

|             |   |
|-------------|---|
| Title       | Activation of peroxisome proliferator-activated receptor-enhances fatty acid oxidation in human adipocytes. |
| Author(s)   | Lee, Joo-Young; Hashizaki, Hikari; Goto, Tsuyoshi; Sakamoto, Tomoya; Takahashi, Nobuyuki; Kawada, Teruo     |
| Citation    | Biochemical and biophysical research communications (2011), 407(4): 818-822                                 |
| Issue Date  | 2011-04-22  |
| URL         | <a href="http://hdl.handle.net/2433/140336">http://hdl.handle.net/2433/140336</a>                           |
| Right       | © 2011 Elsevier Inc.  |
| Type        | Journal Article   |
| Textversion | author  |

Activation of peroxisome proliferator-activated receptor- $\alpha$  enhances fatty acid  
oxidation in human adipocytes

Joo-Young Lee, Hikari Hashizaki, Tsuyoshi Goto, Tomoya Sakamoto, Nobuyuki Takahashi  
and Teruo Kawada \*

*Laboratory of Molecular Function of Food, Division of Food Science and Biotechnology,  
Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan*

\* Corresponding Author: Teruo Kawada, Ph.D.

Laboratory of Molecular Function of Food, Division of Food  
Science and Biotechnology, Graduate School of Agriculture, Kyoto  
University, Uji 611-0011, Japan.

FAX: +81-774-38-3853, E-mail: [fat@kais.kyoto-u.ac.jp](mailto:fat@kais.kyoto-u.ac.jp)

Running Title: Activation of PPAR $\alpha$  enhances FA oxidation in adipocytes.

Keywords: PPAR $\alpha$ , adipocyte, insulin sensitivity, fatty acid oxidation.

## Abstract

Peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) is a key regulator for maintaining whole-body energy balance. However, the physiological functions of PPAR $\alpha$  in adipocytes have been unclarified. We examined the functions of PPAR $\alpha$  using human multipotent adipose tissue-derived stem cells as a human adipocyte model. Activation of PPAR $\alpha$  by GW7647, a potent PPAR $\alpha$  agonist, increased the mRNA expression levels of adipocyte differentiation marker genes such as *PPAR $\gamma$* , *adipocyte-specific fatty acid-binding protein*, and *lipoprotein lipase* and increased both GPDH activity and insulin-dependent glucose uptake level. The findings indicate that PPAR $\alpha$  activation stimulates adipocyte differentiation. However, lipid accumulation was not changed, which is usually observed when PPAR $\gamma$  is activated. On the other hand, PPAR $\alpha$  activation by GW7647 treatment induced the mRNA expression of fatty acid oxidation-related genes such as *CPT-1B* and *AOX* in a PPAR $\alpha$ -dependent manner. Moreover, PPAR $\alpha$  activation increased the production of CO<sub>2</sub> and acid soluble metabolites, which are products of fatty acid oxidation, and increased oxygen consumption rate in human adipocytes. The data indicate that activation of PPAR $\alpha$  stimulates both adipocyte differentiation and fatty acid oxidation in human adipocytes, suggesting that PPAR $\alpha$  agonists could improve insulin resistance without lipid accumulation in adipocytes. The expected effects of PPAR $\alpha$  activation are very valuable for managing diabetic conditions accompanied by obesity, because PPAR $\gamma$  agonists, usually used as antidiabetic drugs, induce excessive lipid accumulation in adipocytes in addition to improvement of insulin resistance.

## 1. Introduction

Metabolic syndrome in an increasing number of people is a serious problem in many countries. Although many factors are involved in the development of metabolic syndrome, a critical factor is obesity accompanied by adipose tissue hypertrophy [1]. Thus, prevention and improvement of obesity is indispensable for treatments of metabolic syndrome. Obesity is due to adipocyte hyperplasia and hypertrophy caused by intake of excess energy. Therefore, it is important for the prevention and improvement of obesity to maintain an adequate balance between energy accumulation and consumption.

Energy accumulation is regulated by peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) in adipose tissues [2]. PPAR $\gamma$  activation induces lipid-accumulation-related genes, such as *fatty acid synthase (FAS)*, *lipoprotein lipase (LPL)*, and *fatty acid transport protein-1 (FATP1)*, and promotes adipocyte differentiation [3]. As a result, the number of adipocytes increases in adipose tissues. Thus, a long duration of PPAR $\gamma$  activation causes severe obesity and insulin resistance [4]. However, because adipocyte differentiation induced by PPAR $\gamma$  activation increases the number of insulin-sensitive adipocytes, short-term or midterm PPAR $\gamma$  activation improves insulin resistance [5]. Indeed, thiazolidinediones (TZDs), potent PPAR $\gamma$  agonists, have been widely used as antidiabetic drugs. On the other hand, energy consumption is regulated by PPAR $\alpha$ , another PPAR isoform, in the liver and skeletal muscle [6]. PPAR $\alpha$  activation upregulates fatty acid oxidation-related genes including *acyl-CoA synthase (ACS)*, *acyl-CoA oxidase (AOX)*, and *carnitine palmitoyl transferase (CPT)* so that fatty acid oxidation is enhanced in both mitochondria and peroxisomes [6]. Because PPAR $\alpha$  activation decreases hepatic and circulating lipid amounts, fibrates, and synthetic PPAR $\alpha$  agonists, improve fatty liver and hyperlipidemia. Therefore, both PPAR isoforms serve as key regulators for the maintenance of whole-body energy balance, although they have opposite functions in energy metabolism.

