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Bixin Activates PPARα and Improves Obesity-Induced Abnormalities of Carbohydrate and Lipid Metabolism in Mice

Tsuyoshi Goto,[†,‡,∥] Nobuyuki Takahashi,[‡,†,∥] Sota Kato,[‡] Young-Il Kim,[‡] Tatsuya Kusudo,[‡] Aki Taimatsu,[‡] Kahori Egawa,[‡] Min-Sook Kang,[‡] Takuro Hiramatsu,[‡] Tomoya Sakamoto,[‡] Taku Uemura,[‡] Shizuka Hirai,[‡] Misato Kobayashi,[§] Fumihiko Horio,[§] and Teruo Kawada*[,†,‡]

[†]Research Unit for Physiological Chemistry, The Center for the Promotion of Interdisciplinary Education and Research, Kyoto University, Kyoto 606-8501, Japan

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

[§]Department of Applied Molecular Bioscience, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

ABSTRACT: Peroxisome proliferator-activated receptor α (PPARα) is a ligand-activated transcription factor that regulates the expression of the genes involved in fatty acid oxidation. PPARα activators induce fatty acid oxidation in the liver, thereby improving lipid and carbohydrate metabolism in obese mice. In this study, the dietary *cis*-carotenoids bixin and norbixin, which are commonly used in the food coloring industry, were found to activate PPARα by luciferase reporter assays using GAL4/PPARα chimeric and full-length PPARα systems. Treatment with bixin and norbixin induced the mRNA expression of PPARα target genes involved in fatty acid oxidation in PPARα-expressing HepG2 hepatocytes. In obese KK-Ay mice, bixin treatment suppressed the development of hyperlipidemia and hepatic lipid accumulation. In the livers of bixin-treated mice, the mRNA levels of PPARα target genes related to fatty acid oxidation were up-regulated. Moreover, bixin treatment also improved obesity-induced dysfunctions of carbohydrate metabolism, such as hyperglycemia, hyperinsulinemia, and hypoadiponectinemia. Glucose tolerance test and insulin tolerance test revealed that glucose intolerance and insulin resistance in KK-Ay obese mice were attenuated by the treatment with bixin. These results indicate that bixin acts as a food-derived agonist of PPARα, and bixin treatment is useful for the management of obesity-induced metabolic dysfunctions in mice.

KEYWORDS: *bixin, energy metabolism, hepatocyte, norbixin, PPARα*

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily, which are activated by small hydrophobic ligands.[1] One PPAR isoform, PPARα, is mostly expressed in tissues with high rates of fatty acid (FA) oxidation and peroxisomal metabolism.[2] In these tissues, PPARα regulates the expression of genes involved in FA oxidation. Synthetic PPARα agonists such as fibrates lower the level of circulating lipids. PPARα agonists are, therefore, commonly used to treat hyperlipidemia and other dyslipidemic states.[2,3] Besides their hypolipidemic effects, PPARα activators also ameliorate obesity-induced dysfunctions of carbohydrate metabolism, including insulin resistance, hyperglycemia, and hyperinsulinemia, in animal models of obesity and type-2 diabetes mellitus.[4,5] Moreover, PPARα activators induce the mRNA expression of adiponectin and its receptors, which enhance insulin sensitivity and suppress inflammation in adipose tissues. Therefore, appropriate spatial and temporal controls of ligand-dependent PPARα activation are important for the treatment of obesity-induced metabolic diseases.

Annatto, obtained from the pericarp of the seeds of *Bixa orellana*, is a natural pigment used extensively in many processed foods.[6,7] This yellow-red dye is a mixture of carotenoids with various bioactivities.[8,9] Thus, it has been used not only as a natural dye but also as a drug for various illnesses, including diabetes mellitus.[10] The main components of this pigment, bixin (one of the oil-soluble carotenoids of *B. orellana* seeds) and norbixin (a water-soluble hydrolyzed derivative of bixin), have antioxidative and anticarcinogenic properties.[11−13] Moreover, annatto extract and norbixin have hypoglycemic effects in nonobese dogs and mice, respectively.[10,14] However, their effects on obesity and obesity-related metabolic disorders such as glucose intolerance, insulin resistance, and hypoadiponectinemia have yet to be fully elucidated, and the molecular mechanisms underlying the hypoglycemic effects of these compounds have not been clarified.

