De novo Expression of Hepatic UCP3 Is Time-Dependently Related with Metabolic Function in Fenofibrate-Treated High Fat Diet Rats

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Uncoupling protein 3 (UCP3) is a mitochondrial protein that is expressed predominantly in skeletal muscle. It may play a role in altering metabolic function. However, its major physiological roles are not fully understood. Recently de novo expression of UCP3 in rat liver by fenofibrate was reported. We also reported previously that fenofibrate-induced de novo expression of UCP3 contributes to reduction of adipose tissue in obese rats. In the present study, we investigated that fenofibrate-induced expression of UCP3 in rat liver is related with metabolic function such as body weight and hepatic lipid content by time-dependent manner in high-fat diet rats. Eight-week-old male Sprague-Dawley rats were randomly divided into two groups; the high fat diet group (HF, n=16) and fenofibrate-treated high fat diet group (HFF, n=16). The mRNA expression of hepatic UCP3 was detected as early as 1 week of fenofibrate treatment by quantitative real-time PCR and the amount of mRNA was increased time-dependently. The mean body weight of the HFF group was significantly less compared with the HF group after 6 weeks of fenofibrate treatment, even though there was no difference of food intake between the two groups. Rectal temperature was increased during 4 to 6 weeks of fenofibrate treatment and body weight was decreased after 6 weeks of treatment. These results were corresponded with the increased amount of the expression of UCP3 mRNA and protein. We suggest that de novo expression of hepatic UCP3 is increased time-dependently with fenofibrate treatment and that the amount of expression is correlated with metabolic function.

Key words : Fenofibrate, high-fat diet, hepatic UCP-3

Introduction

Uncoupling proteins (UCPs) were discovered members of the mitochondrial inner membrane carrier family. There were 5 isoforms (UCP1-5) [16,19,23]. UCP3 is predominantly expressed in skeletal muscle. Mice overexpressing UCP3 are hyperphagic and lean with increased mitochondrial proton conductance [4]. The function of UCP3 was not completely elucidated. Previous evidences indicate that UCP3 may play functional roles unrelated to the basal uncoupling [9]. In conditions of elevated lipid metabolism, UCP3 might export fatty acid anions, related from an excess of acyl-coenzyme A by mitochondrial thioesterase-1, from the mitochondrial matrix to the cytosol [17].

Peroxisome proliferation-activated receptor α (PPARα) was the first member of the PPAR family to be described [11]. It is mainly expressed in tissues with high metabolic activity such as the liver, muscle, adipose tissue and others [2,3]. Fenofibrate is a synthetic ligand for PPARαand it has been used for the treatment of dyslipidemia, especially hypertriglyceridemia [14]. In addition, fenofibrate has been reported to prevent and reduce body weight gain in diet-induced obese rats [7,15]. This effect on body weight has been suggested that it is related with the enhanced lipid catabolism in the liver mainly through PPARs transcriptional induction of the genes for mitochondrial and peroxisomal oxidative enzymes [5]. UCP genes contain functional PPAR-responsive elements [1,20] and they may be targets of fenofibrate. In addition, fenofibrate induced de novo expression of UCP3 in rats [12]. Previously our group reported that fenofibrate decreases adiposity and that hepatic uncoupling by fenofebrate contributes to reduction of adiposity [13,18]

In the present study, we investigated whether the times of expression of UCP3 is related with metabolic function in high-fat diet rats. To evaluate the time dependent relation with de novo gene expression of hepatic UCP3, we simultaneously measured the hepatic UCP3 mRNA expression,

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body weight and body core temperature. Here, we show that the UCP3 expression is time-dependently correlated with body weight and core temperature.

Meterials and Methods

Animal treatment and diet

Male Sprague-Dawley (SD) rats, four-weeks of age (200-225 g, Samtako Bio Korea, Osan, South Korea), were housed at room temperature with 12-h-light/12-h-dark cycle. All of the rats were cared and handled during entire period of experiment by the Guidelines of Animal Experiments by the Korean Academy of Medical Sciences. They were fed with the standard rat diet and tap water *ad libitum* for 4 weeks. After then, all of the rats were randomly divided into two groups. The average body weight between the two groups was not significantly different. The first, the high-fat group, was fed with high fat diet *ad libitum* (n=16, HF). The second, the high-fat with fenofibrate group, was fed with high fat diet, which was mixed with micronized fenofibrate (100 mg/kg, Sigma-ALdrich, St. Louis, MO, USA) (n=16, HFF). The composition of high fat diet was 40% of energy from fat, 20% of energy from protein, and 40% of energy from carbohydrate. Body weight and anal temperature of each rat were monitored weekly. Food intake of each rat was measured daily during the entire experimental period.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis

