Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 1587–1592

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

NO-1886 (ibrolipim), a lipoprotein lipase activator, increases the expression of uncoupling protein 3 in skeletal muscle and suppresses fat accumulation in high-fat diet–induced obesity in rats

Masataka Kusunoki*, Kazuhiko Tsutsumi, Koshi Iwata, Weidong Yin, Takao Nakamura, Hitoshi Ogawa, Tomoko Nomura, Koya Mizutani, Arao Futenma, Keiko Utsumi, Tetsuro Miyata

Medical Clinic, Aichi Medical University, Aichi 261-0005, Japan
Otsuka Pharmaceutical Factory, Inc., Tokushima 772-0801, Japan
School of Medicine, Yamagata University, Yamagata 992-8510, Japan
Nanhua University Medical College, Hunan 421001, PR China
School of Medicine, University of Tokyo, Tokyo 113-0033, Japan

Received 10 November 2004; accepted 7 June 2005

Abstract

Although the lipoprotein lipase (LPL) activator NO-1886 shows antiobesity effects in high-fat–induced obese animals, the mechanism remains unclear. To clarify the mechanism, we studied the effects of NO-1886 on the expression of uncoupling protein (UCP) 1, UCP2, and UCP3 in rats. NO-1886 was mixed with a high-fat chow to supply a dose of 100 mg/kg to 8-month-old male Sprague-Dawley rats. The animals were fed the high-fat chow for 8 weeks. At the end of the administration period, brown adipose tissue (BAT), mesenteric fat, and soleus muscle were collected and levels of UCP1, UCP2, and UCP3 messenger RNA (mRNA) were determined. NO-1886 suppressed the body weight increase seen in the high-fat control group after the 8-week administration (585 ± 39 vs 657 ± 66 g, $P < .05$). NO-1886 also suppressed fat accumulation in visceral (46.9 ± 10.4 vs 73.7 ± 14.5 g, $P < .01$) and subcutaneous (43.1 ± 18.1 vs 68.9 ± 18.8 g, $P < .05$) tissues and increased the levels of plasma total cholesterol and high-density lipoprotein cholesterol in comparison to the high-fat control group. In contrast, NO-1886 decreased the levels of plasma triglycerides, nonesterified free fatty acid, glucose, and insulin. NO-1886 increased LPL activity in soleus muscle (0.082 ± 0.013 vs 0.061 ± 0.016 μmol of free fatty acid per minute per gram of tissue, $P < .05$). NO-1886 increased the expression of UCP3 mRNA in soleus muscle 3.14-fold ($P < .01$) compared with the high-fat control group without affecting the levels of UCP3 in mesenteric adipose tissue and BAT. In addition, NO-1886 did not affect the expression of UCP1 and UCP2 in BAT, mesenteric adipose tissue, and soleus muscle. In conclusion, NO-1886 increased the expression of UCP3 mRNA and LPL activity only in skeletal muscle. Therefore, a possible mechanism for NO-1886's antiobesity effects in rats may be the enhancement of LPL activity in skeletal muscle and the accompanying increase in UCP3 expression.

1. Introduction

We have previously reported that the lipoprotein lipase (LPL) activator NO-1886 suppresses subcutaneous and visceral fat accumulation and shows antiobesity effects in high-fat–induced obese rats and pigs [1,2]. We also suggested that the antiobesity effects of NO-1886 might be the result of the increase in skeletal muscle LPL activity because NO-1886 had no effect on adipose tissue LPL activity [1]. However, this proposed mechanism remained unclear. Some investigators have suggested that increasing LPL activity in adipose tissue results in increased fat accumulation [3,4], whereas others have suggested that increasing LPL activity in skeletal muscle results in decreased fat accumulation [5-7]. It has long been known that uncoupling proteins (UCPs) are responsible for facultative thermogenesis in rodents. UCPs play an important role in energy metabolism and obesity [8,9]. UCP1 expression is restricted to brown adipose tissue (BAT), UCP2 is widely expressed, and UCP3 is found mainly in

* Corresponding author. Medical Clinic, Aichi Medical University, 2-12-1, Higashisakura, Higashi-ku, Nagoya, Aichi 261-0005, Japan.
E-mail address: kusunoki@aichi-med-u.ac.jp (M. Kusunoki).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.06.005