Bixin and norbixin are isoprenoids, one of the largest families of natural products. We reported previously that several isoprenoids have a high potency for activating PPARα.[15−18] Bixin and norbixin are structurally similar to the isoprenoids that activate PPARα. Therefore, in this study, we investigated whether bixin activates PPARα in vitro and in vivo and whether bixin affects obesity-induced metabolic disorders in obese and diabetic KK-Ay mice.

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Table 1. Oligonucleotide Primers Used for mRNA Analysis

gene	forward primer	reverse primer
hACO	TTGGTCAAGGGGACCTGA	AGCCATCCGACATGCTTC
hCPT1	AATCATCAAGAAATGTCGCACGA	AGGCAGAAGAGGTGACGATCG
hCD36/FAT	TATTTGCAGGTCAATCTATGCTGT	TGAGGAAGTGAAATGTACACAGGT
hUCP2	CAAATGAGCTTTGCCTCTGTC	ATGGTCTTGTAGGCATTGACG
h36B4	AAACTGCTGCCTCATATCCGG	TTGTAGATGCTGCCATTGTCGA
mACO	GCACCATTGCCATTCGATACA	CCACTGCTGTGAGAATAGCCGT
mCPT1	CTGTTAGGCCTCAACACCGAAC	*CTGTCATGGCTAGG*CTGTACAT
mCD36/FAT	GATGTGGAACCCATAACTGGATTCAC	GGTCCCAGTCTCATTTAGCCACAGT
mUCP2	CACTTTCCCTCTGGATACCGC	GATCCCTTCCTCTCGTGCAAT
m36B4	TGTGTGTCTGCAGATCGGGTAC	CTTTGGCGGGATTAGTCGAAG

MATERIALS AND METHODS

Materials and Cell Cultures. Unless indicated, all chemicals used, including bixin and norbixin, were purchased from Sigma (St. Louis, MO, USA) or Nacalai Tesque (Kyoto, Japan).

Monkey CV1 kidney cells and human HepG2 hepatic tumor cells were purchased from American Type Culture Collection (Manassas, VA, USA). Both cell lines were maintained in DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin (maintenance medium). Because HepG2 hepatocytes show a low PPARα expression level (data not shown), we used HepG2 cells expressing exogenous human-PPARα in this study.[15] A human PPARα expression vector (pDEST-hPPARα) was transfected into HepG2 hepatocytes using Lipofectamine (Invitrogen, Carlsbard, CA, USA) in accordance with the manufacturer's protocol and as previously described.[15−17] As a negative control, we used empty vector-transfected HepG2 cells. Cell viabilities were determined calorimetrically using CellTiter 96 (Promega, Madison, WI, USA) in accordance with the manufacturer's protocol.

Luciferase Assays. Luciferase assays were performed as previously described.[15−17,19] Briefly, using a GAL4/PPARα chimera system, we transfected p4xUASg-tk-luc (a reporter plasmid), pM-hPPARα (an expression plasmid for a chimeric protein containing a GAL4 DNA binding domain and a human PPARα ligand-binding domain), and pRL-CMV (an internal control for normalizing transfection efficiency) into CV1 cells. For the PPARα full-length system, pDEST-hPPARα, a reporter plasmid (p4xPPRE-tk-luc), and pRL-CMV were transfected. The transfected cells were cultured in OPTI-MEM (Invitrogen) containing each compound for 24 h. Luciferase activities were measured using the dual luciferase system (Promega).