Recently, several reports have shown the involvement of PPAR $\alpha$  in adipocyte functions [7,8]. However, the physiological functions of PPAR $\alpha$  in adipocytes have not been elucidated

yet. Here, we examined the effects of PPAR $\alpha$  activation on adipocyte differentiation to elucidate PPAR $\alpha$ -associated regulation of energy balance between accumulation and consumption in adipocytes.

## **2. Materials and Methods**

### *2.1. Chemicals and cell culture*

GW7647, a PPAR $\alpha$  agonist, was purchased from Sigma (MO, USA) and dissolved in DMSO as a stock solution. All the other chemicals used were from Sigma or Nacalai Tesque (Kyoto, Japan) and guaranteed to be of reagent or tissue-culture grade.

Human multipotent adipose tissue-derived stem cells established by Vernochet et al. [9] were used as a human adipocyte model. The cells were cultured in accordance with a protocol previously described [10]. Human adipocytes were maintained in a maintenance medium (10% FBS and 10 mg/ml penicillin/streptomycin in DMEM) at 37 °C in 5% CO<sub>2</sub>/95% air under a humidified condition. For white adipocyte differentiation, cells were seeded at a high density (25,000 cells/cm<sup>2</sup>). Two days after reaching confluence, the cells were incubated in differentiation medium (DM), which was DMEM/F12 supplemented with 1  $\mu$ M dexamethazone (DEX), 0.1 mM 1-methyl-3-isobutylxanthine (IBMX), 5  $\mu$ g/ml insulin, and 500 nM pioglitazone. Three days after the treatment, the cell culture medium was changed to post-DM, which was DMEM/F-12 medium supplemented with 5  $\mu$ g/ml insulin and 500 nM pioglitazone. The medium was then changed with a fresh one every 2 days until day 12. Cells were treated with the indicated concentration of GW7647 throughout experimental period (from day 0 to day 12). Cells cultured on 6- or 12-well tissue culture plates were prepared for biochemical assays.

To examine the effects of GW7647 on insulin sensitivity, the uptake of [<sup>3</sup>H]-labeled 2-deoxy-d-[1,2-<sup>3</sup>H]-glucose ([<sup>3</sup>H]-labeled 2DG) was measured using human differentiated adipocytes on 24-well plates as previously described [11]. Briefly, 12 days after

differentiation induction in the presence or absence of GW7647, human adipocytes were incubated in DMEM/F-12 for 5 h. After washing with HKR buffer (containing 140 mM NaCl, 5 mM KCl, 1 mM CaCl<sub>2</sub>, 2.5 mM MgSO<sub>4</sub>, and 20 mM HEPES; pH 7.4), the cells were then incubated with 0.5 mL of HKR buffer containing 1% FA-free BSA at 37°C for 20 min in the presence or absence of 100 nM insulin. [<sup>3</sup>H]-labeled 2DG (1 μCi) (American Radiolabeled Chemicals, MO, USA) was added to each well, followed by incubation for 10 min. The cells were washed with cold PBS and solubilized in 0.1 N NaOH for counting the radioactivity of the aqueous solution in a scintillation counter (LS6500, Beckman Coulter, CA, USA).

## 2.2. Quantitative RT-PCR analysis

RNA samples of differentiated human adipocytes were prepared using cells cultured on six-well tissue culture plates 12 days after differentiation induction. Total RNA samples were prepared from cells cultured on six-well tissue culture plates and from mouse fat tissues using Sepasol Super-I (Nacalai Tesque) and an SV total RNA isolation system (Promega, WI, USA), respectively, in accordance with each manufacturer's protocol as previously described [12]. To quantify mRNA expression, PCR analysis was performed using a fluorescence temperature cycler (LightCycler System: Roche Diagnostics, Mannheim, Germany), as described previously [13]. Primer sets were designed using a PCR primer selection program available at the web site of the Virtual Genomic Center from the GenBank database as follows: 5'-TCTCTCCGTAATGGAAGACC-3' and 5'-GTGGGGATGTCTCATAATGC-3' for human *PPAR $\gamma$*  (upstream and downstream, respectively), 5'-GCATGGCCAAACCTAACATGA-3' and 5'-CCTGGCCCAGTATGAAGGAAA-3' for human *adipocyte-specific fatty acid-binding protein* (*aP2*), 5'-GTGGCCAAATAGCACATCCT-3' and 5'-CCGAAAGATCCAGAATTCCA-3' for human *lipoprotein lipase* (*LPL*), 5'-CTCCTTTCCTTGCTGAGGTG-3' and 5'-TCTCGCCTGCAATCATGTAG-3' for human *carnitine palmitoyltransferase-1B* (*CPT-1B*), and 5'-TGCCCAACTGTGCAATGAA-3' and 5'-TCGCAAGAAGGAAGGTACCAA-3' for human *uncoupling protein-1* (*UCP1*). All other

primer sets used in this study were described in our previous reports [13,14]. To compare mRNA expression level among samples, the copy number of each transcript was divided by that of 36B4 showing a constant expression level. All data on mRNA expression level are presented as ratio with respect to the mRNA expression level in a control in each experiment.