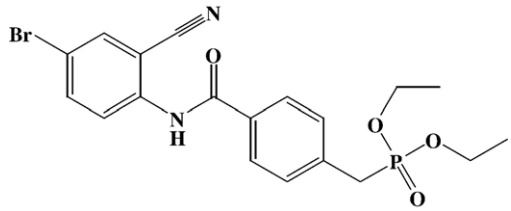

Fig. 1. Chemical structure of NO-1886.

skeletal muscle [10]. To clarify the relationship between skeletal muscle LPL activity and the antiobesity effects of NO-1886, we studied the effects of NO-1886 on the expression of UCP1, UCP2, and UCP3 in rats.

2. Materials and methods

2.1. Materials

Agent NO-1886 (ibrolipim) [4-diethoxyphosphoryl-methyl-*N*-(4-bromo-2-cyanophenyl)benzamide, CAS 133208-93-2; lot C99H74SM] was synthesized in the New Drug Research Laboratory of Otsuka Pharmaceutical Factory, Tokushima, Japan. Glycerol tri[1-^{14}C]oleate (2.2 GBq/mmol) was obtained from Amersham, Cardiff, UK. The chemical structure is shown in Fig. 1. All other chemicals used were high-grade commercially available products.

2.2. Animal experiments

Male Sprague-Dawley rats weighing 500 to 600 g at the age of 8 months were obtained from Japan SLC, Shizuoka, Japan. The animals were maintained under a 12-h light-dark cycle (light cycle from 7:00 AM to 7:00 PM) at a constant temperature of 23°C ± 2°C. Rats were fed for 8 weeks with either high-fat chow (26.7% safflower oil in standard laboratory chow; CRF-1, Oriental Yeast, Tokyo, Japan) containing NO-1886 (NO-1886 group 20.17 kJ/g [4.82 kcal/g]), high-fat chow (high-fat group, 20.17 kJ/g [4.82 kcal/g]), or standard chow (low-fat group, 15.06 kJ/g [3.6 kcal/g]). The rats were housed 1 per cage. The rats were allocated to the high-fat group, NO-1886 group, or low-fat group based on baseline body weight and were given free access to food and tap water. Food consumption and body weight were recorded every 4 weeks. At the end of the experimental period and after a 12-hour overnight fast, the animals were killed by exsanguination under sodium pentobarbital anesthesia. Blood samples were collected from the posterior vena cava for lipid, glucose, and insulin measurements. Visceral fat and subcutaneous fat were removed and weighed. Soleus hind limb muscle, mesenteric white adipose tissue, and scapula BAT were obtained with tongs and maintained in liquid nitrogen for measurement of LPL activity and UCP messenger RNA (mRNA).

2.3. Analytical methods

Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, nonesterified free fatty acid (NEFA), and glucose were determined by conventional enzymatic methods. The cholesterol C-test Wako (Wako Pure Chemical Industries, Osaka, Japan) was used for cholesterol, the Nescote HDL-C kit N (Nippon Shoji, Osaka Japan) for HDL-C, the triglyceride G-test Wako (Wako Pure Chemical Industries) for triglycerides, the NEFA C-test Wako (Wako Pure Chemical Industries) for NEFA, and the glucose CII test Wako for glucose. Insulin was determined by conventional enzyme immunoassay, with the use of the Glazyme insulin-EIA test (Wako Pure Chemical Industries).

2.4. Tissue LPL activity

Soleus heparin-released LPL activity was measured as reported previously [11,12]. A specimen of muscle was homogenized in 50 mmol/L NH_4Cl buffer (pH 8.5) and incubated with buffer containing heparin for 60 minutes at 0°C. The suspension was then centrifuged, and the supernatant was used to measure heparin-released LPL activity. Adipose tissue heparin-released LPL activity was measured