Animal Experiments. The KK-Ay diabetic obese mouse model[20] was used in animal studies. Male KK-Ay mice were purchased from Clea Japan (Tokyo, Japan). The mice were maintained under a constant 12 h light/dark cycle. Four-week-old male mice were used to determine the effects of bixin on the development of a diabetic condition. The mice were maintained for 5 days on a standard diet and then divided into three groups with the same average body weight. Each group was maintained on a high-fat diet (HFD; 60 kcal %-fat, D12492; Research Diets, New Brunswick, NJ, USA) or a HFD containing 0.5 or 1.0% (w/w) bixin for 4 weeks. The energy intake of all the mice was adjusted by controlled feeding. Thus, the levels of food intake for each group were almost the same (average food intakes were 4.23 ± 0.02, 4.24 ± 0.02, and 4.25 ± 0.02 g/day in the control HFD, 0.5% bixin, and 1.0% bixin groups, respectively). Oral glucose tolerance tests (OGTTs) and intraperitoneal insulin tolerance tests (ITTs) were performed on KK-Ay mice fed the experimental diet for 3 weeks. For the OGTT, D-glucose (2 g/kg body weight) was administered using a stomach tube after overnight fasting. For ITT, human insulin (Eli Lilly Japan, Kobe, Japan) was intraperitoneally injected (0.75 unit/kg body weight) into nonfasted animals. Blood samples were collected from the tail vein before and 30, 60, 90, and 120 min after injection. The levels of serum glucose and triacylglyceride (TG) were enzymatically determined using the glucose CII-test and TG E-test kits (Wako Pure Chemicals, Osaka, Japan), respectively. Serum insulin (Morinaga Institute of Biological Science, Yokohama, Japan) and adiponectin (R&D Systems, Minneapolis, MN, USA) concentrations were measured using an ELISA kit. A surrogate index for estimating insulin resistance, the homeostasis model assessment of insulin resistance (HOMA-IR), was calculated from the fasting serum glucose and insulin concentrations as follows: HOMA-IR = $(G_0 \times I_0)/22.5$, where I_0 is insulin (μU/mL) and G_0 is glucose (mmol/L).[21] All of the mice received humane care, as outlined in the *Guide for the Care and Use of Laboratory Animals* (Kyoto University Animal Care Committee in accordance with NIH 86-23; revised 1985; permission no. 17-47)

Liver Histology. Histochemical analysis of the liver was performed as previously described.[22] Briefly, the liver was removed from each animal and fixed in 4% formaldehyde/PBS. The fixed samples were embedded in the optimal cutting temperature (OCT) compound in isopentane cooled with liquid nitrogen. They were cut into 8 μm sections using a cryostat at −30 °C. Liver cryosections were fixed in 50% ethanol for 3−5 min, stained with 2% Sudan III in 70% ethanol for 1 h, and subsequently washed with 50% ethanol and water. Sections were counterstained with Mayer's hematoxylin.

RNA Preparation and Quantification of Gene Expression Levels. For mRNA expression analysis in HepG2 cells, the pDEST-hPPARα-transfected HepG2 cells were cultured on 12-well tissue culture plates as previously described. Briefly, 24 h after transfection, the cells were incubated in maintenance medium supplemented with bixin for another 48 h, and then total RNA samples were prepared and reverse transcribed. To quantify mRNA expression, PCR was performed using a fluorescence temperature cycler (Roche Diagnostics, Mannheim, Germany). All of the primer sets used in the HepG2 study were described in our previous papers.[15−17,23−25] The primers used for our in vivo study are listed in Table 1. To compare mRNA expression levels among the samples, the copy numbers of all the transcripts were divided by the copy number of the human or mouse ribosomal gene 36B4, which shows a constant level of expression in the liver. All mRNA levels were represented as the ratio relative to that of the control in each experiment.

Statistical Analysis. The data are presented as the mean ± SEM. Data involving more than two groups were assessed by one-way ANOVA and Dunnett's multiple-comparison tests. Differences were considered to be significant at $P < 0.05$.

RESULTS

Bixin and Norbixin Activate PPARα in Cell Culture-Based Assay Systems. To determine the effects of bixin and norbixin on PPARα activity, we first performed luciferase assays using both a GAL4/PPARα chimera system and a full-length PPARα system. As shown in Figure 1A, both bixin and norbixin significantly activated PPARα at concentrations >10 μM in the GAL4/PPARα chimera system. In this assay, the luciferase activity induced by 70 μM bixin was modestly greater than that induced by fenofibrate (Feno), a synthetic PPARα agonist. However, bixin (70 μM) did not activate liver-X-receptor (LXR), farnesoid-X-receptor (FXR) (nuclear receptors involved in hepatic lipid metabolism), or retinoid-X-receptor