### *2.3. Biochemical assays*

Cells were washed twice with PBS and collected by scraping with a cell scraper into 25 mM Tris-HCl (pH 7.4) containing 1 mM EDTA. The cell suspension was sonicated for 5 sec at 40 watts with a microson ULTRASONIC CELL DISRUPTOR (Misonix Inc., NY, USA). After centrifugation at 12,800×g for 5 min at 4°C, the supernatants were assayed for GPDH activity as described previously [12]. One unit of GPDH activity is the amount causing the oxidation of 1 μmol of NADH per min. For Oil Red O staining, human adipocytes 12 days after the induction of differentiation were fixed with 10% formaldehyde/PBS and stained with Oil Red O solution (0.5% Oil Red O-isopropyl alcohol/H<sub>2</sub>O (3:2, v/v)) as described previously [15].

To determine intracellular TG amount, human adipocytes were washed with PBS, and placed immediately in lysis buffer containing 50 mM PIPES (pH 6.5) and 1% Triton X-100. The lysate was centrifuged at 10,000 rpm for 5 min, and supernatants were collected. TG content was measured enzymatically using TG E-test Wako (Wako Pure Chemicals, Osaka, Japan).

### *2.4. Fatty acid oxidation measurement*

Eleven days after differentiation induction, human adipocytes were incubated in DMEM containing 0.2 mM palmitic acid, 2.5% FA-free BSA, 200 μM 1-carnitine, and [<sup>14</sup>C]-palmitic acid (1 μCi) (American Radiolabeled Chemicals) for 16 h. Fatty acid (FA) oxidation products were assessed as previously described [16] with modification. Briefly, the labeling medium was collected and centrifuged, and the supernatant was transferred to a 50 mL polypropylene

tube. An uncapped Eppendorf tube containing a piece of filter paper soaked in benzethonium hydroxide was placed inside a 50-mL tube. After the tube was sealed, 200  $\mu$ L of 70% perchloric acid was added to the medium sample to release [ $^{14}$ C]-CO<sub>2</sub>. The tube was then shaken at 37°C for 1 h. The saturated filter paper containing trapped [ $^{14}$ C]-CO<sub>2</sub> was assessed for radioactivity in a liquid scintillation counter (LS6500, Beckman Coulter). The acidified medium was centrifuged twice to remove particulate matter, and 200  $\mu$ L of supernatant was assessed for the amount of [ $^{14}$ C]-labeled acid soluble metabolites (ASMs), which include labeled ketone bodies.

### 2.5. Statistical analysis

The data are presented as means  $\pm$  S.E.M. and statistically analyzed by one-way ANOVA when their variances were heterogeneous and the unpaired t-test. Differences were considered significant at  $P < 0.05$ .

## 3. Results

### 3.1. A PPAR $\alpha$ agonist promoted adipocyte differentiation but not triglyceride accumulation in human adipocytes.

To examine the effects of PPAR $\alpha$  activation on human adipocyte differentiation, GW7647 (a potent PPAR $\alpha$  agonist) was added to the medium and the cells were incubated for 12 days (the concentrations of GW7647 used in this study showed no effect on PPAR $\gamma$  activity). The addition of GW7647 increased mRNA expression levels of adipocyte differentiation marker genes, namely, PPAR $\gamma$ , *aP2*, and *LPL* (Fig. 1A). The mRNA expression level of PPAR $\gamma$  statistically significantly increased and those of other genes tended to increase. The PPAR $\alpha$  mRNA expression level remained constant during the culture period, although the mRNA expression level of PPAR $\alpha$  was 10-50-fold lower than that of PPAR $\gamma$  (data not shown). The activity of GPDH, a biochemical differentiation marker, also increased following



treatment with GW7647 in a dose-dependent manner. The data suggest that GW7647 treatment promotes human adipocyte differentiation. Thus, we examined insulin sensitivity because the promotion of adipocyte differentiation increases insulin sensitivity [5]. As shown in Fig. 2, GW7647 treatment increased the insulin-dependent uptake of glucose. Therefore, we concluded that PPAR $\alpha$  activation promoted the differentiation of human adipocytes.

Next, we studied the effects of PPAR $\alpha$  activation on TG accumulation in human adipocytes because the promotion of adipocyte differentiation increases TG amount in adipocytes. Surprisingly, GW7647 treatment did not increase accumulated TG amount in human adipocytes (Fig. 3). This finding is inconsistent with the observation that PPAR $\alpha$  activation causes adipocyte differentiation, as shown above.

