeletal muscle. UCP1 uncouples oxidative metabolism from ATP synthesis, resulting in the generation of heat; its expression levels are reduced in several rodent models of obesity (Sell et al., 2004). UCP2 expression is induced through activation of PPARα, which is involved in fatty acid oxidation (Mori et al., 2004). In L6 myotubes, overexpression of UCP3 specifically increases fatty acid oxidation (MacLellan et al., 2005). These findings suggest that UCPs may be involved in the energy homeostasis and the interplay between PPARα and UCPs may influence the regulation of obesity.

The objective of the present study was to determine whether fenofibrate treatment prevents diet-induced visceral adiposity and UCPs are involved in this regulation of male mice. Our data demonstrate that fenofibrate treatment increased mRNA expression of UCPs in visceral adipose tissue and skeletal muscle, leading to a reduction in high fat diet-induced visceral adiposity, hypertriglyceridemia, and hyperglycemia in male mice.

MATERIALS AND METHODS

Animals

For all experiments, eight-week-old male wild-type C57BL/6J mice (n=8/group) were purchased from Central Lab Animal (Seoul, Korea) and bred at the Mokwon University with a standard 12-h light/dark cycle. Prior to the administration of special diets, mice were fed standard rodent chow and water *ad libitum*. At the beginning of the study, body weights were 22.5 ± 0.2 g. Mice were divided randomly into two groups, one of which received a high fat diet containing 60 kcal% fat (w/w, Research Diets, New Brunswick, NJ, USA), and another group was fed the same high fat diet supplemented with fenofibrate (0.05%, w/w) for 6 weeks. Animals were sacrificed by cervical dislocation, and tissues were harvested, weighed, snap-frozen in liquid nitrogen, and stored at -80℃ until use. Additional sections of visceral adipose tissue were prepared for histological analyses. Blood was collected from the saphenous vein after a 12-h fast and the plasma was separated and stored at -80℃ until analysis. All animal experiments were approved by the Institutional Animal Care and Use Committees of Mokwon University, and followed National Research Council Guidelines.

Serum levels of triglycerides and glucose were measured using an automatic blood chemical analyzer (CIBA Corning, Oberlin, OH, USA). Levels of free fatty acids were measured using SICDIA NEFAZYME (Shinyang Chemical, Seoul, Korea).

Histological analysis

For hematoxylin and eosin (H&E) staining, visceral adipose tissues were fixed in 10% phosphate-buffered formalin for one day and processed in a routine manner for paraffin sections. Five micrometer-thick sections were cut and stained with H&E for microscopic examination. To quantitate adipocyte number and size, the H&E-stained sections were analyzed using an image analysis system (Image Pro-Plus, Silver Spring, MD, USA).

RT-PCR

Total cellular RNA was prepared using the Trizol reagent (Gibco-BRL, Grand Island, NY, USA). Two μg total RNA was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase and an antisense primer to generate cDNA under standard conditions. The sequences of the sence and antisence primers used for amplification were as follows: UCP2, 5'-ctgagctggtgacctatgac-3' and 5'-caagctgctcaataggtgac-3'; UCP3, 5'-ccatcctgactatggtgcgcaca-3' and 5'-ctgtggcacagaagccagctcc-3'; and β-actin, 5'-tggaa-tcctgtggcatccatgaaac-3' and 5'-taaaacgcagcttaacagaaa-3'. cDNA samples were amplified by PCR in a MJ Research Thermocycler (Waltham, MA, USA). The reaction consisted of 30 cycles of denaturation for 1 min at 94℃, annealing for 1 min at 58℃, and elongation for 1 min at 72℃. The PCR products were analyzed by electrophoresis on a 1% agarose gel. PCR products were quantified from agarose gels using the GeneGenius kit (Syngene, Cambridge, UK).