Obtained liver tissue samples were used for RT-PCR. Total RNA was isolated using TRIzol (Invitrogen, Calsbad, CA, USA). Complementary DNA was synthesized from RNA samples by enhanced AMV reverse transcriptase. An aliquot of the reverse transcription reaction was then used for subsequent PCR amplification with specific primers. The sequences of the sense and antisense primers used for amplification were as follows: *UCP-3,* 5′-GGAGCCATGGCAGTG ACCTGT-3′ and 5′-TGTGATGTTGGGCCAAGTCCC-3′; *beta-actin,* 5′-GTGGTGGTGAAGCTGTAGCC-3′ and 5′-GAGA CCTTCAACACCCC-3′. PCR was performed in Gene Amp PCR system 2400 (Perkin-Elmer, Waltham, MA, USA). Amplification of each gene yielded a single band of the expected size (UCP-3, 179 bp; beta-actin 763 bp). Preliminary experiments were carried out with various amounts of cDNA to determine nonsaturating conditions of PCR amplification for all of the genes studied. Therefore, under these conditions, the RT-PCR method used in this study permitted relative quantification of mRNA. The illuminated bands were quantified by Image Gauge (Fujifilm, Japan). The results for the expression of specific mRNAs were presented relative to the expression of the control gene.

Western blot analysis

Liver tissues from rats of HF and HFF groups were subjected to western blot analysis. Isolated proteins were separated on 12% SDS-polyacrylamide gel and transferred electrophoretically onto nitrocellulose membrane using a mini electroblotter (BioRad Laboratories, Hercules, CA, USA). The membrane was blocked with 5% non-fat milk for 1 hr, and then incubated for 2 hr in anti-rat UCP-3 antibody (R&D systems, USA). After washing with TBST, the membrane was incubated for 1 hr with secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunoreactive bands were visualized with ECL Plus™ Western Blotting Detection Reagents (GE Healthcare, Pittsburgh, PA, USA). β-actin (42 kDa; Sigma-ALdrich) was used as the internal control.

Measurement of rectal temperature

After 12 hours fasting, all rats inhaled ether and were injected with ketamine (200 mg/kg) into the peritoneum. After the anesthesia administered, the flexible thermometer probe (Temperature controller, DX4-PSSNR, KSC 1614, HANYOUNG, Korea) was placed into the rectal canal. Digital body temperatures were monitored and measured. We evaluated the rectal temperatures two times on different days under the same conditions.

Histological analysis

We sacrificed 2 rats per group weekly. The tissue of liver was collected and weighed. After that, the tissue was rapidly frozen in liquid nitrogen and then was stored at -70^{o}C until further assay. Hepatic tissues were removed and fixed in 10% neutral buffered formalin, and embedded in paraffin. The sections were stained with hematoxylin and eosin (H & E).

Results

Expression of UCP3 mRNA in liver

We evaluated de novo expression of hepatic UCP3 mRNA

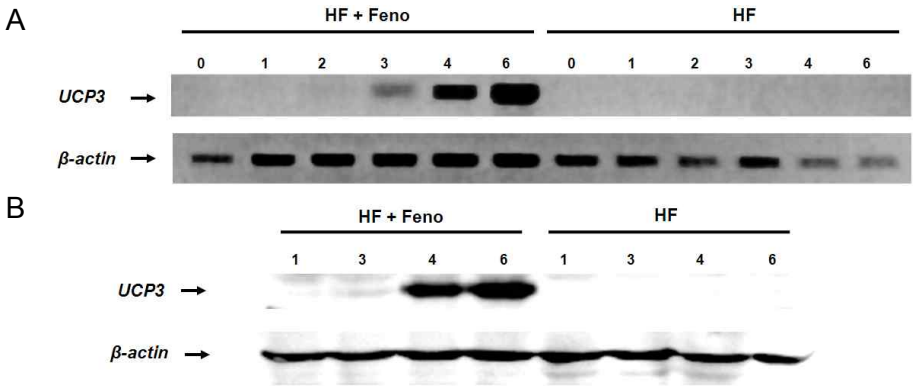

Fig. 1. Expression analysis of hepatic UCP3. A: RT-PCR of UCP3 in liver. Hepatic UCP3 was de novo expressed in liver from 2 weeks of fenofibrate treatment and strongly expressed on 6 weeks of fenofibrate treatment. However, there was no expression of hepatic UCP3 from rats of HF group. B: Immunoblotting of hepatic tissues. Protein expression of hepatic UCP3 is corresponded with mRNA expression.

