RESEARCH ARTICLE

Auraptene regulates gene expression involved in lipid metabolism through PPARα activation in diabetic obese mice

Nobuyuki Takahashi[1,2], Mari Senda[1]*, Shan Lin[1], Tsuyoshi Goto[1,2], Masamichi Yano[3], Takao Sasaki[4], Shigeru Murakami[5] and Teruo Kawada[1,2]*

[1] Laboratory of Molecular Function of Food, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Uji, Kyoto, Japan
[2] Research Unit for Physiological Chemistry, The Center for the Promotion of Interdisciplinary Education and Research, Kyoto University, Kyoto, Japan
[3] Department of Citriculture, National Institute of Fruit Tree Sciences, Ministry of Agriculture, Forestry and Fisheries, Shimizu, Shizuoka, Japan
[4] ARKRAY, Inc., Kyoto, Japan
[5] Taisho Pharmaceutical Co. Ltd., Toshima-ku, Tokyo, Japan

Received: June 15, 2011
Revised: August 4, 2011
Accepted: August 15, 2011

Scope: Peroxisome proliferator-activated receptor-α (PPARα) is a key regulator of circulating lipid level. Thus, various food-derived compounds that activate PPARα as agonists have been screened and characterized.

Methods and results: We investigated the effects of auraptene, a citrus-derived compound serving as a PPARα agonist in vitro, on abnormalities in lipid and glucose metabolisms. In high-fat-diet (HFD)-fed KK-Ay diabetic obese mice, auraptene treatment suppressed hyperlipidemia and triglyceride accumulation in the liver and skeletal muscle, and increased the mRNA expression levels of the PPARα target genes involved in fatty acid oxidation in the liver and skeletal muscle. Moreover, the adipocyte size in the auraptene-treated mice was significantly smaller than that in the control HFD-fed mice resulting in the improvement of HFD-induced hyperglycemia and abnormalities in glucose tolerance.

Conclusions: These findings indicate that auraptene activates PPARα also in vivo and its treatment may improve abnormalities in lipid and glucose metabolisms, suggesting that auraptene is a valuable food-derived compound for managing metabolic disorders.

Keywords:
Auraptene / Lipid metabolism / Metabolic syndrome / PPAR

1 Introduction

Obesity and its related metabolic disorders such as hyperglycemia and hyperlipidemia are serious medical problems [1]. These diseases are due to abnormalities in glucose and lipid metabolisms. Thus, the normalization of these metabolic abnormalities is indispensable for the treatment of obesity and its related disorders. Recently, various drugs have been widely used for the normalization of such metabolic abnormalities, for example, fibrates and thiazolidinediones (TZD) for the treatment of hyperlipidemia and hyperglycemia, respectively. These drugs target the regulation of the activities of the peroxisome proliferator-activated receptor (PPAR) [2, 3]. This transcriptional factor is classified as a nuclear receptor, which is activated by specific ligands to up-/down-regulate the mRNA expression of target genes in various cells [4]. PPAR has three isoforms (PPARα,

Correspondence: Dr. 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
E-mail: fat@kais.kyoto-u.ac.jp
Fax: +81-774-38-3752

Abbreviations: ***ACO,*** *acyl-CoA oxidase*; ***ACS,*** *acyl-CoA synthase*; ***CPT1,*** *carnitine palmitoyl transferase-1*; **HFD,** high fat diet; **NEFA,** nonesterified FA; **OGTT,** oral glucose tolerance test; **PPAR,** peroxisome proliferator-activated receptor; **TG,** triacylglycerol; **WAT,** white adipose tissue

*These authors contributed equally to this work

PPARγ, and PPARδ), which show specific ligand dependences and tissue distributions [5]. PPARα is mainly expressed in the liver and skeletal muscle, whose activation induces the mRNA expression of several genes involved in fatty acid (FA) oxidation to reduce circulating lipid level. Thus, fibrates, which are potent PPARα agonists, are widely used as anti-hyperlipidemic drugs [6]. Therefore, it is generally accepted that the control of PPARα activation is crucial for hyperlipidemia treatment.