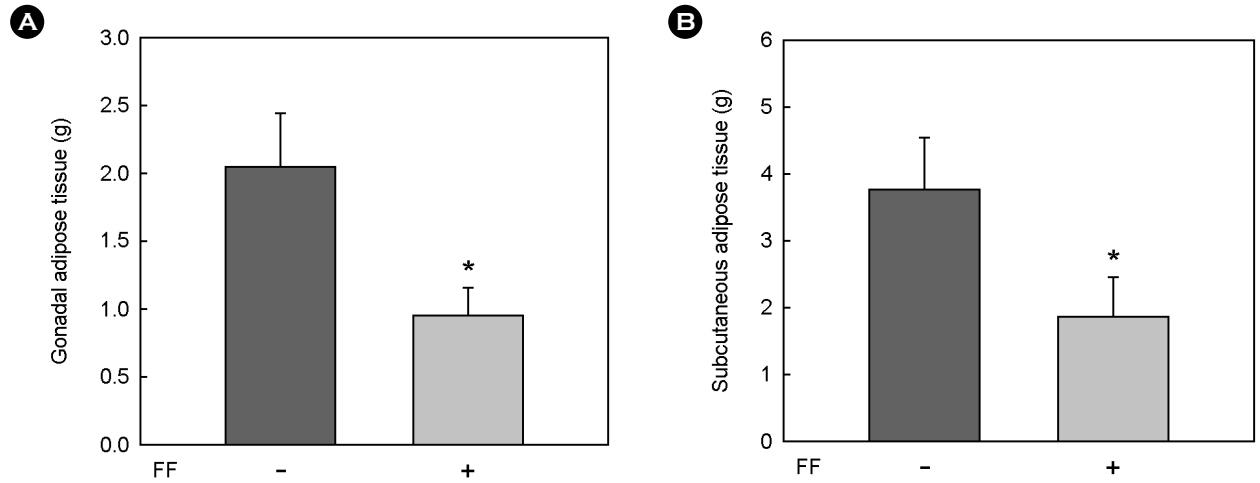

Fig. 1. Regulation of gonadal (A) and subcutaneous (B) adipose tissue weights after fenofibrate treatment. Adult male mice (n=8/group) received a high fat diet with or without fenofibrate (FF; 0.05% w/w) for 6 weeks. All values are expressed as the mean ± SD. Adipose tissues were measured at the end of the study. *$P < 0.05$ compared with high fat group. −, high fat diet alone; +, high fat diet with fenofibrate.

Statistical analysis

Unless otherwise noted, all values are expressed as mean ± standard deviation (SD). All data were analyzed by the unpaired, Student's *t*-test for significant differences between the mean values of each group using SigmaPlot 2001 (SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Our results demonstrated that fenofibrate decreased adipose tissue mass and visceral adipocyte size as well as serum lipids and glucose in high fat diet-induced obese mice and that this process may be mediated by increasing the transcriptional expression of UCPs in visceral adipose tissue and skeletal muscle.

Regulation of adipose tissue mass and adipocyte size by fenofibrate

Administration of fenofibrate to male mice for 6 weeks decreased both gonadal and subcutaneous adipose tissue weights in mice given a high fat diet supplemented with fenofibrate as compared to mice fed the high fat diet (Fig. 1). Visceral and subcutaneous adipose tissue weights were decreased by 53% and 51%, respectively. These results are supported by our previous reports showing that treatment of mice with fenofibrate for 7 weeks significantly decreased high fat diet-induced increases in body weight and visceral fat mass by 17.6% and 44%, respectively (Jeong and Yoon, 2009). In contrast, our previous study also showed that fenofibrate did not decrease body weight and fat mass in female mice, suggesting that these effects of fenofibrate on obesity may be exerted with sexual dimorphism and seem to be influenced by female sex hormones (Yoon et al., 2002, 2003; Jeong et al., 2004b; Jeong et al., 2005, 2007; Jeong and Yoon, 2007; Yoon, 2009).

In addition to the effect of fenofibrate on fat mass, histological analysis showed that fenofibrate caused a 42% decrease in the size of adipocytes in visceral adipose tissue in mice fed the high fat diet (Fig. 2). The average size of visceral adipocytes in the high fat diet-fed obese mice was 3,625 ± 506 μm^2, whereas adipocyte size was 2,094 ± 551 μm^2 in fenofibrate-treated obese mice (Fig. 2B). The number of adipocytes in a fixed area was increased by 85% in fenofibrate-treated obese mice compared with high fat diet fed-obese mice (Fig. 2C). These results show that fenofibrate increases small adipocytes, while decreasing large adipocytes in visceral adipose tissue, suggesting that fenofibrate may convert larger adipocytes into smaller adipocytes, leading to the inhibition of visceral adipocyte hypertrophy. It is known that hypotrophic adipocytes produce and secrete molecules, such as free fatty acids,