Table 1
Food intakes of low-fat–fed rats and NO-1886–treated high-fat–fed rats

Group	n	Food intake (g)			Food intake (kcal [kJ])		
		Before	4 wk	8 wk	Before	4 wk	8 wk
Low fat[a]	6	17.6 ± 3.6	18.0 ± 4.9*	18.4 ± 2.6*	63.4 ± 12.9 [265.3 ± 54.0]	64.8 ± 17.6 [271.1 ± 73.6]	66.2 ± 9.4 [277.0 ± 39.3]
High fat[b]							
Control	6	18.5 ± 4.9	13.7 ± 1.5	14.3 ± 1.5	66.6 ± 17.6 [278.6 ± 73.6]	66.0 ± 7.2 [278.6 ± 30.1]	68.9 ± 7.2 [288.3 ± 30.1]
NO-1886	6	19.1 ± 2.6	14.0 ± 1.7	14.8 ± 2.8	68.7 ± 9.4 [287.4 ± 39.3]	67.5 ± 8.1 [282.4 ± 33.9]	71.3 ± 13.5 [298.3 ± 56.5]

Data are expressed as means ± SD.
Before indicates before starting high-fat diet.
[a] Low-fat chow: 15.06 kJ/g (3.6 kcal/g).
[b] High-fat chow: 20.17 kJ/g (4.82 kcal/g).
* $P < .05$, significantly different from high-fat control group.

Table 2
Plasma lipid, glucose, and insulin levels in NO-1886–treated high-fat–fed rats after 8 weeks of administration

Group	n	Total cholesterol (mg/dL)	HDL-C (mg/dL)	Triglyceride (mg/dL)	NEFA (μEq/L)	Glucose (mg/dL)	Insulin (μU/mL)
Low fat	6	59 ± 14	51 ± 11	122 ± 9*	373 ± 9*	149 ± 7**	53 ± 13*
High fat							
Control	6	73 ± 19	52 ± 7	161 ± 32	516 ± 98	171 ± 12	91 ± 27
NO-1886	6	126 ± 20**	118 ± 8**	83 ± 12**	368 ± 41**	152 ± 5*	58 ± 20*

Data are expressed as means ± SD.
* $P < .05$, significantly different from high-fat group.
** $P < .01$, significantly different from high-fat group.

as described earlier [13]. Because the high-fat content and fragility of the adipose precluded making a tissue homogenate, a specimen of adipose tissue weighing 100 mg was minced into small pieces and placed in Krebs-Ringer bicarbonate buffer (pH 7.4) in the presence of heparin for 60 minutes at 37°C. The incubation medium was then assayed for LPL activity.

2.5. RNA preparation and real-time reverse transcription–polymerase chain reaction

Total RNA was extracted from a 30-mg tissue sample of soleus muscle, mesenteric adipose tissue, and BAT by the QIAGEN RNeasy mini kit (QIAGEN, Tokyo, Japan). The extracted total RNAs were dissolved in 100 μL of RNase-free water and stored at −80°C until use.

Real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) was performed to determine the relative expression levels of the rat UCP1, UCP2, and UCP3 in tissue. The TaqMan probe consisted of an oligonucleotide with a 5′ reporter dye and a downstream 3′ quencher dye. The fluorescent reporter dye, FAM (6-carboxy-fluorescein), was covalently linked to the 5′ end of the oligonucleotide. This reporter dye was quenched by TAMRA (6-carboxy-tetramethyl-rhodamine) typically located at the 3′ end. Fluorescence quenching depended on the spatial proximity of the reporter and quencher dyes. Oligonucleotide primers and TaqMan probes were used as reported by Xiao et al [14]. The RT-PCR reaction was performed in a 30-μL volume reaction using TaqMan One-Step RT-PCR Master Mix Reagents kit (Applied Biosystems, Foster City, CA) in 96-well plates for the rat UCPs and the endogenous control, GAPDH mRNA. TaqMan assay reaction buffer contained 1× Master Mix reagents, 1× MultiScribe and RNase Inhibitor Mix (TaqMan One-Step RT-PCR Master Mix Reagents), 300 nmol/L forward primer, 900 nmol/L reverse primer, 200 nmol/L TaqMan probe, and 50 ng total RNA. However, rodent GAPDH mRNA reaction buffer contained all the above, except for the following differences: 50 nmol/L forward primer, 50 nmol/L reverse primer, and 50 nmol/L TaqMan probe. Reverse transcription conditions were 48°C for 30 minutes and 95°C for 10 minutes for 1 cycle; PCR conditions were 95°C for 15 seconds and 60°C for 1 minute for 40 cycles on an ABI PRISM 7700 Sequence Detector System (Applied Biosystems). The results for expression of mRNA were presented relative to the expression of the control gene, GAPDH mRNA.