We have identified and characterized many food-derived compounds that activate PPARα [7–12]. Auraptene is a citrus-fruit-derived compound mainly present in the peel [13]. We have reported that this compound stimulates FA uptake in HepG2 hepatocytes by inducing the mRNA expressions of FA-oxidation-related genes such as *acyl-CoA synthase* (*ACS*), *acyl-CoA oxidase* (*ACO*), and *carnitine palmitoyl transferase-1* (*CPT1*) [12]. In the present study, we examined the in vivo effects of auraptene on the development of diabetes symptoms in a diabetic obese model, KK-Ay mouse.

2 Materials and methods

2.1 Chemicals and animals

Auraptene (>98%) was purchased from LKT Lab (MN, USA). All the other chemicals used were from Sigma (MO, USA) or Nacalai Tesque (Kyoto, Japan) and guaranteed to be of reagent or tissue-culture grade.

Four-wk-old male diabetic obese KK-Ay/Ta Jcl mice were purchased from Nippon CLEA (Osaka, Japan). All the mice were housed separately at 24±1°C under a 12-h light/dark cycle. High-fat-diet (HFD) consisting of 60% (kcal%) fat was purchased from Research Diet (MO, USA). After 1 wk of adaptation period, the KK-Ay mice were given HFD-containing auraptene (0.1 or 0.2%). The number of mice in each group was eight. A control group fed HFD was pair-fed with the 0.2% auraptene-treated group. For the oral glucose tolerance test (OGTT), the mice were fasted for more than 16 h before the test and then orally loaded with glucose (1.5 mg/g body weight). Blood samples were collected from the caudal vein and centrifuged with heparin. The animal care procedures and methods were approved by the Animal Care Committee of Kyoto University.

2.2 Determination of biochemical parameters

The plasma levels of glucose, nonesterified FA (NEFA), and triacylglycerol (TG) were determined by the glucose CII-test Wako (Wako, Osaka, Japan), NEFA C-test Wako (Wako), and triacylglycerol E-test Wako (Wako), respectively, according to the manufacturer's protocols. The plasma levels of insulin, leptin, and adiponectin were determined using the ultrasensitive rat insulin and mouse leptin kits (Morinaga Institute of Biological Science, Kanagawa, Japan) and Quintikine mouse adiponectin kit (R&D Systems, MN, USA), respectively, according to the manufacturer's protocols.

2.3 RNA preparation and real-time fluorescence monitoring RT-PCR analysis

Total RNA was prepared from tissues using Qiazol lysis reagent (Qiagen, CA, USA) in accordance with manufacturer's instructions. By using M-MLV reverse transcriptase (Invitrogen, CA, USA), the total RNA was reverse transcribed in accordance with the manufacturer's instructions using a thermal cycler (Takara PCR Thermal Cycler SP: Takara Shuzo Co., Shiga, Japan). To quantify mRNA expression, real-time RT-PCR was performed with a LightCycler System (Roche Diagnostics, Mannheim, Germany) using SYBR Green fluorescence signals, as described previously [7, 14]. The oligonucleotide primers of mouse PPAR target genes were designed using a PCR primer selection program shown in the website of the Virtual Genomic Center from the GenBank database, as previously described [8, 9, 12]. To compare the mRNA expression level among the samples, the copy numbers of all transcripts were divided by that of mouse *36B4* showing a constant expression level. The mRNA expression level was represented as the ratio relative to that of the control in each experiment.

2.4 Statistical analysis

The data were presented as means±SEM and statistically analyzed using unpaired *t*-test, Welch *t*-test, and one-way ANOVA when variances were heterogeneous. Differences were considered significant at $p<0.05$.

3 Results

3.1 Auraptene suppressed the development of diabetic symptoms in diabetic obese KK-Ay mice

To examine the effects of auraptene on the development of diabetic symptoms, 5-wk-old KK-Ay mice were fed with HFD containing 0.1 or 0.2% auraptene for 4 wk. Auraptene treatment showed no significant change in body weight (Table 1). Only the weight of subcutaneous white adipose tissue (WAT) decreased in the 0.2% auraptene-treated mice, whereas those of epididymal, mesenteric, and retroperitoneal WATs only tended to decrease. The livers were relatively enlarged in both the 0.1 and 0.2% auraptene-treated mice, although the differences were not statistically significant. Plasma TG level decreased in the auraptene-treated mice as shown in Fig. 1A, although the level was not dose-dependent. Plasma NEFA level showed no significant change at the end of this experiment, as shown in Fig. 1B. These results suggest that hepatic PPARα is activated

