Bitter Gourd (Momordica charantia) Extract Activates Peroxisome Proliferator-Activated Receptors and Upregulates the Expression of the Acyl CoA Oxidase Gene in H411EC3 Hepatoma Cells

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Peroxisome proliferator-activated receptor α • Stable transfection • Bitter gourd • Hypolipidemic

Abstract
Peroxisome proliferator-activated receptor α (PPARα) is a ligand-dependent transcription factor that regulates the expression of genes involved in lipid metabolism and transport. Ligands/activators of PPARα, like fibrate-type drugs, may have hypolipidemic effects. To identify food that contains activators of PPARα, a transactivation assay employing a clone of CHO-K1 cells stably transfected with a (UAS)₄-tk-alkaline phosphatase reporter and a chimeric receptor of Gal4-rPPARα LBD was used to screen ethyl acetate (EA) extracts of a large variety of food materials. It was found that the EA extract of bitter gourd (Momordica charantia), a common oriental vegetable, activated PPARα to an extent that was equivalent to or even higher than 10 μM Wy-14643, a known ligand of PPARα. This extract also activated PPARγ to a significant extent which was comparable to 0.5 μM BRL-49653. The activity toward PPARα was mainly in the soluble fraction of the organic solvent. The EA extract prepared from the whole fruit showed significantly higher activity than that from seeds or flesh alone. The bitter gourd EA extract was then incorporated into the medium for treatment of a peroxisome proliferator-responsive murine hepatoma cell line, H411EC3, for 72 h. Treated cells showed significantly higher activity of acyl CoA oxidase and higher expressions of mRNA of this enzyme and fatty acid-binding protein, indicating that the bitter gourd EA extract was able to act on a natural PPARα signaling pathway in this cell line. It is thus worth further investigating the PPAR-associated health benefits of bitter gourd.

Introduction
Peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors that control energy homeostasis through genomic action [41]. As other members of the steroid hormone nuclear receptor superfamily do, PPARs have a common modular structure including a DNA-binding domain and a ligand-binding domain (LBD) [9, 36]. Upon activation by a ligand, PPAR heterodimerizes with the RXR and promotes transcription of its target genes through binding to a peroxisome proliferator-responsive element (PPRE) [9, 41]. Three subtypes of this receptor exist, namely PPARα...
(NR1C1), PPARδ (NR1C2) and PPARγ (NR1C3), which display tissue-selective expression patterns and distinct biological functions. PPARα is predominantly expressed in the liver, heart, kidney, and skeletal muscle where it controls fatty acid catabolism. PPARγ is highly expressed in brown and white adipose tissue where it triggers cellular differentiation, promotes lipid storage, and modulates the action of insulin. PPARδ is ubiquitously expressed and controls brain lipid metabolism and fatty acid-induced adipogenesis and preadipocyte proliferation [9, 41]. Recent reports have further demonstrated a role of PPAR in regulating glucose homeostasis, cellular differentiation and apoptosis, and cancer development as well as in controlling the inflammatory response [7, 19].

Being the ligand/activator of PPARα, fibrate-type hypolipidemic drugs can induce the expression of genes that participate in aspects of lipid catabolism such as fatty acid uptake and binding, fatty acid oxidation (in microsomes, peroxisomes, and mitochondria), and lipoprotein assembly and transport [36]. These are PPARα target genes since PPRE has been identified in the promoter region of these genes. Using a PPARα-deficient mouse model, the hypolipidemic action of fibrates was found to be mediated by this receptor [31]. The results indicated that the PPARα ligand/activator may lower liver and/or blood lipids by upregulating PPARα target genes, which in turn enhance the oxidation of fatty acids [36]. Likewise, thiazolidinediones are PPARγ ligands, and the antidiabetic effects of this type of drug are believed to be mediated by PPARγ [3, 43]. Substantial attempts are currently being made to develop new therapeutic agents for hyperlipidemia, insulin resistance, or atherosclerosis by screening agonists/antagonists of PPARs [33, 41]. Attention has especially been paid to compounds that serve as ligands for both the α- and γ-subtypes of PPAR [33, 41]. A similar approach was thus employed in this study for the purpose of identifying PPARα activators from food materials.