### *3.2. Activation of PPAR $\alpha$ enhanced fatty acid oxidation in human adipocytes.*

To clarify this inconsistency, we measured the mRNA expression levels of fatty acid oxidation-related genes. GW7647 treatment increased the mRNA expression levels of *CPT-1B* and *AOX*, which are rate-limiting enzymes in mitochondria and peroxisomes, respectively (Fig. 4A). Interestingly, the mRNA expression level of *UCP1*, which is involved in energy consumptions, also increased. The induced expression of *CPT-1B* was abrogated by GW6471, a synthetic PPAR $\alpha$  antagonist (Fig. 4B), indicating that the induction of this gene is dependent on PPAR $\alpha$  activity. Moreover, the evaluation of CO<sub>2</sub> and ASM releases using [<sup>14</sup>C]-palmitic acid revealed that GW7647 treatment increased the amounts of released CO<sub>2</sub> and ASMs, which are products of fatty acid oxidation, in human adipocytes (Figs. 4C and D). These findings indicated that PPAR $\alpha$  activation increases fatty acid oxidation in human adipocytes in addition to the enhancing adipocyte differentiation.

## **4. Discussion**

PPAR $\gamma$  regulates adipocyte differentiation [17]. This regulation is mediated by the

induction of the mRNA expression of PPAR $\gamma$  target genes. When an agonist binds to the ligand-binding domain of PPAR $\gamma$ , a heterodimer of PPAR $\gamma$  and RXR is recruited onto the PPAR-response element (PPRE) inducing mRNA expression [18]. Target genes such as *aP2*, *LPL*, and *GLUT4* have PPRE in their own promoter regions. However, PPRE bound by PPAR $\gamma$  cannot be distinguished from that bound by PPAR $\alpha$ , although affinities to other PPAR isoforms might differ in each PPRE. Recently, it has been reported that synthetic PPAR $\alpha$  agonists such as fenofibrate and bezafibrate induce *adiponectin* mRNA expression in mouse 3T3-L1 adipocytes; *adiponectin* is a typical PPAR $\gamma$  target gene in adipocytes [8]. On the other hand, the sequence of the PPRE of the promoter of human AOX, which is a PPAR $\alpha$  target gene in hepatocytes, has been widely used in luciferase assay for all PPAR isoforms [19,20]. Thus, there is a possibility that each PPAR isoform can induce the mRNA expression of target genes known to be induced by other PPAR isoforms, although it has not yet been elucidated how the specificities of isoforms or tissues are manifested. In this study, we showed that PPAR $\alpha$  activation increased the mRNA expression levels of adipocyte marker genes controlled by PPAR $\gamma$  such as *aP2* and *LPL*, whose promoters have PPRE. The findings suggest that PPAR $\alpha$ , as well as PPAR $\gamma$ , regulates adipocyte differentiation by inducing the mRNA expression of adipocyte differentiation-related genes, which have PPRE in their promoters.

Generally, PPAR $\alpha$  activation enhances fatty acid oxidation in peripheral tissues such as the liver, heart, and skeletal muscle [21]. The enhancement of fatty acid oxidation contributes to the effects of PPAR $\alpha$  activation on hyperlipidemia. Many groups including us have reported that treatment of a PPAR $\alpha$  agonist suppresses weight gain of adipose tissues [22,23]. It has been considered that the enhancement of fatty acid oxidation is a cause of the suppression of adipose tissue weight gain. However, we showed here that treatment with a PPAR $\alpha$  agonist decreases TG accumulation in human adipocytes. In addition to the increase in the mRNA expression levels of genes involved in fatty acid oxidation, the treatment with GW7647 significantly increased amounts of released CO<sub>2</sub> and ASMs, which are products of

fatty acid oxidation, in human adipocytes. The findings indicate that PPAR $\alpha$  activation enhances energy consumption in adipocytes, as well as, in hepatocytes and myocytes. It is unknown why only PPAR $\alpha$  can induce fatty acid oxidation in adipocytes although PPAR $\alpha$  activation, as well as, PPAR $\gamma$  activation, can also induce adipocyte differentiation. A simple possibility is that the PPRE sequences of fatty-acid-oxidation-related genes show higher affinities to PPAR $\alpha$  than to PPAR $\gamma$ . Generally, a PPAR $\alpha$ -induced response is greater than a PPAR $\gamma$ -induced response in luciferase assay using an AOX-derived PPRE reporter in our experiments [12]. Although further investigations are necessary to elucidate the isoform specificity, the finding that the activation of PPAR $\alpha$ , not PPAR $\gamma$ , enhanced fatty acid oxidation in human adipocytes suggests the validity of the use of PPAR $\alpha$  agonists for the management of diabetic conditions. Although PPAR $\gamma$  agonists such as TZDs improve insulin resistance, long-term treatment with these agonists induces adipocyte hypertrophy causing severe insulin resistance [24]. However, PPAR $\alpha$  agonists could improve insulin resistance without adipocyte hypertrophy, because they do not enhance the TG accumulation in adipocytes, as we showed here. So far, several reports have demonstrated that PPAR $\alpha$  agonists improve insulin resistance in human clinical trials [8,25]. Therefore, treatment with a PPAR $\alpha$  agonist is a better choice for the management of obesity-induced diabetes.