Table 1. Weights of body, WATs, and other organs of auraptene-fed mice

	HFD	HFD+AUR (0.1%)	HFD+AUR (0.2%)
Food intake (g/day)	3.97±0.04	4.00±0.02	4.02±0.02
Energy intake (kcal/day)	20.71±0.19	20.90±0.12	20.99±0.12
Body weight (g)	40.30±0.77	40.03±0.42	39.02±0.92
Tissue weight (g)			
Subcutaneous	1.31±0.07	0.99±0.04	0.87±0.09
Epididymal	1.84±0.05	1.85±0.07	1.71±0.15
Mesenteric	1.22±0.16	0.97±0.05	0.94±0.05
Retroperitoneal	0.70±0.03	0.69±0.03	0.59±0.05
Total WAT	5.07±0.14	4.50±0.13	4.11±0.27
Skeletal muscle	0.24±0.01	0.24±0.01	0.23±0.01
Liver	1.71±0.04	1.75±0.08	1.84±0.07
BAT	0.23±0.01	0.20±0.01	0.19±0.01
Kidney (g/100 g BW)	0.49±0.01	0.51±0.01	0.54±0.01
Subcutaneous	3.23±0.14	2.50±0.10	2.22±0.19
Epididymal	4.60±0.04	4.65±0.13	4.42±0.25
Mesenteric	3.01±0.39	2.45±0.11	2.39±0.19
Retroperitoneal	1.73±0.05	1.73±0.05	1.51±0.09
Total WAT	12.6±0.19	11.3±0.19	10.5±0.50
Skeletal muscle	0.60±0.01	0.60±0.02	0.58±0.02
Liver	4.28±0.07	4.36±0.23	4.75±0.21
BAT	0.59±0.02	0.51±0.01	0.50±0.01
Kidney	1.24±0.05	1.26±0.01	1.37±0.04

Food intakes, body weights (BW), mesenteric, renal, subcutaneous, and epididymal white adipose tissue (WAT) weights, skeletal muscle, liver, and kidney weights, and brown adipose tissue (BAT) weights of KK-Ay mice not treated (high-fat diet, HFD; 0%) and treated with AUR (HFD+AUR 0.1 or 0.2%) for 4 wk while on HFD. The values are means±SEM of 5–6 samples. $^{*}p<0.05$ and $^{**}p<0.01$, compared with each HFD control (0%).

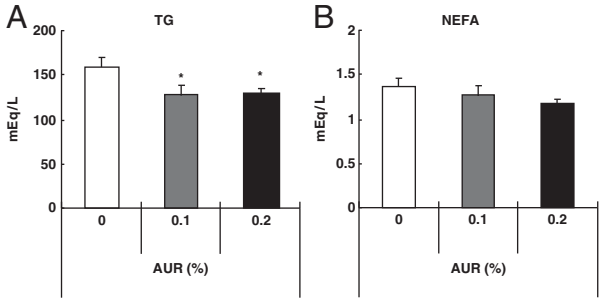

Figure 1. Serum levels of triglyceride (TG) and free fatty acid (NEFA). Fasting serum levels of TG (A) and NEFA (B) are shown. They were measured after 4 wk of HFD feeding. The values are means±SEM of 5–6 samples. $^{*}p<0.05$, compared with each vehicle control.

resulting in a decrease in plasma TG level under HFD feeding.

3.2 Auraptene induced the mRNA expression of PPAR target genes in the liver

To elucidate the details of the auraptene effects, we examined TG accumulation and the mRNA expression levels of PPARα target genes in the liver and skeletal muscle. The Oil Red O staining of the liver revealed that hepatic TG accumulation was suppressed in the auraptene-treated mice as shown in Fig. 2A–C. TG accumulation in the skeletal muscle was also suppressed in addition to that of the liver (Fig. 2D and E). Moreover, the hepatic mRNA expression levels of PPARα target genes (*FAT/CD36*, *ACS*, *ACO*, and *CPT1*) were increased by auraptene treatment, although the change in *ACO* mRNA level was not statistically significant, as shown in Fig. 3. Similarly, the mRNA expression levels of the same genes in the skeletal muscle except the *FAT/CD36* mRNA level were also significantly increased by the auraptene treatment (Fig. 4). These results indicate that auraptene treatment increases the mRNA expression levels of PPARα target genes in the liver and skeletal muscle, suggesting that the induction of PPARα target genes suppresses the HFD-induced increase in serum TG level and TG accumulation in the liver and skeletal muscle.