PPAR activators of food/diet origin may provide health benefits without toxicity concerns, as long as the food/diet is consumed in a reasonable amount on a regular basis. A number of PPAR activators that are of food/diet origin have been identified. Conjugated linoleic acids (CLA) [29] and phytic acid [11, 12] are well-known examples. Although the mechanism of action is not fully understood, CLA [4] has health benefits such as antiatherogenic, antidiabetic, and anticarcinogenic effects, while phytic acid induces brown adipocyte differentiation and UCP-1 transcription [35]. The hypolipidemic effect of fish oil [8] has been attributed, at least in part, to the activation of PPARα. Oxidized lipids in oxidized frying oil can activate PPARα, upregulate some PPARα target genes, and reduce plasma and liver triglycerides in rats [5].

In this study, we screened a large number and variety of food materials, and found that bitter gourd (Momordica charantia) contains PPARα and γ activator(s) that can be extracted by an organic solvent. The activating effect on natural PPARα was further demonstrated in H4IIEC3, a peroxisome proliferator-responsive murine hepatoma cell line.

Materials and Methods

Expression and Reporter Vectors

The chimeric receptor constructs used were pBK-CMV-Gal4-rPPARα-ligand-binding domain (Gal4-rPPARαLBD) and the pBK-CMV-Gal4-rPPARγ-ligand-binding domain (Gal4-rPPARγLBD), respectively. The reporter gene was pBK-CMV-(UAS)₄-tk-AP and the antibiotic genes were pSV₂-neomycin and pKSV-hygromycin. These were gifts from Dr. J.A. Gustaffson, Department of Medical Nutrition, Karolinska Institute, Huddinge, Sweden. The correct in-frame fusions were confirmed by sequencing.

Cell Culture and Stable Transfection

Procedures for the establishment of cell lines stably transfected with the Gal4-rPPARα LBD chimeric receptor and (UAS)₄-tk-AP reporter have been described previously [15]. Briefly, CHO-K1 cells (American Type Culture Collection, Rockville, Md., USA) were grown at 37°C in the presence of 5% CO₂ in Ham’s F-12 medium supplemented with 10% fetal bovine serum (Gibco BRL, Rockville, Md., USA). Using Lipofectamine™ 2000 (Gibco BRL) according to the manufacturer’s instructions, cells were first transfected with the reporter construct and pSV₂-Neo. Transfected cells were selected in the presence of 0.8 mg/ml medium of G418 (neomycin). The colonies grown were pooled to form a reporter clone mix and further transfected with the chimeric receptor construct pBK-CMV-Gal4-rPPARα and pKSV-Hygro, and then selected with 0.8 mg/ml medium of hygromycin. About 150 clones were picked, expanded, and tested for their responsiveness to 50 μM/l/Wy-14643 (Cayman Chemicals, Ann Arbor, Mich., USA), a known activator of PPARα. Among the 10 responsive clones, clone 77 showed the maximal induction of reporter gene AP activity, and was used for the following experiments. The expression of PPARα-LBD in cells of clone 77 was confirmed by RT-PCR.

Food Extracts for Testing

Tested food materials were chosen from vegetables, fruits, nuts, traditional Chinese herbs, and microbial products. Fresh vegetables included bitter gourd (Momordica charantia L.), onion, celery, sweet potato leaves, and rice seedlings; fruits included lychee (litchi or Litchi chinensis Sonn) and longan (Nephelium longana Camb or Eugenia longana Lam); nuts included freshly roasted peanuts and sesame seeds; dried traditional Chinese herbs included ricinus seed (Ricini semen), lotus seed plummule (Nelumbo nucifera Gaertn), and Hawthorn (Crataegi fructus), and microbial products included Monascus
**ankha** (dried powder of Chinese red yeast rice), *Ganoderma tsugae*, lactic acid bacteria, and desalted pressed residue of soy sauce mash. Tested food materials were extracted as previously described [16, 44], but only the ethyl acetate (EA) extract was used for testing in this study. This is because ligands of PPARs are known to be relatively hydrophobic. Briefly, samples were homogenized in a Waring blender with a minimal amount of double-distilled water, and homogenates were filtered through several layers of gauze. The residues were collected, freeze-dried, ground up, and extracted with EA (1:30, w/v) by stirring overnight at room temperature. The EA solutions were filtered, and the filtrates were evaporated in a rotary evaporator (Bn-col, St. Paul, Minn., USA) immediately before use.