Table 3
Heparin-released LPL activities in mesenteric adipose tissue and soleus muscle in NO-1886–treated high-fat–fed rats after 8 weeks of administration

Group	n	Soleus muscle	Mesenteric adipose tissue	Visceral fat
Low fat	6	0.088 ± 0.014*	0.133 ± 0.101**	4.225 ± 1.750**
High fat				
Control	6	0.061 ± 0.016	0.418 ± 0.032	30.52 ± 7.608
NO-1886	6	0.082 ± 0.018*	0.439 ± 0.040	20.98 ± 4.502*

We determined the heparin-releasable LPL activity in both soleus muscle and adipose tissue. LPL activity is given in micromoles of free fatty acid per minute per wet gram of tissue for soleus muscle and mesenteric adipose tissue and in micromoles of free fatty acid per minute per wet total visceral fat for the visceral fat. Data are expressed as means ± SD.
* $P < .05$, significantly different from high-fat control group.
** $P < .01$, significantly different from high-fat control group.

2.6. Statistical analysis

The data were analyzed using StatView (version 5) statistical analysis software supported by SAS Institute (Cary, NC). Data are expressed as means ± SD. Comparisons among the 3 groups were analyzed for statistical significance using 1-way analysis of variance followed by Dunnett test multiple comparisons. P values less than .05 were considered significant. Correlation analysis was performed using Spearman test.

3. Results

3.1. Effects of NO-1886 on body weight gain and food consumption

The body weight of the high-fat group was significantly increased compared with the low-fat group after 4 weeks of feeding and remained significantly increased for the duration of the experiment. Addition of NO-1886 to the high-fat diet reduced the weight gain, such that the NO-1886 group had a statistically significantly lower body weight ($P < .05$) from 4 weeks to the end of the experiment compared with the high-fat group (low fat: 503 ± 32, high fat: 602 ± 57, NO-1886: 533 ± 32 at 4 weeks; low fat: 518 ± 36, high fat: 657 ± 66, NO-1886: 585 ± 39 at 8 weeks). High-fat feeding resulted in a decrease in the

food intake; however, energy intake was not affected. There was no significant difference in food intake (grams and kilojoules) between the high-fat group and NO-1886 group (Table 1).

3.2. Effects of NO-1886 on visceral fat and subcutaneous fat weight

The control group had a 2.3- and 1.6-fold increase in visceral and subcutaneous fat weight, respectively, compared with the low-fat group. Treatment with NO-1886 significantly suppressed the increase in accumulation of visceral fat and subcutaneous fat ($P < .05$) compared with high-fat control group (visceral fat weight, low fat: 32.3 ± 8.6, high fat control: 73.7 ± 14.5, NO-1886: 46.9 ± 10.4; subcutaneous fat weight, low fat: 41.8 ± 18.4, high fat: 68.9 ± 18.8, NO-1886: 43.1 ± 18.1).

3.3. Effects of NO-1886 on plasma lipid, glucose, and insulin levels

Plasma lipid, glucose, and insulin levels are shown in Table 2. Plasma triglyceride, NEFA, glucose, and insulin levels in the high-fat group were higher than in the low-fat group. NO-1886 increased plasma total cholesterol and HDL-C and decreased triglyceride, NEFA, glucose, and insulin levels.