3.3 Auraptene inhibited hypertrophy of adipocytes induced by HFD

Recently, it has been reported that PPARα activation improves insulin resistance by enhancing hepatic lipid metabolism [15, 16]. Thus, to examine the effects of auraptene on adipose tissues, we measured adipocyte

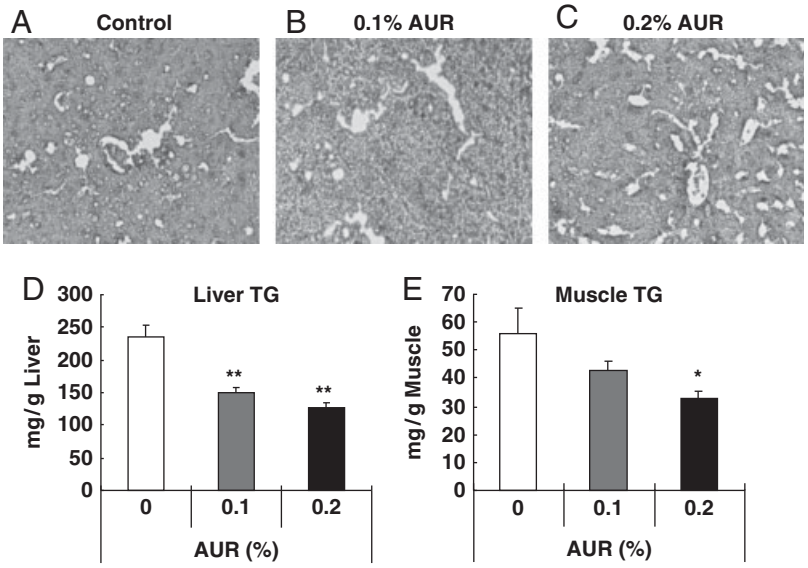

Figure 2. Triglyceride (TG) accumulation in liver and skeletal muscle. (A–C) Isolated livers were fixed in 10% formalin/PBS for more than 24 h and embedded. Liver sections were cut into 10-μm-thick sections. The liver sections were stained with Oil Red O and Mayers's hematoxylin. Bar shown in each photograph, 50 μm. Livers and skeletal muscles were harvested from the mice treated with or without auraptene. After lipid extraction, hepatic (D) or skeletal muscular (E) TG levels were measured by using an enzymatic colorimetric assay. The values are means ± SEM of 5–6 samples. $^{*}p<0.05$ and $^{**}p<0.01$, compared with each control.

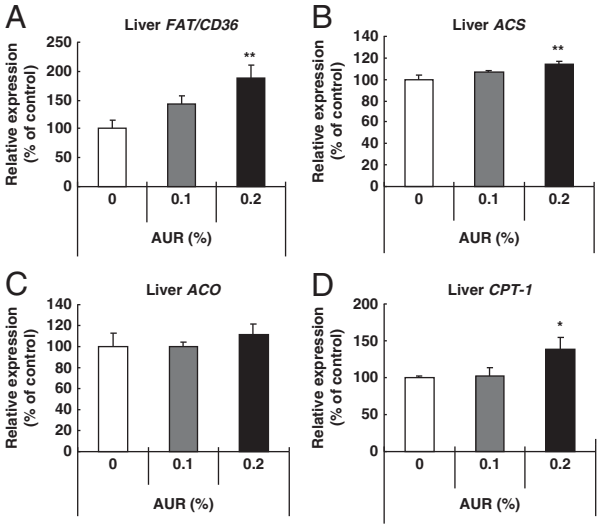

Figure 3. mRNA expression levels of PPARα target genes in liver. Expression levels of PPARα target genes: *FAT/CD36* (A), *ACS* (B), *ACO* (C), and *CPT-1* in liver of auraptene-treated KK-Ay mice. The mRNA expression level of the control was set at 100% and relative mRNA expression levels were presented as fold induction with respect to that of the control. The values are means ± SEM of 5–6 samples. $^{*}p<0.05$ and $^{**}p<0.01$, compared with each control.