**Transactivation Experiments and Reporter Gene Assay**

For the transactivation experiments, the EAEs of tested samples were dissolved in a minimal amount of ethanol or dimethyl sulfoxide (DMSO). They were then diluted to appropriate concentrations with Ham's F-12 medium containing 10% serum replacement (TCM, Celox, St. Paul, Minn., USA) immediately before use. The procedure of the transactivation assay has previously been described [5]. Briefly, stably transfected cells were seeded in 96-well plates and incubated with medium containing Wy-14643 and the tested food extracts or vehicles (DMSO or ethanol) for 48 h. Culture medium was then collected and assayed for AP activity using CSPDP © (Tropix, Applied Biosystems, Foster City, Calif., USA) as the substrate and Sapphire-II as the enhancer. Chemiluminescence was measured in a luminometer (Wallac 1420 Victor² multiple label counter; Perkin Elmer, Turku, Finland). The viability of treated cells was checked by the MTT (Sigma, St. Louis, Mo., USA) assay. Data reported are confined to those for which treatment did not significantly change the cell viability. Fold changes of activity were calculated by taking the AP activity of vehicle-treated cells as 1.

**Distribution of the PPARa Activator in Bitter Gourd**

To examine the distribution of the PPARa activator in different parts of bitter gourd, the flesh, seeds, and whole fruit of green bitter gourd were, respectively, extracted and compared for their PPARa activation ability using the transactivation assay described above. To compare the activity of four different varieties of bitter gourd, whole fruits of white, green, pearl-shaped, and wild bitter gourd (fig. 2b) were extracted as described above and tested using the transactivation assay.

**Extraction of the PPARa Activator of Bitter Gourd**

Green bitter gourd whole fruit was extracted by several procedures, and the extracts obtained were tested for their PPARa-activating potential using the transactivation assay described above. Fresh green bitter gourd whole-fruit samples were homogenized in a Waring blender with a minimal amount of double-distilled water, and homogenates were filtered through several layers of gauze. The juice was further centrifuged at 10,500 g at 4°C for 30 min, and the precipitate was collected and added to the filtered residue. The clear supernatant of juice and the residue were freeze-dried. The dried residue was ground up and extracted with EA (1:30, w/v) by stirring overnight at room temperature. The EA solutions were filtered, and the filtrates were evaporated in a rotary evaporator (Buchi) to remove the solvent. The EAEs were weighed and stored at -20°C. The yield of bitter gourd EA extract in this extraction procedure was ~1.8 g/kg fresh whole fruit. In a set of experiments, EA was replaced by n-hexane. Alternatively, sample material was sliced, freeze-dried, and directly extracted with EA, and the EAE was partitioned using a saturated NaCl aqueous solution. On the other hand, sample material was directly extracted using methanol, and then the methanol extract was sequentially partitioned using n-hexane, EA, and n-butanol.

** transient Transfections**

Cells transfected with the reporter construct (reporter clone mix obtained in the first stage of stable transfection as described above) were used in this experiment. Nearly confluent cells were seeded in 96-well plates and transfected with 0.2 μg of the chimeric receptor construct pBK-CMV-Gal4-PPARα or pBK-CMV-Gal4-PPARγ using 1.2 μl of Lipofectamine 2000 reagent in 100 μl serum-free medium OPTI-MEM® (Gibco BRL) per well. After 5 h, the medium was changed to Ham's F-12 medium containing 10% serum replacement and vehicle (ethanol or DMSO), 10 μM Wy-14643, 0.5 μM BRL-49653 or appropriate concentrations of wild bitter gourd whole-fruit EA extract. Medium was collected 48 h later and analyzed for alkaline phosphatase activity as described above.