In conclusion, we showed here that PPAR $\alpha$  activation by GW7647 treatment increased the mRNA expression levels of adipocyte differentiation marker genes, GPDH activity, and insulin-dependent glucose uptake level in human adipocytes. However, a significant increase in the amount of accumulated TG was not observed under the same conditions. On the other hand, PPAR $\alpha$  activation enhanced fatty acid oxidation through the induction of mRNA expression of genes involved in fatty acid oxidation. These findings indicate that PPAR $\alpha$  activation improves insulin sensitivity without accumulation of TG, which is observed in PPAR $\gamma$  agonist treatment, suggesting that PPAR $\alpha$  agonists are valuable for improvement of diabetic conditions accompanied by adipocyte hypertrophy.

## **Acknowledgements**

Authors thank to Dr. C. Dani and Dr. E.Z. Amri (Universite' de Nice) for valuable discussion and to S. Shinotoh and Y. Tada for secretarial and technical supports, respectively. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sport, Science and Technology of Japan (22228001 and 22380075).

## References

- [1] P.J. Miranda, R.A. DeFronzo, R.M. Califf, J.R. Guyton, Metabolic syndrome: definition, pathophysiology, and mechanisms, *Am. Heart J.* 149 (2005) 33-45.
- [2] B.M. Spiegelman, J.S. Flier, Adipogenesis and obesity: rounding out the big picture. *Cell* 87 (1996) 377-389.
- [3] N. Takahashi, T. Goto, S. Hirai, T. Uemura, T. Kawada, Genome science of lipid metabolism and obesity, *Forum Nutri.* 61 (2009) 25-38.
- [4] S. Rocchi, F. Picard, J. Vamecq, et al., A unique PPAR-gamma ligand with potent insulin-sensitizing yet weak adipogenic activity, *Mol. Cell* 8 (2001) 737-747.
- [5] R.K. Semple, V.K. Chatterjee, S. O'Rahilly, PPAR-gamma and human metabolic disease, *J. Clin. Invest.* 116 (2006) 581-589.
- [6] S. Mandard, M. Müller, S. Kersten, Peroxisome proliferator-activated receptors-alpha target genes, *Cell. Mol. Life Sci.* 61 (2004) 393-416.
- [7] A. Tsuchida, T. Yamauchi, S. Takekawa, Y. Hada, Y. Ito, T. Maki, T. Kadowaki, PPAR-alpha activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPAR-alpha, PPAR-gamma, and their combination, *Diabetes* 54 (2005) 3358-3370.
- [8] A. Hiuge, A. Tenenbaum, N. Maeda, M. Benderly, M. Kumada, E.Z. Fisman, D. Tanne, Z. Matas, T. Hibuse, K. Fujita, H. Nishizawa, Y. Adler, M. Motro, S. Kihara, I. Shimomura, S. Behar, T. Funahashi, Effects of PPAR ligands, bezafibrate and fenofibrate, on adiponectin level, *Arterioscler. Thromb. Vasc. Biol.* 27 (2007) 635-641.
- [9] C. Vernochet, S. Azoulay, D. Duval, R. Guedj, F. Cottrez, H. Vidal, G. Ailhaud, C. Dani, Human immunodeficiency virus protease inhibitors accumulate into cultured human adipocytes and alter expression of adipocytokines, *J. Biol. Chem.* 280 (2005) 2238-2243.
- [10] A. M. Rodriguez, C. Elabd, F. Delteil, J. Astier, C. Vernochet, P. Saint-Marc, J. Guesnet, A. Guezennec, E.Z. Amri, C. Dani, G. Ailhaud, Adipocyte differentiation of multipotent