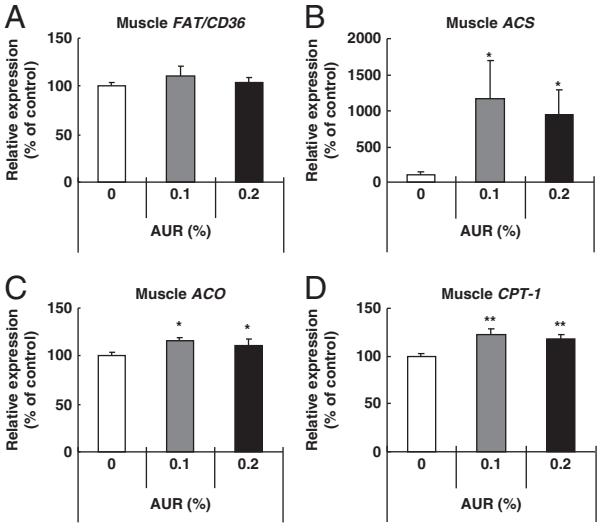

Figure 4. mRNA expression levels of PPARα target genes in skeletal muscle. Expression levels of PPARα target genes: *FAT/CD36* (A), *ACS* (B), *ACO* (C), and *CPT-1* in skeletal muscle of auraptene-treated KK-Ay mice. The mRNA expression level of the control was set at 100% and relative mRNA expression levels were presented as fold induction with respect to that of the control. The values are means ± SEM of 5–6 samples. $^{*}p<0.05$ and $^{**}p<0.01$, compared with each control.

size in the WAT of auraptene-treated mice. The auraptene treatment significantly reduced the average size of adipocytes (Fig. 5) and increased the number of smaller adipocytes in comparison to that in the case of the control HFD-fed mice. Because small adipocytes show high adiponectin expression levels [16], we measured adiponectin expression level in WAT. As shown in Fig. 5E, the mRNA expression level of adiponectin in adipocytes increased in the auraptene-treated mice, although the plasma adiponectin level only tended to increase (Fig. 6F). Moreover, the auraptene treatment significantly suppressed the increase in fasting blood glucose level at the end of the experimental period (Fig. 6A). A decrease in plasma glucose level was observed during the experimental period in the 0.2% auraptene-treated mice (Fig. 6B). OGTT showed good glucose tolerance in auraptene-treated mice (Fig. 6C). Moreover, plasma insulin level also decreased with the auraptene treatment, as shown