**Treatment of the H4IIEC3 Cell Line with Bitter Gourd EA Extract**

H4IIEC3 cells (American Type Culture Collection) are Reuber rat hepatoma cells that offer a good in vitro model system of the induction of peroxisomes and peroxisomal β-oxidation enzymes by peroxisome proliferators. H4IIEC3 cells were maintained at 37°C in the presence of 5% CO2 in Dulbecco's modified Eagle's medium supplemented with 20% horse serum (Gibco) and 5% fetal bovine serum (Gibco). Near confluence, cells were seeded at a density of 60,000 cells/ml into 10-cm dishes and grown for 24 h in the same medium under 5% CO2 at 37°C. The EAE of the dried water-insoluble fraction of wild bitter gourd whole fruit was dissolved in a minimal amount of absolute ethanol and diluted with the medium to a concentration of 50, 100, or 150 μg/ml, respectively, and used to treat these cells. Blank dishes received vehicle (medium containing 0.1 or 0.3% absolute ethanol) only. Cells treated with medium containing 50 μM Wy-14643 or 500 μM clofibrate served as positive controls. After 72 h of treatment, cells were harvested for analysis of acyl CoA oxidase (ACO) activity, and expressions of mRNA of ACO and fatty acid-binding protein (FABP).

**ACO Activity Assay and Northern Blot Analysis**

Harvested cell pellets were homogenized, and the postnuclear supernatant fraction was isolated and analyzed for ACO activity according to the method of Small et al. [38]. To determine the mRNA of ACO and FABP by Northern blot, total RNA was extracted from the harvested cell pellet using trizol reagent (Life Technologies, Rockville, Md., USA). Twenty micrograms of extracted RNA from each treated cell sample was separated by electrophoresis in denaturing formaldehyde agarose (1%) gel, transferred to a nylon membrane (Gene Screen Plus; DuPont, Boston, Mass., USA), and cross-linked to the membrane by UV irradiation. The blots were prehybridized at 42°C for 3 h in the hybridization buffer excluding the probe but containing salmon sperm DNA. It was then hybridized at 42°C for 12–16 h with 32p-labeled cDNA probes of ACO and FABP, respectively. To correct for possible differences in transfer and loading, blots were also hybridized with labeled β-actin probe as an internal control. Afterward, blots were washed at the appropriate stringency to remove nonspecific binding and were exposed to X-OMAT AR film (Kodak, Rochester, N.Y., USA). Signals were analyzed quantitative-
Data reported are expressed as the mean ± standard deviation of triplicate wells, and are representative of at least three separate experiments with similar results. The significance of difference between each treatment was analyzed by one-way ANOVA using SAS® (SAS 8.1, Cary, N.C., USA) software.

Results

Cell Clones Obtained from Stable Transfection

After successive transfection with the (UAS)4-AP reporter and Gal4-rPPARα LBD chimeric receptor, about 160 clones of cells that grew in the presence of 0.8 mg/ml hygromycin were picked, expanded, and tested for their responsiveness to Wy-14643, a known PPARα ligand. Ten clones were found to be responsive and further expanded. Figure 1a shows the dose-dependent expression of the AP reporter gene of 1 of these clones, clone 77, in response to Wy-14643. This cell clone also responded to a number of known PPARα activators including common and oxidized fatty acids, CLA, and phytanic acid in a manner similar to the results reported in the literature (data not shown). Therefore, clone 77 was considered to be acceptable and was used in the later transactivation assay for estimating the PPARα-activating potential of various food extracts. Throughout batches of experiments in which different passages of clone 77 were used, the folds of activation of 10 μM Wy-14643 ranged from 2 to 4. The folds of activation of 10 μM Wy-14643 were highest when a vial of an early passage of clone 77 cells was thawed, but the value decreased gradually as the passage number increased. Cells were discarded when the folds of activation of 10 μM Wy-14643 approached 2.

The PPARα-Activating Ability of Tested Food Extracts

Using cells of clone 77, the EAEs of food materials were tested for their transactivation of PPARα. Most of the tested samples showed low or moderate activity (data not shown). However, the extract of bitter gourd showed such a high activity that the maximum fold of activation was comparable to that of 10 μM of Wy-14643. The dose-dependent activation of bitter gourd EA extract on PPARα is shown in figure 1b, and the EC50 was estimated to be 70 μg/ml. The maximal fold of activation was attained by 90 μg/ml of green bitter gourd EA extract.