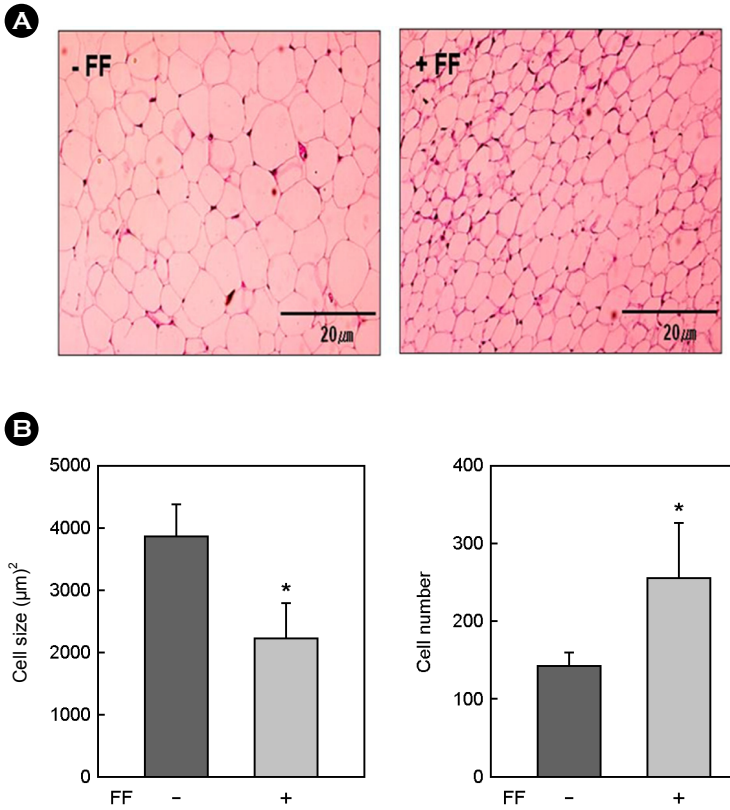

Fig. 2. Histology of visceral white adipose tissue. Adult male mice (n=8/group) received a high fat diet with or without fenofibrate (FF; 0.05% w/w) for 6 weeks. (A) Representative hematoxylin and eosin-stained sections (5 μm thick) of visceral adipose tissue. Adipocyte size from high fat plus FF groups was smaller than that from high fat groups. The size (B) and number (C) of adipocytes in a fixed area (1,000,000 μm^2) were measured. All values are expressed as the mean ± SD. *$P < 0.05$ compared with high fat group. −, high fat diet alone; +, high fat diet with fenofibrate.

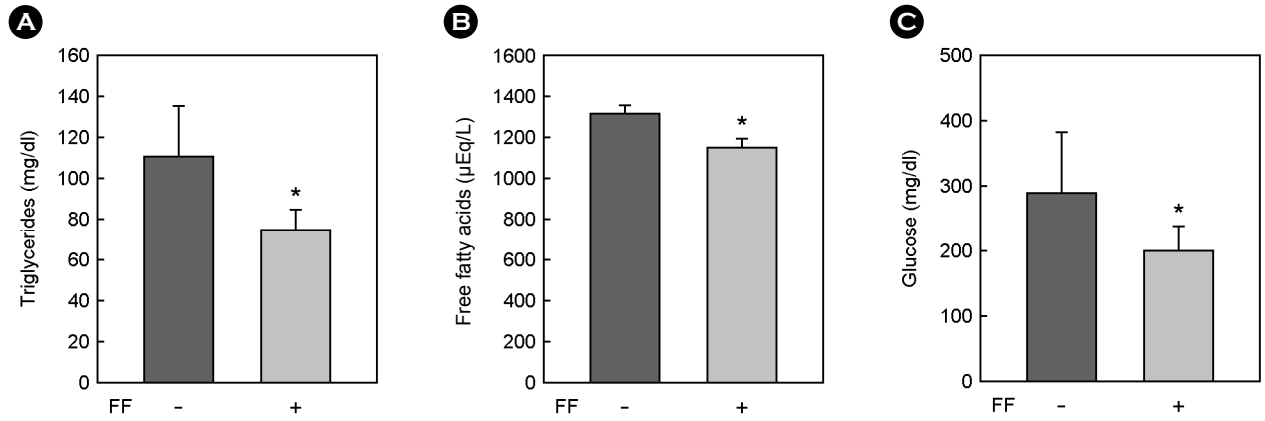

Fig. 3. Changes in circulating triglycerides, free fatty acids, and glucose by fenofibrate. Adult male mice (n=8/group) received a high fat diet with or without fenofibrate (FF; 0.05% w/w) for 6 weeks. Serum concentrations of triglycerides (A), free fatty acids (B), and glucose (C) were measured and all values are expressed as the mean ± SD. *$P < 0.05$ compared with high fat group. −, high fat diet alone; +, high fat diet with fenofibrate.

leptin, and tumor necrosis factor α, which are implicated in the development of insulin resistance (Okuno et al., 1998; Kubota et al., 1999; Kadowaki, 2000). Thus, fenofibrate may alleviate insulin resistance, at least in part, due to its ability to reduce adipocyte size.