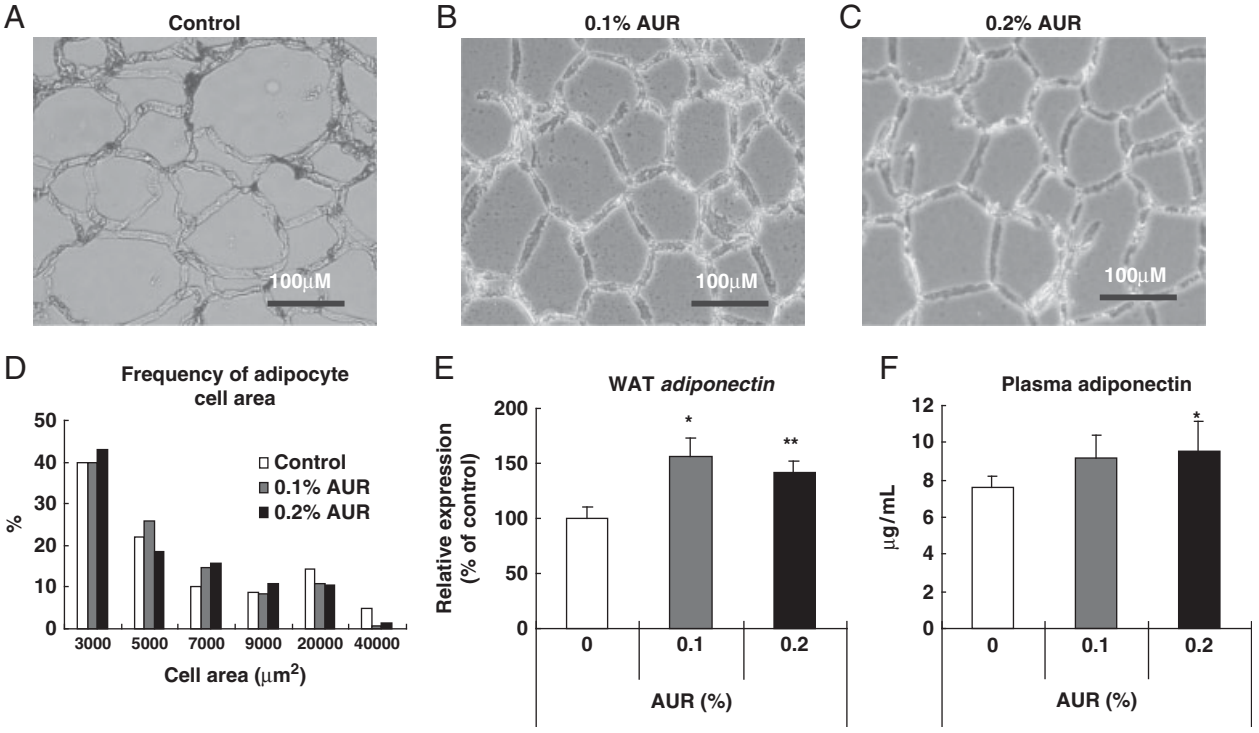

Figure 5. Adipocyte size and adiponectin expression levels of auraptene-treated mice. (A–C) Histological analyses of epididymal adipose tissue of KK-Ay mice fed auraptene. Scale bars: 100 μm. Adipose tissue sections were cut into 10-μm-thick sections. (D) Size distribution of adipocytes in epididymal fat tissue of KK-Ay mice. Data are means from analyses of five sections from each of the five mice. (E and F) Expressions of adiponectin at mRNA levels of adipose tissues (E) and at plasma protein levels (F). For mRNA quantification, the value of the control HFD-fed mice was set at 100 % and the relative value was presented as fold induction with respect to that of the control HFD-fed mice. The values are means ± SEM of 5–6 mice. $^{*}p < 0.05$, compared with control diet-fed mice.

in Fig. 6D. These results indicate that auraptene treatment suppresses hyperglycemia development in KK-Ay mice under HFD feeding.

4 Discussion

In the present study, auraptene treatment decreased serum and hepatic TG levels (82 and 54%, respectively, in the 0.2% auraptene-treated mice as shown in Figs. 1A and 2D). This effect is likely to be mediated by the increase in the mRNA expression levels of PPARα target genes such as *FAT/CD36*, *ACS*, *ACO*, and *CPT1*, which are involved in FA uptake and/or oxidation (Fig. 3). The increase in PPARα target gene expression level by the auraptene treatment was observed in PPARα-expressing HepG2 hepatocytes, as demonstrated in our previous study in which auraptene activates PPARα in luciferase reporter assay and induces mRNA expression levels of fatty-acid-oxidation-related genes including *CPT1A*, *ACO*, and *ACS* [12]. Moreover, our previous study has demonstrated that treatment with 0.2% bezafibrate, a potent PPARα agonist, decreases plasma and hepatic TG levels (62 and 73%, respectively) in the same protocol using KK-Ay mice [17]. In addition, the auraptene treatment induced the mRNA expression levels of PPARα target genes in the skeletal muscle resulting in the suppression of TG accumulation in the skeletal muscle (Figs. 2E and 4), although the induction levels in the skeletal muscle were lower than those in the liver. Thus, it is suggested that these regulations of mRNA expression levels contribute to the decrease in circulating and accumulated lipid levels in HDF-fed KK-Ay mice. Finally, although PPARα agonists including fibrates generally cause the increase in liver weight, auraptene did not as shown in Table 1. This is a beneficial effect of auraptene in comparison to other PPARα agonists.