3.4. Effects of NO-1886 on tissue LPL activity

The LPL activity in mesenteric adipose tissue and soleus muscle is shown in Table 3. Soleus muscle LPL activity was reduced by the high-fat diet. However, NO-1886 administration resulted in LPL activity returning to normal levels (Table 3). Mesenteric adipose tissue LPL activity in rats fed

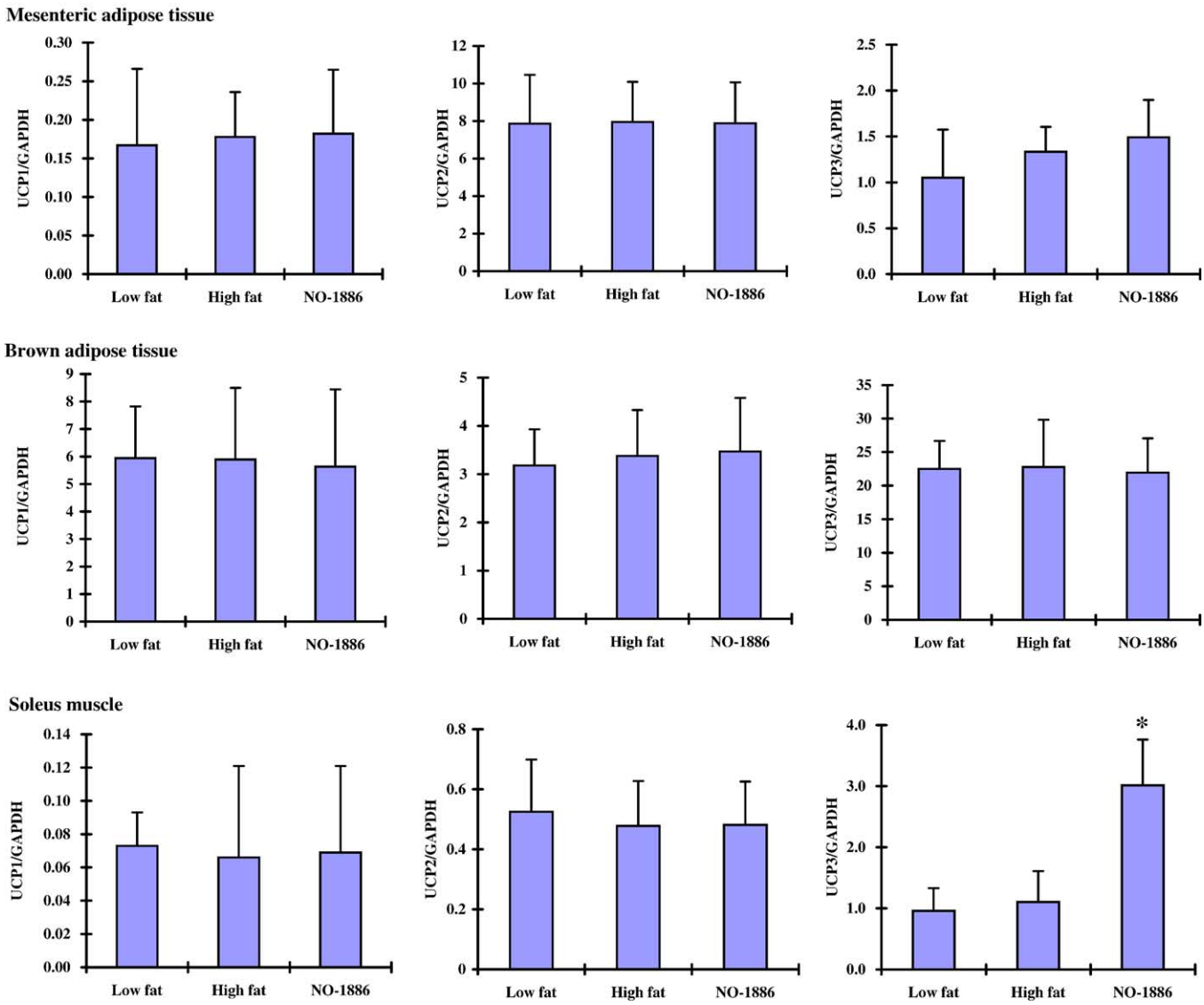

Fig. 2. Effects of NO-1886 on UCP1, UCP2, and UCP3 mRNA. NO-1886 was mixed with a high-fat chow to supply a dose of 100 mg/kg to 8-month-old male Sprague-Dawley rats. Total RNA was isolated from mesenteric adipose tissue, BAT, and skeletal muscle. Tissue UCP levels were determined by real-time quantitative RT-PCR. Data are expressed as means ± SD (n = 6). Low fat indicates standard chow group; high fat, high-fat chow group; NO-1886, high-fat chow containing NO-1886 group. * $P < .01$, significantly different from the value in the respective high-fat group.