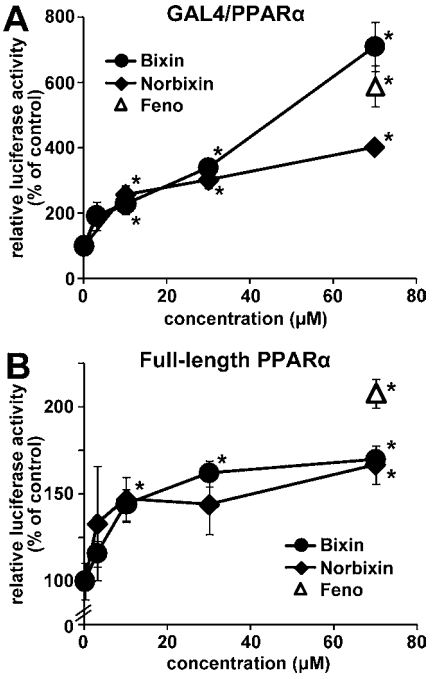

Figure 1. Effects of bixin and norbixin on PPARα activity in cultured cells. Effects of bixin and norbixin on PPARα activity were observed in luciferase reporter assays using the GAL4/PPARα chimera system (A) and the PPARα full-length system (B). CV1 monkey kidney cells were transfected with pM-PPARα, p4xUASg-tk-luc, and pRL-CMV (A) or pDEST-hPPARα, p4xPPRE-tk-luc, and pRL-CMV (B). The cells were incubated in vehicle control-, bixin-, norbixin-, or Feno-containing medium for another 24 h after the transfection. The activity of the vehicle control was set at 100%, and the relative luciferase activities obtained are presented as fold induction with respect to the activity in the vehicle control. Values are the mean ± SEM of the data from three or four samples. *, $P < 0.05$ compared with vehicle controls.

(RXR) (a heterodimeric partner of PPARα) in the chimeric system (data not shown). This suggests that the agonistic activity of bixin is specific to PPARα. Moreover, to elucidate the activities of bixin and norbixin as activators for PPARα, a PPRE (PPAR response element)-luciferase/PPARα full-length system was used for the ligand assay. Bixin and norbixin (70 μM) activated full-length PPARα by approximately 1.7- and 1.6-fold over the vehicle control, respectively (Figure 1B). In this system, Feno showed greater activity than both bixin and norbixin. Bixin and norbixin, at the concentrations used in this study, did not affect the viability of CV1 cells (data not shown). These data indicate that bixin and norbixin are novel ligands for the activation of PPARα.

Next, to determine whether the direct addition of bixin affects the mRNA expression of PPARα target genes in hepatocytes, we treated PPARα-expressing HepG2 cells with bixin. Bixin significantly induced the mRNA expression of PPARα target genes involved in fatty acid oxidation, such as acyl-CoA oxidase (*ACO*), carnitine palmitoyl transferase 1 (*CPT1*), fatty acid translocase/CD36 (*FAT/CD36*), and uncoupling protein-2 (*UCP2*) in PPARα-expressing HepG2 cells (Figure 2). The expression levels of *ACO*, *CPT1*, *CD36/FAT*, and *UCP2* were induced by 70 μM bixin by 1.9-, 3.3-, 1.4-, and 1.4-fold, respectively. The degree of up-regulation was similar to, or a little weaker than, that induced by Feno. However, no bixin-dependent induction of PPARα target genes was observed in the empty-vector-transfected control HepG2 cells (data not shown). In addition, bixin at the concentrations used in this study did not affect the viability of HepG2 cells (data not shown). These data indicate that treatment with bixin induces the mRNA expression of PPARα target genes in PPARα-expressing HepG2 cells.

Treatment with Bixin Improves Dyslipidemia and Hepatic Steatosis by Regulating the Expression of Genes Involved in Hepatic Lipid Metabolism in HFD-Fed KK-Ay Mice. To determine the in vivo effects of bixin on the development of obesity-related metabolic dysfunctions, we treated HFD-fed obese KK-Ay mice with bixin. After 5 days of prefeeding with a normal diet, the mice were fed a HFD with or without 0.5 or 1.0% bixin under pair-fed conditions for 4 weeks. The weights of the body, white adipose tissue (WAT), liver, spleen, and kidney showed no significant differences between the control HFD and the bixin-containing HFD groups (data not shown). However, serum triacylglyceride (TG) concentrations significantly decreased in the bixin-treated mice, as shown in Figure 3A (24% decrease in 1.0% bixin-fed mice compared with the control mice). Sudan III staining showed that TG accumulation was suppressed in the bixin-treated mice, although the livers of the control mice showed a high TG content. The suppression was more apparent in the mice treated with 1.0% bixin than in those treated with 0.5% bixin. These results indicate that bixin treatment suppresses the obesity-induced development of hyperlipidemia and hepatic TG accumulation.