by fenofibrate via RT-PCR and western blotting. As shown in Fig. 1A, the expression of UCP3 mRNA in the liver was newly induced by fenofibrate after 2 weeks of fenofibrate treatment. The expression of UCP3 was increased gradually until 6 weeks of fenofibrate treatment. The protein expression of UCP3 was newly induced followed by mRNA expression and gradually increased until 6 weeks of fenofibrate treatment (Fig. 1B).

Food intake and change of body weight

The mean amount of daily food consumption was 11.4±0.22 g in HF group, 11.8±0.30 g in HFF group. There was no significant difference between the two groups even though daily food intake of fenofibrate treated group seemed to be slightly increased (Fig. 2A). The changes of mean body weight of the two groups are presented in Fig. 2B. Body weights of both groups were increased as a similar pattern during initial 6 weeks of fenofibrate treatment. After 6 weeks of fenofibrate treatment, the body weight of HFF group was decreased for 2 weeks and maintained for more two weeks. In HF group, the rate of increasing weight was blunted after 6 weeks to 10 weeks. The mean body weight of HFF group was significantly decreased after 6 weeks of fenofibrate treatment.

Body core temperatures

The body core temperature was measured to confirm the relationship between the hepatic UCP3 de novo expression and the increase of body energy expenditure as expressed by increase of body core temperatures. The rectal temperature of HF group was slightly higher at 2 weeks of fenofibrate treatment compared to HFF group. In HFF group, however, the temperature was increased at 4 weeks of fenofibrate treatment and remained at higher state compared to

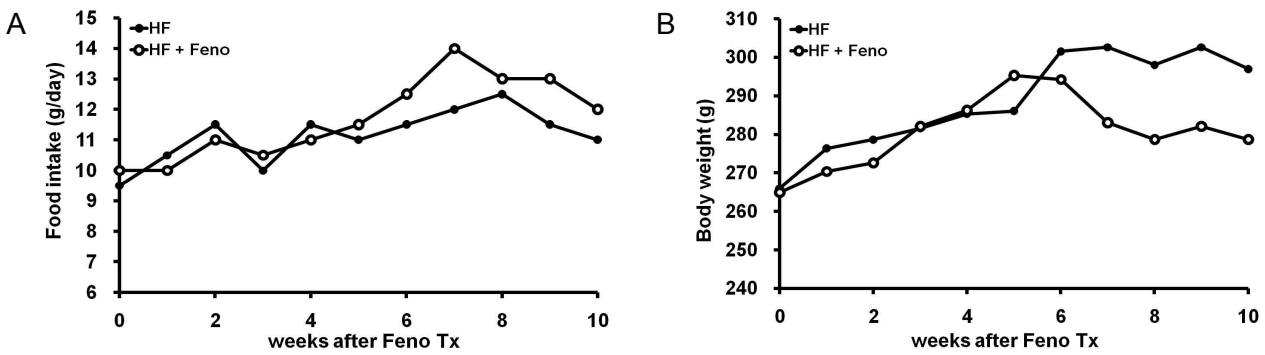

Fig. 2. Changes of daily food intake and body weight. A: Daily food intake of HF and HFF groups seems to be different after 6 weeks of fenofibrate treatment. However, there is no significant difference between two groups. B: Body weight gain is significantly suppressed and decreased on 6 weeks of fenofibrate treatment. These changes are corresponded with hepatic UCP3 expression.

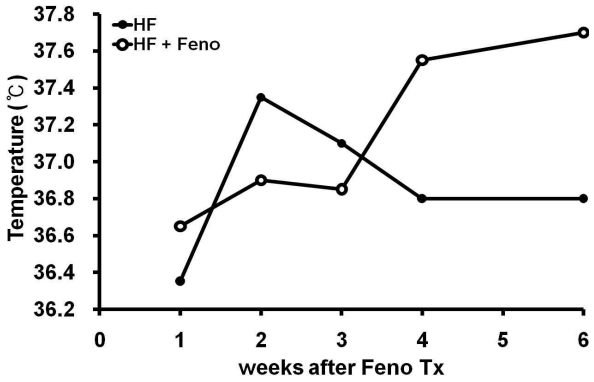

Fig. 3. Changes of body core temperature. Body core temperature was measured through rectum. Rectal temperature was increased from 4 weeks and maintained to 6 weeks in fenofibrate treated group. This result reflected increased expression of hepatic UCP3 induced by fenofibrate.