with a high-fat diet increased 3.1-fold compared with the low-fat group. Treatment with NO-1886 had no effect on the adipose tissue increase of LPL activity caused by the high-fat diet when expressed per gram of tissue. Given the reduced visceral adipose tissue mass and assuming that the LPL results in mesenteric adipose tissue can be generalized to visceral adipose tissue, overall LPL activity in visceral adipose tissue is significantly reduced by NO-1886 (Table 3).

3.5. Effects of NO-1886 on mRNA levels of UCP1, UCP2, and UCP3 in rat tissue

The mRNA levels of UCP1, UCP2, and UCP3 in mesenteric adipose tissue, BAT, and soleus muscle were not affected by the high-fat diet. NO-1886 administration caused a 3.14-fold increased in UCP3 mRNA levels in soleus muscle compared with the high-fat group. However, NO-1886 administration did not affect UCP3 mRNA levels in mesenteric adipose tissue and BAT. NO-1886 had no effects on UCP1 and UCP2 mRNA in mesenteric adipose tissue, BAT, and soleus muscle (Fig. 2).

4. Discussion

NO-1886 is known to be an LPL activator that increases LPL activity in primary cultured adipose and skeletal muscle cells [15]. NO-1886 prevents the development of atherosclerosis by increasing LPL activity, reducing plasma triglycerides, and elevating HDL-C [12,16]. In addition, Kusunoki et al [1] have reported that NO-1886 prevents fat accumulation in both visceral and subcutaneous adipose tissue in high-fat–induced obese rats without affecting food intake. Furthermore, Hara et al [17] have reported that NO-1886 causes a reduction in respiratory quotient in fructose-fed rats, and Doi et al [18] have reported that NO-1886 accelerates the expression of fatty acid oxidation–related enzymes, resulting in a reduction of respiratory quotient. However, the mechanism for the antiobesity effects of NO-1886 remained unclear. To clarify the mechanism, we studied the effects of NO-1886 on the expression of UCP1, UCP2, and UCP3 in rats.

In this study, high-fat feeding caused the elevation of plasma triglycerides, NEFA, glucose, and insulin levels in rats. However, the administration of NO-1886 normalized the plasma triglyceride, NEFA, glucose, and insulin levels. In addition, NO-1886 increased plasma total cholesterol and HDL-C. These results may indicate that NO-1886 improved the insulin resistance in the high-fat–fed rats.

Furthermore, NO-1886 suppressed fat accumulation in visceral and subcutaneous tissues, resulting in a decrease in body weight after the 8-week administration in the high-fat–induced obese rats. NO-1886 increased LPL activity in skeletal muscle, decreased LPL activity in total visceral fat, and increased the expression of UCP3 mRNA in skeletal muscle compared with the high-fat group, but had no effects on skeletal muscle UCP1 and UCP2.

Kratky et al [19] reported that the expression levels of UCP1 and UCP2 in BAT and in skeletal muscle were not affected by variations in tissue LPL activities and that UCP3 mRNA levels were induced in mice with high levels of LPL in skeletal muscle. In this study, there was a positive correlation between skeletal muscle LPL activity and UCP3 mRNA ($r = 0.621$, $P = .029$) in the high-fat and NO-1886 groups. Therefore, our results may indicate that NO-1886 increases the expression of UCP3 mRNA in skeletal muscle by increasing the LPL activity in skeletal muscle. However, UCP3 mRNA levels in the high-fat group were similar to the low-fat group. This may indicate that the elevation of the normal UCP levels resulted in a decrease the fat accumulation.