The auraptene treatment also suppressed hyperglycemia in the HFD-fed KK-Ay mice, as shown in Fig. 6. Many reports have indicated that PPARα agonists such as fibrates improve insulin resistance in obese mice [15, 16, 18, 19]. The improvement is thought to be partly mediated by the suppression of the increase in the number of hypertrophied adipocytes, which show insulin resistance. In the present study, the size of adipocytes in the auraptene-treated mice decreased (Fig. 5). The decrease in the size of adipocytes enhanced the expression level of adiponectin, an adipocytokine improving insulin resistance in peripheral tissues [20]. An increase in adiponectin expression was

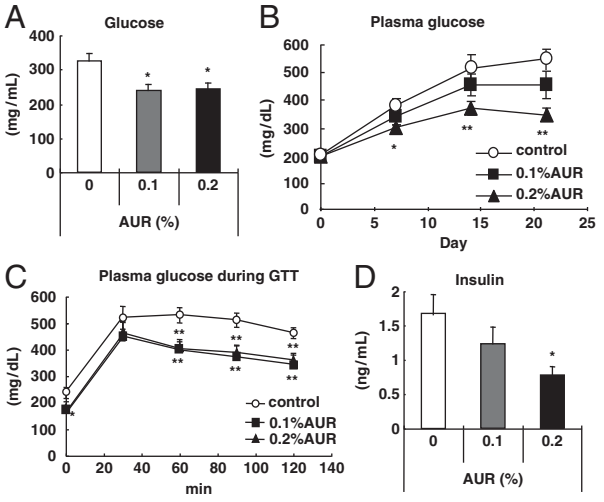

Figure 6. Plasma glucose and insulin levels of auraptene-treated mice. (A and B) Fasting blood levels of glucose at end point of and during experimental period (4 wk). Oral GTT results (C) and serum insulin levels (D) are also shown. Obese diabetic KK-Ay mice were fed the control HFD with or without 0.1 or 0.2% auraptene for 4 wk under pair-fed condition. Serum levels of glucose and insulin were measured by enzymatic colorimetric assay, as described in Section 2. A protocol of GTT was also described in Section 2. The values are means ± SEM of 5–6 mice. $^{*}p<0.05$ and $^{**}p<0.01$, compared with control diet-fed mice.

observed in both mRNA and protein levels in the auraptene-fed mice. There are many studies that demonstrated that the treatment with a PPARα agonist such as fibrates increases the expression level of adiponectin causing an improvement in insulin resistance [21, 22], although its mechanism has not yet been completely elucidated. Moreover, the anti-diabetic effects of auraptene were confirmed from the results that the auraptene treatment also improved the abnormality of glucose tolerance under HFD-fed conditions (Fig. 6C). Therefore, it can be suggested that auraptene may be valuable functional food-derived compound for managing diabetic conditions.

In conclusion, auraptene feeding decreases circulating lipid level and suppresses lipid accumulation in the liver and skeletal muscle under HFD feeding. The effects are likely to be mediated by the increase in the mRNA expression levels of FA-oxidation-related genes in the liver and skeletal muscle. In addition, auraptene treatment also suppresses increases in plasma glucose and insulin levels and improves the abnormality of glucose tolerance induced by HFD feeding. These findings suggest that auraptene improves the abnormalities of lipid and glucose metabolisms under HFD feeding through PPARα activation.

The authors thank Dr. Hajime Ohigashi and Dr. Akira Murakami (Kyoto University) for valuable advice on auraptene and Sayoko Shinotoh and Yukiko Tada for secretarial and technical assistances, 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).

The authors have declared no conflict of interest.

5 References

[1] Miranda, P. J., DeFronzo, R. A., Califf, R. M., Guyton, J. R., Metabolic syndrome: definition, pathophysiology, and mechanisms. *Am. Heart J.* 2005, *149*, 33–45.

[2] Mandard, S., Müllar, M., Kersten, S., Peroxisome proliferator-activated receptors-alpha target genes. *Cell. Mol. Life Sci.* 2004, *61*, 393–416.

[3] Rocchi, S., Picard, F., Vamecq, J., Gelman, L. et al., A unique PPAR-gamma ligand with potent insulin-sensitizing yet weak adipogenic activity. *Mol. Cell* 2001, *8*, 737–747.

[4] Evans, R. M., Barish, G. D., Wang, Y. X., PPARs and complex journey to obesity. *Nat. Med.* 2004, *10*, 1–7.