HF group (37.7°C *vs.* 36.8°C) (Fig. 3).

Histological analysis of the liver

The liver tissues of HF groups showed slight hepatic steatosis on 1 week of high fat diet, gradually progressed on 3 weeks and presented severe hepatic steatosis around portal area on 6 weeks of high fat diet (Fig. 4A, C, E). In contrast to HF group, the liver tissues of HFF goups showed nearly normal architecture from 1 to 6 weeks of high fat diet with fenofibrate (Fig. 4B, D, F). As time goes by, the liver of HF group shows moderate to severe fatty changes at 3 and 6 weeks of high fat diet. Fenofibrate treatment, however, induced UCP3, improved hepatic steatosis and nearly normal appearance at 6 weeks even high fat diet feeding.

Discussion

In this study, we found that de novo expression of UCP3 by fenofibrate is timely interrelated with body weight and body core temperature in high fat diet rats. De novo expression of UCP3 in the liver by fenofibrate has been already reported that fenofibrate treatment (320 mg/kg) for 2 months induced de novo expression of UCP3 in high-fat diet fed rats [19]. However, there was little report about time-dependent effect of fenofibrate treatment on UCP3 de novo expression in rat liver. In the present study, we presented that fenofibrate (100 mg/kg) and high-fat diet induced hepatic UCP3 expression as early as 2 week of fenofibrate treatment. We investigated that much less dose of fenofibrate (100 mg/kg) compared to the dose of previous report

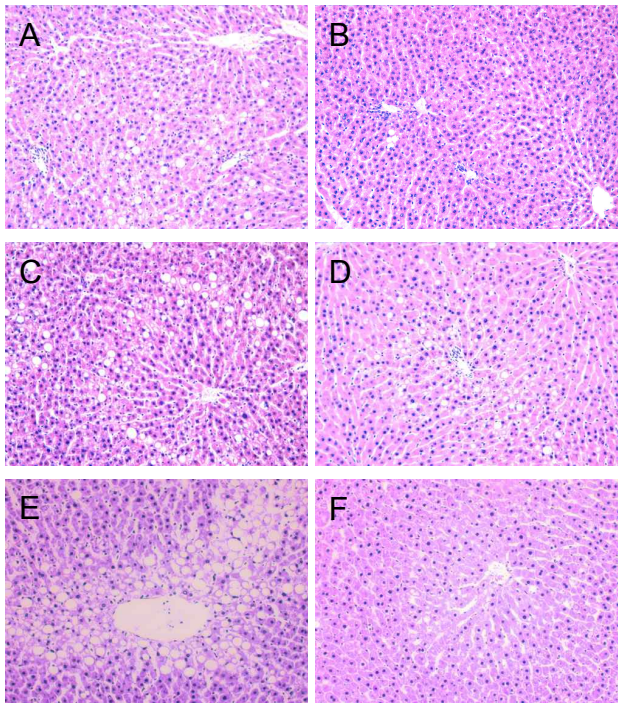

Fig. 4. Histological analysis of hepatic tissues of HF and HFF groups (H-E stain, x40). The livers of HF group show slight fatty change (C) to severe hepatic steatosis (E) from 1, 3 and 6 weeks (A, C and E, respectively). In contrast to HF group, the livers of HFF group show no fatty change on 1, 3 and 6 weeks (B, D and F, respectively)

(320 mg/kg) also could induce de novo expression of hepatic UCP3 mRNA. The amount of the UCP3 expression in liver was increased more and more as time went by.

Fenofibrate, and other PPARα agonists, were reported prevented weight gain and even decreased body weight and fat mass in models of genetic or diet-induced obesity in rodents [15,21,22]. In our previous report, we also reported that fenofibrate treatment decreased visceral adiposity and improve insulin sensitivity in obese type 2 diabetic rats [13]. The mechanisms of PPARα-induced reduction in weight gain were not fully known yet. One of the suggested mechanisms was that a reduction in fat mass and consequential decrease in leptin production may ultimately induce hypophagia and the resultant decrease in weight gain [21]. In this study there was significantly less weight gain in fenofibrate treated group nonetheless there was no difference in food intake.