Weigle et al [20] reported that skeletal muscle UCP3 mRNA increased because of the increase in plasma NEFA caused by fasting and lipid emulsion. Intralipid plus heparin infusion and induction of UCP3 may be linked to the use of NEFA. However, NO-1886 did not increase plasma NEFA concentration. Rather, NO-1886 administration caused a decrease in plasma NEFA in this study. This may indicate that NO-1886 accelerates fatty acid oxidation [18]. On the other hand, Kratky et al [19] reported that the response of UCP3 mRNA expression to variations in LPL activity in skeletal muscle was independent of feeding status and plasma NEFA concentration. These findings may indicate that NEFA, as products from chylomicrons and very-low-density lipoprotein hydrolysis by LPL, increases UCP3 expression by promoting the increased uptake of NEFA in skeletal muscle. Therefore, our results may indicate that NO-1886 increases the expression of UCP3 mRNA by increasing uptake of NEFA in skeletal muscle by increasing LPL activity in skeletal muscle.

Clapham et al [21] reported that transgenic mice, which overexpress human UCP3 in skeletal muscle, weighed much less than their wild-type littermates and that magnetic resonance imaging showed a striking reduction in adipose tissue mass. In this study, NO-1886 administration caused a reduction in body weight and adipose tissue mass by inducing UCP3 mRNA in skeletal muscle and by increasing LPL activity in skeletal muscle. These results are in accordance with the report of Clapham et al [21] on overexpressing UCP3 in mouse skeletal muscle. However, NO-1886 decreased plasma insulin levels. Insulin increases the LPL activity and fat accumulation in adipose tissue [22]. Therefore, additional studies are necessary to clarify the relationship between plasma insulin levels and suppression of fat accumulation of NO-1886.

Skeletal muscle is the largest single tissue in humans and animals, making up more than 40% of the total body mass in young adults [23]. UCP3 expression is found mainly in skeletal muscle [10]. Therefore, the increase of UCP3 in skeletal muscle may induce the antiobesity effects.

Recently, Kitajima et al [7] reported on transgenic rabbits overexpressing human LPL transgene. In these LPL transgenic rabbits fed with a high-fat diet, LPL activity increased

in skeletal muscle but did not increase in adipose tissue, resulting in a significant reduction of visceral and subcutaneous fat compared with control rabbits. These results show that the increased LPL activity in skeletal muscle may induce the reduction of body adipose tissue.

Kim et al [24] reported that transgenic mice with skeletal muscle-specific overexpression of LPL had a 3-fold increase in muscle triglyceride content and were insulin-resistant. However, in this study, NO-1886 decreased the plasma glucose and insulin levels and decreased fat accumulation in high-fat–induced obese rats. These results may indicate that NO-1886 improved the insulin resistance. In addition, Kitajima et al [7] reported that the increase in skeletal muscle LPL activity induced the improvement of insulin resistance without increasing muscle triglyceride contents. The reason for the difference between our results and the results of Kim et al [24] is unknown.

However, there are different points between our studies and the study of Kim et al [24]. We used rats, Kitajima et al [7] used rabbits, and Kim et al [24] used mice. In addition, in our study and that of Kitajima et al [7], the animals were fed with a high-fat diet (obesity), but in the study of Kim et al [24], the animals were not fed with a high-fat diet (not obesity). It is necessary to clarify the difference of the results between our study and that of Kim et al [24] regarding insulin resistance in further studies.

In summary, the results of our study indicate that NO-1886 increases skeletal muscle LPL activity and increases UCP3 mRNA in skeletal muscle, resulting in suppression of fat accumulation in high-fat–induced obese rats. These results indicate that the main mechanism for the antiobesity effect seen after NO-1886 administration may be the increase in UCP3 expression in skeletal muscle.

References

[1] Kusunoki K, Tsutsumi K, Nakamura T, et al. The lipoprotein lipase activator, NO-1886, suppresses fat accumulation and insulin resistance in rats fed a high-fat diet. Diabetologia 2000;43:875-80.

[2] Yin W, Liao D, Wang Z, et al. NO-1886 inhibits size of adipocytes, suppresses plasma levels of tumor necrosis factor–α and free fatty acids, improves glucose metabolism in high-fat/high-sucrose-fed miniature pigs. Pharmacol Res 2004;49:199-206.

[3] Kern PA, Ong JM, Saffarin B, et al. The effects of weight loss on the activity and expression of adipose tissue lipoprotein lipase in very obese humans. N Engl J Med 1990;322:1053-9.