We next measured the mRNA expression of PPARα target genes in the liver. Bixin treatment increased the mRNA levels of *ACO* (Figure 4A), the rate-limiting enzyme in peroxisomal β-oxidation, in the liver. The levels were 1.4- and 1.5-fold higher in the 0.5 and 1.0% bixin-fed mice, respectively. Moreover, bixin treatment also increased the levels of *CPT1* (Figure 4B), *FAT/CD36* (Figure 4C), and *UCP2* mRNA (Figure 4D) in the liver. In the 1.0% bixin-fed mice, the expressions of *CPT1*, *FAT/CD36*, and *UCP2* were induced by 2.5-, 1.9-, and 1.4-fold, respectively. These results show that bixin induces the expression of PPARα target genes in the liver.

Bixin Treatment Improves Carbohydrate Metabolism Deficiencies in HFD-Fed KK-Ay Mice. Finally, to determine the effects of bixin on carbohydrate metabolism in vivo, we examined the insulin sensitivity of bixin-fed mice. As shown in Table 2, a 4 week administration of a HFD resulted in high serum glucose and insulin levels, suggesting that these mice developed a diabetic condition. However, the addition of bixin to the diet decreased the levels of serum glucose and insulin by 22 and 32%, respectively, in the 1.0% bixin-fed-mice. This led to a lower homeostasis model assessment of insulin resistance (HOMA-IR), a simple surrogate index for insulin resistance,[21] in the bixin-treated mice (51% decrease in the 1.0% bixin diet group). In addition, the level of serum adiponectin, an adipocytokine that improves insulin resistance,[26] increased in the bixin-fed mice (Table 2). Moreover, we carried out an oral glucose tolerance test (OGTT) (Figure 5A) and insulin tolerance test (ITT) (Figure 5B) in KK-Ay mice fed each experimental diet for 3 weeks. Serum glucose levels during both tests decreased more rapidly in the 1.0% bixin-fed mice than in the pair-fed control mice (Figure 5). These results demonstrate a reduction in the glucose intolerance and insulin resistance induced by obesity in the bixin-fed mice.

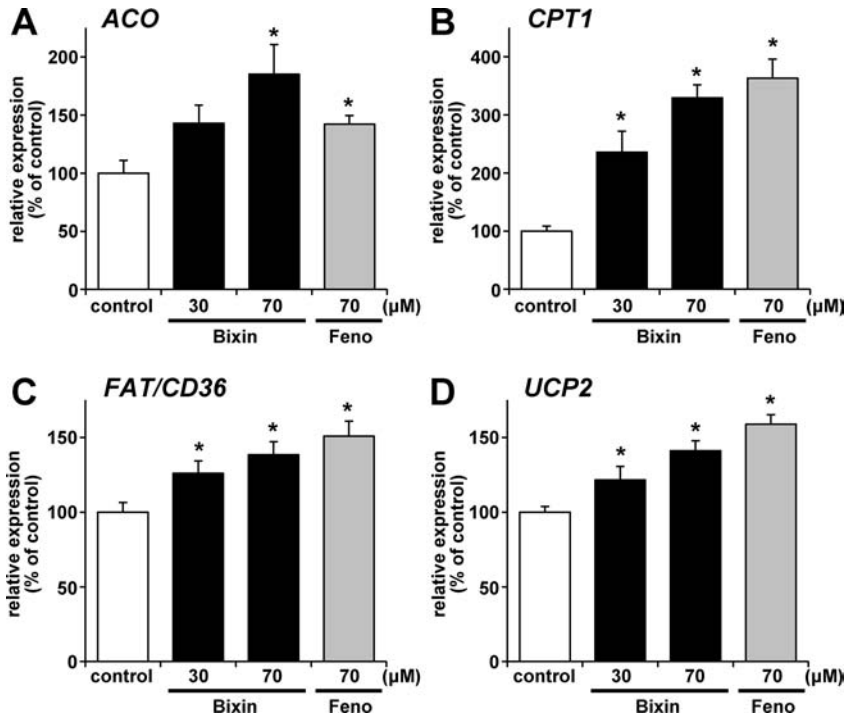

Figure 2. Effects of bixin and norbixin on PPARα target gene expression in PPARα-expressing HepG2 hepatocytes. PPARα-transfected HepG2 cells were treated with vehicle control, bixin (30 or 70 μM), or Feno (70 μM) for 48 h to assess the effect on the expression of *ACO* (A), *CPT1* (B), *FAT/CD36* (C), and *UCP2* (D) mRNA. The expression level of each gene was measured by real-time RT-PCR. mRNA expression levels were normalized to the mRNA expression of the ribosomal *36B4* gene. Expression levels in the cells treated with the vehicle control were set at 100%, and the relative expression levels obtained are presented as fold changes with respect to the level in cells treated with the vehicle control. All values are the mean ± SEM of three or four samples. ∗, $P < 0.05$ compared with vehicle controls.