Dissipating excess energy in liver is a possible therapeutic approach to high-fat diet induced obesity or metabolic disorders. UCP1 was originally identified in brown adipose

tissue and demonstrated to mediate non-shivering thermogenesis. UCP1 plays an important role in mediating cold exposure-induced thermogenesis and is also a likely regulator of diet-induced thermogenesis [6,8]. Enhanced UCP1 expression in the liver increased energy expenditure, decreased body weight, and reduced fat in high-fat diet-induced diabetes and obesity [10]. Alternatively, UCP3 predominantly expressed in muscle, is involved in energy homeostasis [19]. UCP3 may play functional roles not strictly related to the uncoupling. UCP3 can act as a FFA translocator and a role as a regulator of lipids as fuel substrate has been hypothesized in situations where lipid metabolism predominates [9]. De novo expression of UCP3 in liver, provided evidence that UCP3 and mitochondrial thioesterase-1 may concur in regulating fatty acid metabolism and enhancing energy dissipation in liver mitochondria [12]. In this study, we demonstrated that the de novo expression of UCP3 by fenofibrate treatment was related with increase of rectal temperature and less weight gain in high-fat fed rats. This result was similar with dissipating excess energy in the liver by enhanced UCP1 expression in previous report [10]. It might provide more comprehension of the physiological role of UCP3.

Fenofibrate also improves insulin resistance in diet-induced obesity animal models and in subjects with diabetes [7,13]. Enhanced expression of UCP1 in liver resulted in markedly improved insulin resistance and decreased blood glucose level in diabetes associated with diet-induced obese mice. In this study, de novo expression of hepatic UCP3 by fenofibrate treatment showed no significant effect on peripheral blood glucose level by high-fat diet fed rats. One of the possible causes of the result is that the high-fat fed rats consumed less amount of food than normal chow diet fed control group.

In conclusion, our results indicate that de novo expression of hepatic UCP3 induced by fenofibrate treatment timely increased with metabolic function such as decreasing body weight and increasing body core temperature and prevented hepatic steatosis.

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초록 : 고지방 섭취한 쥐에서 페노파이브레이트 복용에 의한 간 UCP3 발현 기간과 대사변화 관계

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Uncoupling protein 3 (UCP3)는 골격근에서 주로 발현되는 미토콘드리아 단백질이다. 생체의 대사기능에 관여할 것으로 알려져 있지만, 아직 그 역할에 대하여 깊이 연구되어 있지 않다. 최근에 고지혈증 치료제인 페노파이브레이트를 처치한 쥐의 간에서는 UCP3가 새로이 발현 유도된다는 것이 알려졌으며, 본 연구자들은 새로이 발현되는 UCP3가 비만쥐의 지방조직을 감소시키며, 인슐린 감수성을 증가시킨다는 보고를 하였다. 본 연구에서는 이러한 새로이 발현되는 UCP3의 시간에 따른 발현 양상과 대사 기능의 연관 관계를 규명하고자 하였다. 8주령 흰쥐를 두 군으로 나누어, 한 군은 고지방 식이를 실시하고(HF군), 다른 군은 고지방 식이와 페노파이브레이트(HFF군)를 함께 복용토록 하였다. 10주 동안 식이와 약물을 처리하면서 1, 3, 6 주에 간조직을 분석하였으며, 매주 먹이량, 체중, 체온을 측정하였다. 페노파이브레이트에 의하여 간에서 새로이 발현되는 UCP3는 2주부터 시작하여 3, 6주에 아주 높은 발현 양상을 보였다. 두 군 간의 먹이량의 차이는 없는 반면, 페노파이브레이트 처리군에서 6주부터 시작하여 체중증가가 억제되며, 오히려 감소되는 경향을 보였다. 체온은 페노파이브레이트 처리군에서6주부터 상승하기 시작하여, UCP3의 발현에 의한 간에서의 upcoupling 현상에 따른 발열현상으로 추측할 수 있다. 이상의 결과들로 유추해 볼 때, UCP3 발현이 3-6주부터 많은 양이 생성되어 생체의 대사 기능에 영향을 미치며, 이러한 시기와 연관되어 체중, 체온의 변화와 관련이 있는 것으로 생각한다.