- cells established from human adipose tissue, *Biochem Biophys Res Commun* 315 (2004) 255-263.
- [11] T. Uemura, T. Goto, M.S. Kang, N. Mizoguchi, S. Hirai, J.Y. Lee, Y. Nakano, J. Shono, S. Hoshino, K. Taketani, N. Tsuge, T. Narukami, M. Makishima, N. Takahashi, T. Kawada, Diosgenin, the main aglycon of fenugreek, inhibits LXR-alpha activity in HepG2 cells and decreases plasma and hepatic triglycerides in obese, diabetic mice, *J. Nutr.* 141 (2011) 17-23.
- [12] N. Takahashi, T. Kawada, T. Yamamoto, T. Goto, A. Taimatsu, N. Aoki, H. Kawasaki, K. Taira, K.K. Yokoyama, Y. Kamei, T. Fushiki, Overexpression and ribozyme-mediated targeting of transcriptional coactivators CBP and p300 revealed their indispensable roles in adipocyte differentiation through the regulation of PPAR-gamma, *J. Biol. Chem.* 277 (2002) 16906-16912.
- [13] N. Takahashi, T. Kawada, T. Goto, T. Yamamoto, A. Taimatsu, N. Matsui, K. Kimura, M. Saito, M. Hosokawa, K. Miyashita, T. Fushiki, Dual action of isoprenols from herbal medicines on both PPAR-gamma and PPAR-alpha in 3T3-L1 adipocytes and HepG2 hepatocytes, *FEBS Lett.* 514 (2002) 315-322.
- [14] T. Goto, N. Takahashi, S. Kato, K. Egawa, S. Ebisu, T. Moriyama, T. Fushiki, T. Kawada, Phytol directly activates PPAR-alpha and regulates gene expression involved in lipid metabolism in PPAR-alpha-expressing HepG2 hepatocytes, *Biochem. Biophys. Res. Commun.* 337 (2005) 440-445.
- [15] N. Takahashi, T. Goto, A. Taimatsu, K. Egawa, S. Kagoh, T. Kusudo, T. Sakamoto, C. Ohyan, J.Y. Lee, Y.I. Kim, T. Uemura, S. Hirai, T. Kawada, Bixin regulates mRNA expression involved in 3T3-L1 adipocytes through PPAR-gamma activation, *Biochem. Biophys. Res. Commun.* 390 (2009) 1372-1376.
- [16] T. Goto, J.Y. Lee, A. Teraminami, Y.I. Kim, S. Hirai, T. Uemura, H. Inoue, N. Takahashi, T. Kawada, Activation of PPAR-alpha stimulates both differentiation and fatty acid oxidation in adipocytes, *J Lipid Res.* In press.

- [17] R.M. Evans, G.D. Barish, Y.X. Wang, PPARs and complex journey to obesity, *Nat. Med.* 10 (2004) 1-7.
- [18] B. Desvergne, W. Wahli, Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* 20 (1999) 649-688.
- [19] M.S. Kang, S. Hirai, T. Goto, K. Kuroyanagi, Y. Ezaki, N. Takahashi, T. Kawada, Dehydroabietic acid, herbal terpenoid, acts as ligands for PPARs in macrophages to regulate inflammation, *Biochem. Biophys. Res. Commun.* 369 (2008) 333-338.
- [20] N. Takahashi, K.M. Kang, K. Kuroyanagi, T. Goto, S. Hirai, K. Ohyama, J.Y. Lee, R. Yu, M. Yano, T. Sasaki, S. Murakami, T. Kawada, Auraptene, a citrus fruit compound, regulates gene expression as a PPAR-alpha agonist in HepG2 hepatocytes, *BioFactors* 33 (2008) 25-32.
- [21] P. Lefebvre, G. Chinetti, J.C. Fruchart, B. Staels, Sorting out the roles of PPAR-alpha in energy metabolism and vascular homeostasis, *J. Clin. Invest.* 116 (2006) 571-580.
- [22] C. Knauf, J. Rieusset, M. Foretz, P.D. Cani, M. Uldry, M. Hosokawa, E. Martinez, M. Bringart, A. Waget, S. Kersten, B. Desvergne, S. Gremlich, W. Wahli, J. Seydoux, N.M. Delzenne, B. Thorens, R. Burcelin, PPAR-alpha-null mice have increased white adipose tissue glucose utilization, GLUT4, and fat mass: Role in liver and brain, *Endocrinol.* 147 (2006) 4067-4078.
- [23] M.S.Kang, S. Hirai, T. Goto, K. Kuroyanagi, I.Y. Kim, K. Ohyama, T. Uemura, J.Y. Lee, T. Sakamoto, Y. Ezaki, R. Yu, N. Takahashi, T. Kawada, Dehydroabietic acid, a phytochemical, acts as ligand for PPARs in macrophages and adipocytes to regulate inflammation of mouse adipose tissues. *BioFactors* 35 (2009) 442-448.
- [24] C. Fernandes-Santos, R.E. Carneiro, L. de-Souza-Mendonca, M.B. Aguila, C.A. Mandarim-de-Lacerda, Pan-PPAR agonist beneficial effects in overweight mice fed a high-fat high-sucrose diet, *Nutrition* 25 (2009) 818-827.
- [25] S. Ogawa, K. Takeuchi, K. Sugimura, M. Fukuda, R. Lee, S. Ito, T. Sato, Bezafibrate reduces blood glucose in type-2 diabetes mellitus, *Metabol.* 49 (2000) 331-334.

## Figure legends

**Fig. 1. Effects of PPAR $\alpha$  agonist (GW7647) on differentiation of human adipocytes.** (A) mRNA expression levels of adipocyte differentiation marker genes (*PPAR $\gamma$* , *aP2*, and *LPL*) in human adipocytes incubated with 100 nM GW7647 for 12 days. The value of a vehicle control was set at 100% and relative value is presented as fold induction with respect to that of each vehicle control. The values are means  $\pm$ S.E.M. of 3-4 samples. \* $P$ <0.05 compared with each vehicle control. (B) GPDH activity in GW7647-treated human adipocytes. \* $P$ <0.05 and \*\* $P$ <0.01 compared with each vehicle control.