Circulating levels of lipids and glucose

During high fat feeding, lipids accumulated in adipose tissue are largely obtained from circulating triglycerides. In agreement with the decreases in fat mass and adipocyte

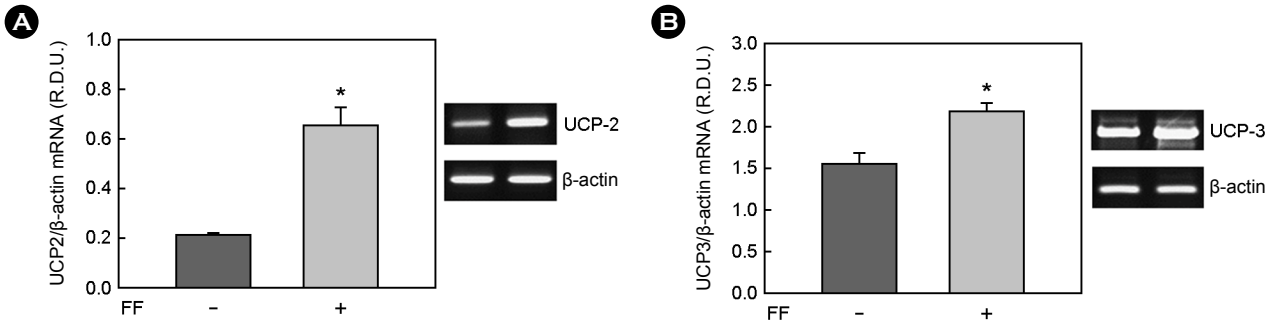

Fig. 4. The mRNA expression levels of visceral adipose tissue UCP2 (A) and skeletal muscle UCP3 (B). Adult male mice (n=8/group) received a high fat diet with or without fenofibrate (FF; 0.05% w/w) for 6 weeks. RNA was extracted from visceral adipose tissue and skeletal muscle, and mRNA levels of UCP2, UCP3, and β-actin were measured as described in the Materials and Methods. All values are expressed as the mean ± SD of R.D.U. (relative density units) using β-actin as a reference. **P* < 0.05 compared with high fat group. Representative PCR bands from one of three independent experiments are shown. −, high fat diet alone; +, high fat diet with fenofibrate.

size following fenofibrate treatment, serum levels of free fatty acid and triglycerides levels decreased by 13% and 34% in fenofibrate-treated mice compared with those in untreated mice (Fig. 3A and B). Thus, fenofibrate can normalize high fat diet-induced hyperlipidemia. Fenofibrate also caused a decrease in glucose levels by 32% in high fat diet-fed animals (Fig. 3C). These results indicate that fenofibrate, by reducing circulating free fatty acids, may improve insulin resistance in obese mice since circulating free fatty acids inhibits glucose uptake and utilization by muscle (Boden et al., 1994; Roden et al., 1996).

Expression of UCP2 in visceral adipose tissue and UCP3 in skeletal muscle

To determine whether the reduction of adipose tissue mass and adipocyte size can be induced by changes in fenofibrate-mediated UCP expression, we measured the mRNA levels of UCP2 in visceral adipose tissue and UCP3 in skeletal muscle. The fenofibrate-treated, high fat diet-fed mice exhibited substantially higher mRNA levels of UCP2 and UCP3 by 208% and 41%, respectively, compared with high fat diet-fed mice (Fig. 4). These results suggest that fenofibrate may decrease adipose tissue metabolism, in part, through visceral adipose UCP2 and skeletal muscle UCP3. PPARα regulates the expression of UCPs, which contain PPRE in their promoters (Lentes et al., 1999; Acin et al., 1999). It is reported that UCPs play an important role in the regulation of energy metabolism in mammals. Transgenic mice overexpressing UCP3 in their skeletal muscle exhibit increased fatty acid oxidation and are resistant to diet-induced obesity (Clapham et al., 2000; Wang et al., 2003; Hesselink et al., 2003; Choi et al., 2007). Reductions in body weight and adiposity by fenofibrate are associated with elevation of hepatic UCP2 expression (Srivastava et al., 2006). According to Cabrero et al. (1999), the PPAR activator bezafibrate also increases UCP3 mRNA levels in white adipose tissue and skeletal muscle in rats. The potent PPARα activator Wy14,643 as well as bezafibrate increase UCP3 mRNA levels in primary culture of rat preadipocytes (Cabrero et al., 2000). These results indicate that fenofibrate-activated PPARα may be involved in antiobesity by regulating UCP2 in visceral adipose tissue and UCP3 in skeletal muscle.

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