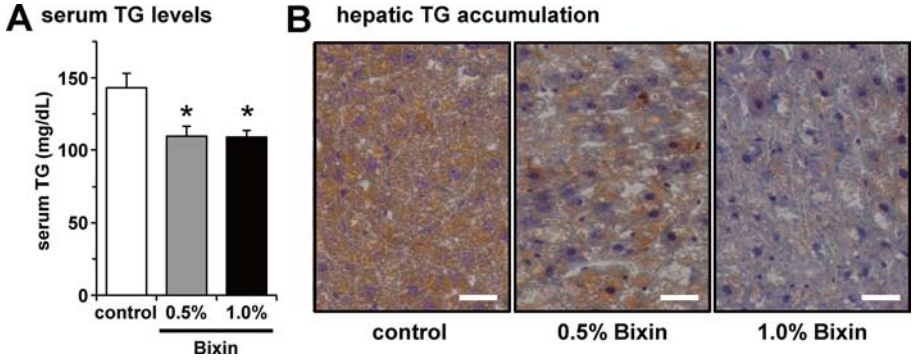

Figure 3. Effects of bixin on the serum and hepatic TG levels in HFD-fed KK-Ay mice. Serum TG amounts were determined using the TG E-test as described under Materials and Methods (A). All values are the mean ± SEM of five or six samples. ∗, $P < 0.05$ compared with the control group. Isolated livers were fixed in 4% formaldehyde for >24 h and then embedded. Liver sections were cut at 8 μm thickness. The liver sections were stained with Sudan III and hematoxylin (B). The bar shown in each photograph indicates 30 μm.

DISCUSSION

Many natural compounds act as PPARα agonists.[15−18,23] Many of these compounds have similar structures and belong to the isoprenoid family, which includes carotenoids and is one of the largest families of natural products. In this study, bixin and norbixin, both of which belong to this family, activated PPARα in luciferase reporter assays using both chimeric GAL4/PPARα and full-length PPARα systems. Furthermore, bixin induced PPARα target genes involved in fatty acid oxidation in PPARα-overexpressing HepG2 cells. These results indicate that bixin and norbixin can function as novel, naturally occurring PPARα ligands and regulate hepatic lipid metabolism, at least in vitro. Bixin could not induce the mRNA expression of PPARα target genes in the empty-vector-transfected HepG2 cells, which expressed PPARα at a very low level. Moreover, LXR, FXR, and RXR were not activated by bixin in luciferase assays. LXR and FXR regulate hepatic lipid metabolism, and RXR is a heterodimeric partner of PPARα. These results suggest that PPARα is critical for the induction of these genes by bixin treatment. Various potent synthetic PPARα agonists have been used as therapies for hyperlipidemia,[2] and herbs with a high content of isoprenoids, such as basil, rosemary, oregano, and sage, have been used as herbal therapies for dyslipidemia.[27] Therefore, bixin and norbixin were expected to improve hyperlipidemia through the induction of PPARα target gene expression in the liver.

Considering the results of our in vitro studies, we examined the in vivo effects of bixin by using obese KK-Ay mice fed a