**Fig. 2. Effects of GW7647 on insulin sensitivity.** Insulin-dependent glucose uptake into human adipocytes incubated with GW7647 for 12 days. The value of a vehicle control was set at 100% and the relative value is presented as fold induction with respect to that of the vehicle control. The values are means  $\pm$ S.E.M. of 3-4 samples. \* $P$ <0.05 and \*\* $P$ <0.01 compared with vehicle controls.

**Fig. 3. Effects of GW7647 on lipid accumulation.** (A) Oil Red O staining of a vehicle control and GW7647-treated human adipocytes 12 days after differentiation induction. Scale bars indicate 200  $\mu$ M. (B) TG amounts in the GW7647-treated human adipocytes. The value of a vehicle control was set at 100% and the relative value is presented as fold induction with respect to that of the vehicle control. Total TG amount in each well is calculated using concentrations of lipid-extracted solutions. The values are means  $\pm$ S.E.M. of 3-4 samples.

**Fig. 4. Effects of GW7647 on fatty acid oxidation.** (A) mRNA expression levels of adipocyte differentiation marker genes (*CPT-1B*, *AOX*, and *UCPI*) in human adipocytes incubated with 100 nM GW7647 for 12 days. (B) Effects of GW6471 (a PPAR $\alpha$  antagonist) on the induction of *CPT-1B* mRNA expression. GW7647 and GW6471 (an agonist and antagonist, respectively) were added to the culture, which was then incubated for 12 days. (C)



CO<sub>2</sub> release and (D) acid-soluble metabolite (ASM) release in GW7647-treated human adipocytes. The value of a vehicle control was set at 100% and the relative value was presented as fold induction with respect to that of the vehicle control. The values are means  $\pm$ S.E.M. of 3-4 samples. \* $P$ <0.05 and \*\* $P$ <0.01 compared with each vehicle control.