[5] Takahashi, N., Goto, T., Hirai, S., Uemura, T., Kawada, T., Genome science of lipid metabolism and obesity. *Forum Nutr.* 2009, *61*, 25–38.

[6] Lefebvre, P., Chinetti, G., Fruchart, J. C., Staels, B., Sorting out the roles of PPAR-alpha in energy metabolism and vascular homeostasis. *J. Clin. Invest.* 2006, *116*, 571–580.

[7] Takahashi, N., Kawada, T., Goto, T., Yamamoto, T., Dual action of isoprenols from herbal medicines on both PPAR-gamma and PPAR-alpha in 3T3-L1 adipocytes and HepG2 hepatocytes. *FEBS Lett.* 2002, *514*, 315–322.

[8] Takahashi, N., Kawada, T., Goto, T., Kim, C. S. et al., Abietic acid activates PPAR-gamma in RAW264. Macrophages and 3T3-L1 adipocytes to regulate gene expression involved in inflammation and lipid metabolism. *FEBS Lett.* 2003, *550*, 190–194.

[9] Goto, T., Takahashi, N., Kato, S., Egawa, K. et al., Phytol directly activates PPAR-alpha and regulates gene expression involved in lipid metabolism in PPAR-alpha-expressing HepG2 hepatocytes. *Biochem. Biophys. Res. Commun.* 2005, *337*, 440–445.

[10] Kuroyanagi, K., Kang, M. S., Goto, T., Hirai, S. et al., Citrus auraptene acts as an agonist for PPARs and enhances adiponectin production and MCP-1 reduction in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.* 2008, *366*, 219–225.

[11] Kang, M. S., Hirai, S., Goto, T., Kuroyanagi, K. et al., Dehydroabietic acid, herbal terpenoid, acts as ligands for PPARs in macrophages to regulate inflammation. *Biochem. Biophys. Res. Commun.* 2008, *369*, 333–338.

[12] Takahashi, N., Kang, M. S., Kuroyanagi, K., Goto, T. et al., Auraptene, a citrus fruit compound, regulates gene expression as a PPAR-alpha agonist in HepG2 hepatocytes. *Biofactors* 2008, *33*, 25–32.

[13] Genovese, S., Epifano, F., Auraptene: A natural biologically active compound with multiple targets. *Curr. Drug Targets* 2011, *12*, 381–386.

[14] Takahashi, N., Kawada, T., Yamamoto, T., Goto, T. et al., 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.* 2002, *277*, 16906–16912.

[15] Tsuchida, A., Yamauchi, T., Takekawa, S., Hada, Y. et al., 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* 2005, *54*, 3358–3370.

[16] Hiuge, A., Tenenbaum, A., Maeda, N., Benderly, M. et al., Effects of PPAR ligands, bezafibrate and fenofibrate, on adiponectin level. *Arterioscler. Thromb. Vasc. Biol.* 2007, *27*, 635–641.

[17] Kang, M. S., Hirai, S., Goto, T., Kuroyanagi, K. et al., Dehydroabietic acid, a phytochemical, acts as ligand for PPARs in macrophages and adipocytes to regulate inflammation of mouse adipose tissues. *Biofactors* 2009, *35*, 442–448.

[18] Lee, J. Y., Hashizaki, H., Goto, T., Sakamoto, T. et al., Activation of PPAR-alpha enhances fatty acid oxidation in human adipocytes. *Biochem. Biophys. Res. Commun.* 2011, *407*, 818–822.

[19] Goto, T., Teraminami, A., Lee, J. Y., Hirai, S. et al., Activation of PPAR-alpha stimulated both differentiation and fatty acids oxidation in adipocytes. *J. Lipid Res.* 2011, *52*, 873–884.

[20] Yamauchi, T., Kamon, J., Waki, H., Murakami, K. et al., The mechanisms by which both heterozygous PPAR-gamma deficiency and PPAR-gamma agonist improve insulin resistance. *J. Biol. Chem.* 2001, *276*, 41245–41254.

[21] Kadowaki, T., Yamauchi, T., Adiponectin and adiponectin receptors. *Endocr. Rev.* 2005, *26*, 439–451.

[22] Iwaki, M., Matsuda, M., Maeda, N., Funahashi, T. et al., Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes.* 2003, *521*, 655–1663.