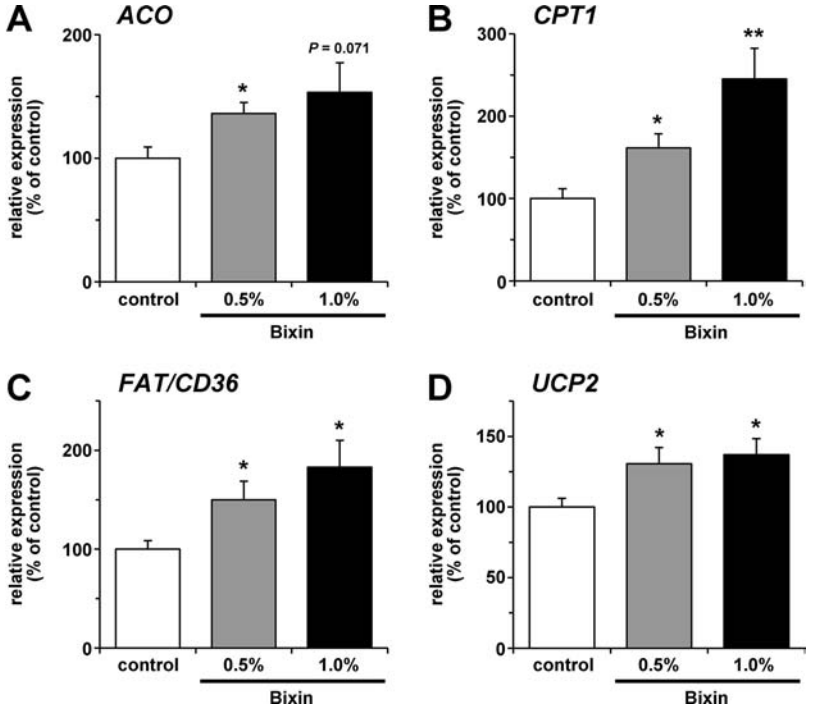

Figure 4. Effects of bixin on the expression of PPARα target genes in the livers of HFD-fed KK-Ay mice. The expression levels of PPARα target genes in the livers of the control and bixin-fed mice were measured by real-time RT-PCR. The mRNA levels of the target genes were normalized to the mRNA levels of the ribosomal *36B4* gene. The expression levels in the control HFD-fed mice were set at 100%, and the relative expression levels are presented as fold inductions with respect to the level in the control. *ACO* (A), *CPT1* (B), *FAT/CD36* (C), and *UCP2* (D) mRNA expression levels in the liver. Results are shown as the mean ± SEM of five or six animals per group. ∗, $P < 0.05$, and ∗∗, $P < 0.01$, compared with the control group.

Table 2. Effects of Bixin Treatment for 4 Weeks on Serum Glucose, Insulin, and Adiponectin Levels and HOMA-IR in HFD-Fed KK-Ay Mice[a]

	control	0.5% bixin	1.0% bixin
serum glucose (mg/dL)	399.3 ± 24.3	359.1 ± 18.8	310.8 ± 27.2 a
serum insulin (ng/mL)	19.1 ± 1.6	15.8 ± 1.8	12.9 ± 1.8 a
HOMA-IR	471.9 ± 63.4	356.1 ± 50.8	232.1 ± 20.3 b
serum adiponectin (μg/mL)	8.8 ± 1.5	11.6 ± 0.4 a	13.6 ± 0.9 b

[a]Each value is the mean ± SEM of five or six animals per group. HOMA-IR was calculated as described under Materials and Methods. a, $P < 0.05$, and b, $P < 0.01$, compared with the control group.

HFD for 4 weeks. As expected, bixin treatment increased the mRNA expression of genes involved in fatty acid β-oxidation (*ACO* and *CPT1*), fatty acid uptake (*FAT/CD36*), and energy consumption (*UCP2*) in the liver. Notably, it was previously reported that PPARα regulates the expression of these genes in the liver.[15,28] Here, bixin treatment was also found to improve obesity-related dyslipidemia and hepatic steatosis. Following the same protocol as in the present study, we previously showed that the hepatic mRNA expression of *ACO*, *CPT1*, *FAT/CD36*, and *UCP2* increased in KK-Ay mice fed a HFD containing 0.2% bezafibrate, a well-known PPARα agonist (6.8-, 1.4-, 3.6-, and 8.4-fold increases, respectively, overexpression in the control HFD-fed mice; unpublished data). Thus, the in vivo effects of bixin observed in the present study were similar to or weaker than those of bezafibrate, although the in vitro effects of bixin were similar to those of Feno, another well-known PPARα agonist. These results indicate that bixin is a beneficial, food-derived compound for controlling in vivo PPARα activity, although its effects are weaker than those of synthetic PPARα agonists. However, further investigation using PPARα-knockout mice is needed to demonstrate the PPARα-specificity of bixin activity in vivo.