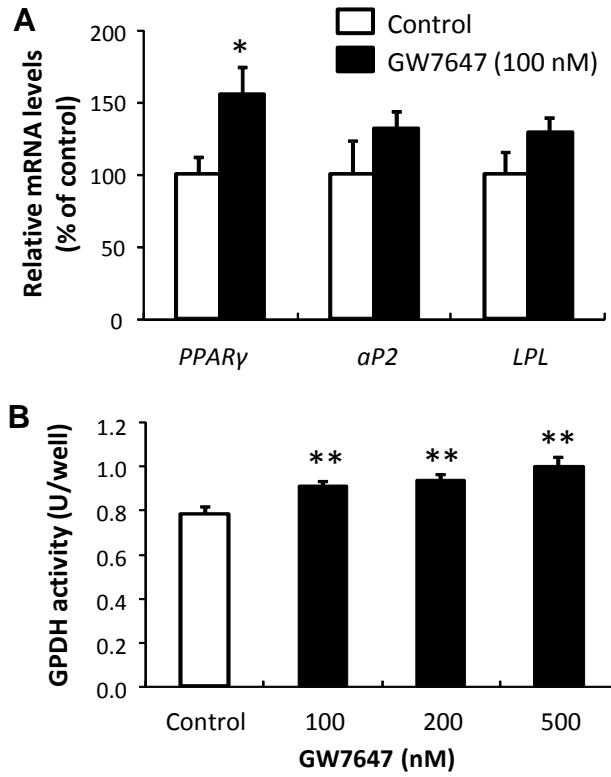


Figure 1

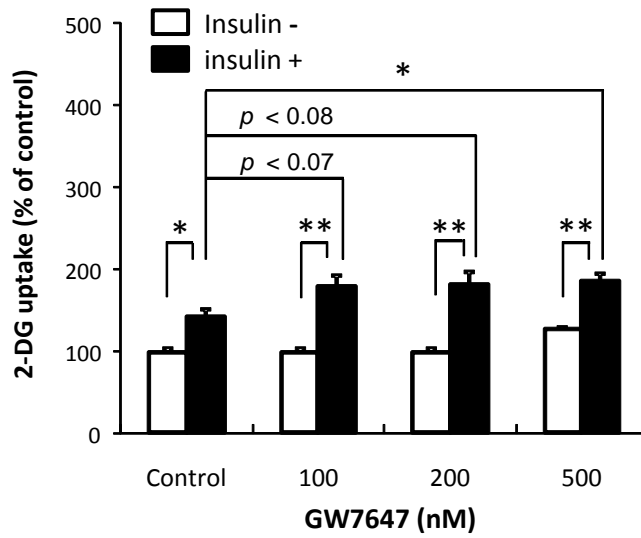


Figure 2

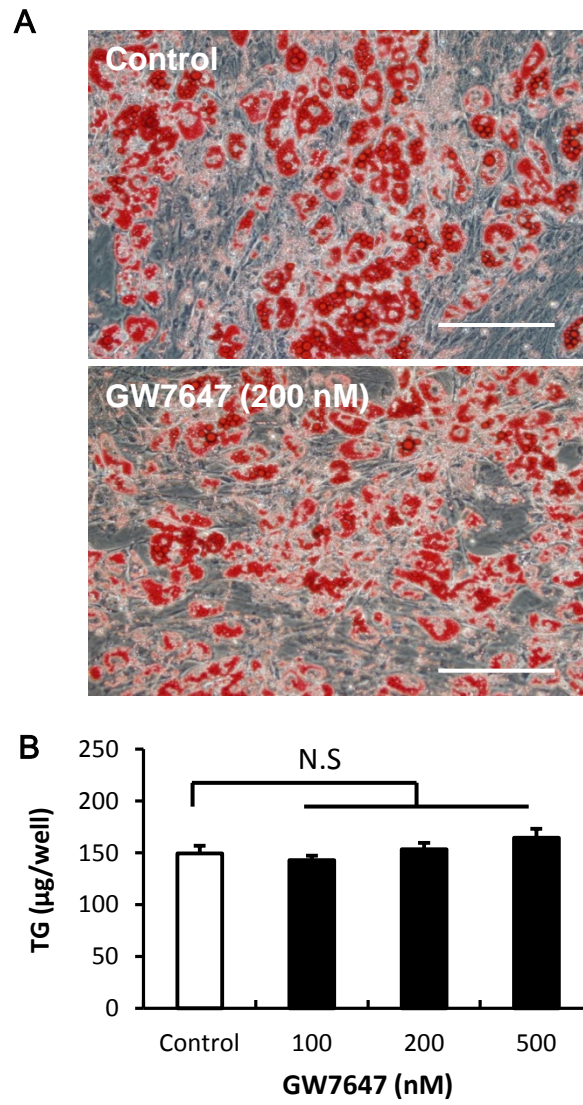


Figure 3

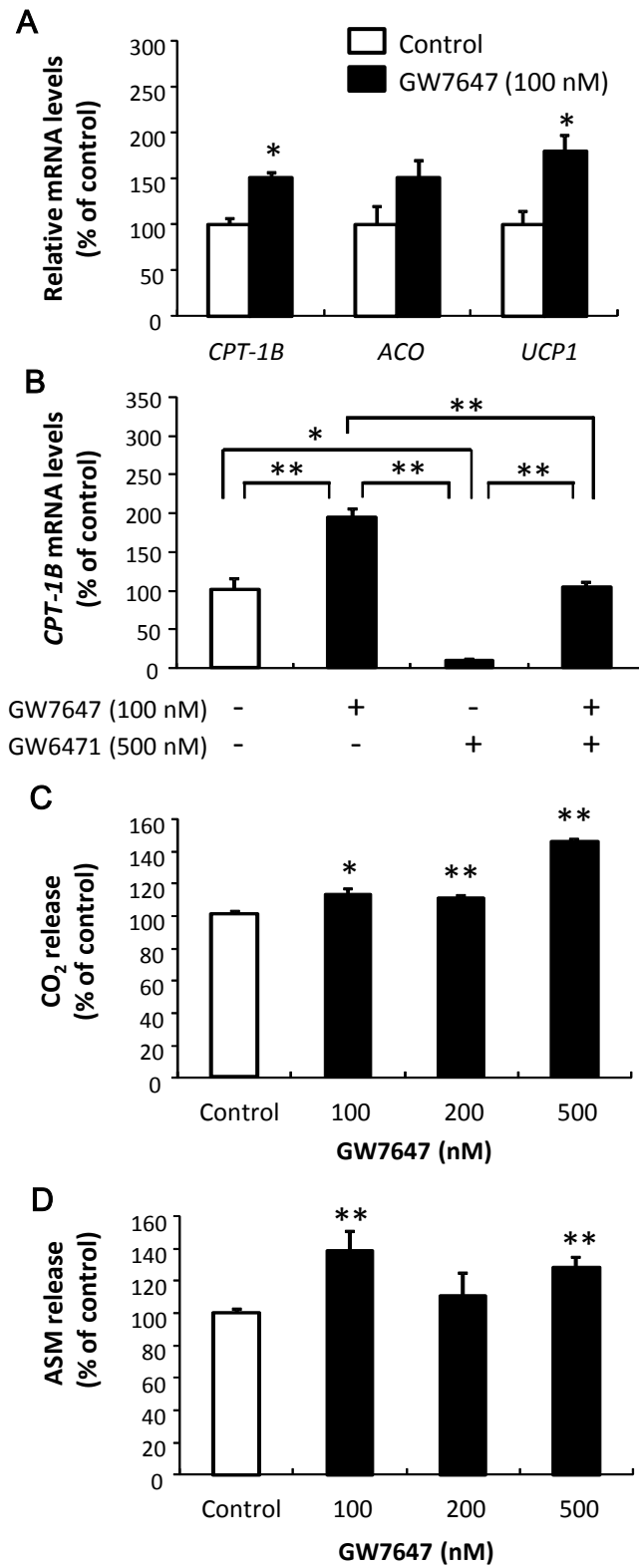


Figure 4