[4] Bessesen DH, Robertson AD, Eckel RH. Weight reduction increases adipose but decreases cardiac LPL in reduced-obese Zucker rats. Am J Physiol 1991;261:E246-51.

[5] Levak-Frank S, Radner H, Walsh A, et al. Muscle-specific overexpression of lipoprotein lipase causes a severe myopathy characterized by proliferation of mitochondria and peroxisomes in transgenic mice. J Clin Inves 1995;96:976-86.

[6] Jensen DR, Schlaepefer IR, Morin CL, et al. Prevention of diet-induced obesity in transgenic mice overexpressing skeletal muscle lipoprotein lipase. Am J Physiol 1997;273:R683-9.

[7] Kitajima S, Morimoto M, Liu E, et al. Overexpression of lipoprotein lipase improves insulin resistance induced by a high-fat diet in transgenic rabbits. Diabetologia 2004;47:1202-9.

[8] Boss O, Muzzin P, Giacobino J-P. The uncoupling proteins, a review. Eur J Endocrinol 1998;139:1-9.

[9] Ricquier D, Bouillaud F. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem J 2000;345:161-79.

[10] Schrauwen P, Hesselink M. UCP2 and UCP3 in muscle controlling body metabolism. J Exp Biol 2002;205:2275-85.

[11] Murase T, Uchimura H. A selective decline of post-heparin plasma hepatic triglyceride lipase in hypothyroid rats. Metabolism 1980; 29:797-801.

[12] Tsutsumi K, Inoue Y, Shima A, et al. The novel compound NO-1886 increases lipoprotein lipase activity with resulting elevation of high density lipoprotein cholesterol, and long-term administration inhibits atherogenesis in the coronary arteries of rats with experimental atherosclerosis. J Clin Invest 1993;92:411-7.

[13] Murase T, Yamada N, Matsuzaki F, et al. The in vivo effects of growth hormone on adipose tissue lipoprotein lipase in rats. Life Sci 1981; 28:7199-201.

[14] Xiao XQ, Grove KL, Grayson BE, et al. Inhibition of uncoupling protein expression during lactation: role of leptin. Endocrinology 2004;145:830-8.

[15] Hagi A, Hirai I, Kohri H, et al. The novel compound NO-1886 activates lipoprotein lipase in primary cultured adipose and skeletal muscle cells. Biol Pharm Bull 1997;20:1108-10.

[16] Chiba T, Miura S, Sawamura F, et al. Antiatherogenic effects of a novel lipoprotein lipase-enhancing agent in cholesterol-fed New Zealand white rabbits. Arterioscler Thromb Vasc Biol 1997;17: 2601-8.

[17] Hara T, Cameron-Smith D, Cooney GJ, et al. The action of a novel lipoprotein lipase activator, NO-1886, in hypertriglyceridemic fructose-fed rats. Metabolism 1998;47:149-53.

[18] Doi M, Kondo Y, Tsutsumi K. Lipoprotein lipase activator NO-1886 (ibrolipim) accelerates the mRNA expression of fatty acid oxidation-related enzymes in rat liver. Metabolism 2003;52:1547-50.

[19] Kratky D, Strauss JG, Zechner R. Tissue-specific activity of lipoprotein lipase in skeletal muscle regulates the expression of uncoupling protein 3 in transgenic mouse models. Biochem J 2001;355:647-52.

[20] Weigle DS, Selfridge LE, Schwartz MW, et al. Elevated free fatty acids induced uncoupling protein 3 expression in muscle. Diabetes 1998;47:298-302.

[21] Clapham JC, Arch JRS, Chapman H, et al. Mice overexpressing human uncoupling protein–3 in skeletal muscle are hyperphagic and lean. Nature 2000;406:415-8.

[22] Eckel RH. Adipose tissue lipoprotein lipase. In: Borensztajn J, editor. Lipoprotein lipase. Chicago (Ill): Evener Publishers; 1987. p. 79-132.

[23] Murray RK. Muscle and the cytoskeleton. In: Murray RK, editor. Harper's biochemistry. 25th ed. New York: McGraw-Hill; 2000. p. 715-36.

[24] Kim JK, Fillmore JJ, Chen Y, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A 2001;98:7522-7.