In the present study, we showed that bixin treatment improves obesity-induced dysfunctions of carbohydrate metabolism, such as glucose intolerance, insulin resistance, hyperglycemia, and hyperinsulinemia. PPARα plays an important role in carbohydrate metabolism, in addition to its role in lipid metabolism. Severe hypoglycemia has been observed in PPARα-deficient mice under fasting conditions.[29] Moreover, PPARα agonists have been shown to improve obesity-induced insulin resistance, hyperglycemia, and hyperinsulinemia in animal models of obesity and type-2 diabetes mellitus.[4,5] However, the molecular mechanism of these effects has not been fully clarified, partly because of the enhanced fatty acid clearance from insulin-sensitive organs such as the liver and skeletal muscles.[30,31] Thus, PPARα activation in the liver and the subsequent improvement of hepatic steatosis in bixin-treated mice may ameliorate dysfunctions of carbohydrate metabolism. Recently, PPARα agonists have been shown to directly and transcriptionally increase adiponectin levels via adipose PPARα.[32,33] In fact, the serum adiponectin level in the bixin-treated mice was significantly elevated in the present study. Because adiponectin is an adipocytokine that improves insulin resistance,[26] the bixin-induced elevation of the circulating adiponectin levels contributes to the improvement of insulin resistance. Furthermore, we found that UCP1 protein expression levels were significantly up-regulated in the brown adipose tissue of mice fed a diet containing bixin (data not shown). Encoded by a PPARα target gene, UCP1 plays a

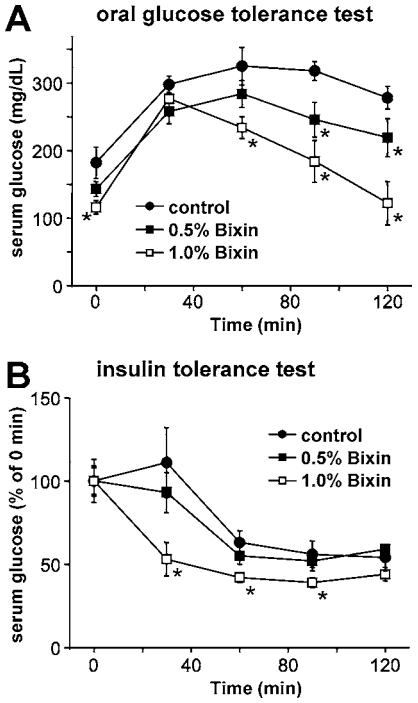

Figure 5. Effects of bixin on obesity-induced glucose intolerance and insulin resistance in HFD-fed KK-Ay mice. An oral glucose tolerance test (OGTT) and an intraperitoneal insulin tolerance test (ITT) were performed on KK-Ay mice fed a HFD with or without bixin for 3 weeks. Blood glucose curves during the OGTT (A) and ITT (B) on KK-Ay mice fed each experimental diet are shown. Glucose (2 g/kg body weight) was orally administered to 16-h-fasted mice of each group; insulin (0.75 unit/kg body weight) was intraperitoneally injected into nonfasted mice of each group. Each bar represents the mean ± SEM of five or six animals per group. *, $P < 0.05$ compared with the control group.

crucial role in nonshivering thermogenesis in brown adipose tissue.[34,35] These results give rise to the possibility of bixin-dependent activation of PPARα not only in the liver but also in white and brown adipose tissues.

Bixin is commonly used as a natural color pigment in various foods. Therefore, the bioactivities and toxicities of this natural compound have been well studied. For example, the effects of bixin on oxidative stress and carcinogenesis have been examined.[11−13] Recently, oxidative stress was found to correlate with insulin resistance.[36] Therefore, the antioxidative effect of bixin might contribute to the antidiabetic effect observed in the present study, at least partially. In addition, the antioxidative and antitumor proliferation effects of bixin are intensified when used in combination with other carotenoids.[11] Thus, it will be worthwhile to determine the combined effects of bixin and other carotenoids on diabetes and obesity.

From the results of the present study, we conclude that bixin serves as an agonist for PPARα not only in vitro but also in vivo. In the in vivo experiments, bixin activated hepatic PPARα, resulting in the improvement of obesity-induced metabolic disorders. These findings suggest that bixin could be used as a functional food-derived compound for regulating hepatic lipid metabolism.

AUTHOR INFORMATION

Corresponding Author

*Postal address: Laboratory of Molecular Function of Food, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Uji 611-0011, Japan. Phone: +81-774-38-3751. Fax: +81-774-38-3752. E-mail: fat@kais.kyoto-u.ac.jp.

Author Contributions

‖These authors equally contributed to this work.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ACO, acyl-CoA oxidase; CPT, carnitine-palmitoyl CoA transferase; FAT, fatty acid translocase; HFD, high-fat diet; HOMA-IR, homeostasis model assessment of insulin resistance; ITT, insulin tolerance test; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; TG, triacylglyceride; UCP, uncoupling protein; WAT, white adipose tissue

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