Distribution of the PPARα Activator in Bitter Gourd

Although the EA extract prepared from seed or flesh significantly activated PPARα, bitter gourd whole-fruit EA extract showed still higher activity (p < 0.05) than the seed or flesh extract (fig. 2a). At concentrations of 100 or
Fig. 3. Transactivation of Gal4-rPPARα (a) and Gal4-rPPARγ (b) by wild bitter gourd EA extract (BGEAE) in transient transfection experiments. Folds of activation were calculated by taking the AP activity of the vehicle (ethanol) as 1. Values are the mean ± SD of triplicate wells from 1 experiment. Data shown are representative of 3 separate experiments with similar results. Wy-14643 (10 μM) and BRL-49653 (0.5 μM) were used as positive controls. Values not sharing the same letter significantly differ from one another by one-way ANOVA and Duncan’s multiple range test (p < 0.05).

Fig. 2. Distribution of the PPARα activator in bitter gourd (BG). CHO-K1 cells stably transfected with the Gal4-rPPARα receptor chimera and the AP reporter (clone 77) were used to determine the transactivation of PPARα by EA extracts of seeds, flesh, or whole fruit of green bitter gourd (a), EA extract of 4 varieties of bitter gourd (b), and the green bitter gourd water-soluble fraction and EA extract of the water-insoluble fraction (c). Folds of activation were calculated by taking the AP activity of the vehicle (ethanol)-treated cells as 1. Values are the mean ± SD of triplicate wells from 1 experiment. Data shown are representative of 3 separate experiments with similar results. Wy-14643 (10 μM) was included as a positive control. Values not sharing the same letter significantly differ from one another by one-way ANOVA and Duncan’s multiple range test (p < 0.05).

150 μg/ml, the EA extract of four varieties of bitter gourd all showed significant activation of PPARα that was comparable to that of 10 μM of Wy-14643 (fig. 2b). But the EA extract of white bitter gourd had a significantly lower value for the maximal fold of activation (p < 0.05) compared to those of the remaining three varieties of bitter gourd.

**Extraction of the PPARα Activator from Bitter Gourd**

As shown in figure 2c, the water-soluble fraction of bitter gourd did not activate PPARα at all, whereas the EA extract of the dried water-insoluble fraction showed significant activation which was comparable to 10 μM of Wy-14643. The fold of activation of the n-hexane extract of the dried water-insoluble fraction did not significantly differ from that of the EA extract.

Alternatively, green bitter gourd whole fruit was freeze-dried and directly extracted with EA, and the EAE was partitioned using a saturated NaCl aqueous solution. This salt solution-washed EA extract also activated PPARα to an extent comparable to the EA extract from the original extraction procedure (residue from the water extraction subsequently extracted by EA). When bitter gourd was extracted with methanol and sequentially partitioned using n-hexane, EA, and n-butanol, only the n-hexane fraction activated PPARα to an extent comparable to the EA extract obtained from the original procedure (data not shown). These results indicated that the PPARα activator in bitter gourd is preferentially extracted by relatively non-polar organic solvents, such as EA and n-hexane.

**Transient Transfection with the PPARα or PPARγ Chimeric Receptor**

The transient transfection approach was also employed to test PPAR activation of the bitter gourd EA extract since the folds of activation observed in the transactivation assay using our stably transfected clone 77 cells were relatively low compared to data reported in the literature using transient transfection. The result with the PPARα chimeric receptor was very similar to that of the transactivation assay using our stable cell clone 77, but the fold of activation was higher and comparable to some reported data. Wild bitter gourd EA extract showed equivalent (100 μg/ml) or higher (150 μg/ml) activation of PPARα compared to that of 10 μM Wy-14643 (fig. 3a). Excitingly, wild bitter gourd EA extract also significantly activated PPARγ. As shown in figure 3b, the fold of activation of 150 μg/ml wild bitter gourd EA extract was equivalent to that of 0.5 μM BRL-49653, a well-known PPARγ ligand.
**Fig. 4.**

**a** The ACO activity of H4IIEC3 cells treated with clofibrate, Wy-14643 (Wy), or EA extract of wild bitter gourd (BGEAE) for 72 h.

**b** Northern blot analysis for mRNA of ACO and FABP in H4IIEC3 cells treated with Wy or BGEAE for 72 h. Wy: 50 μM; BGEAE: 50, 100 or 150 μg/ml. Total RNA (20 μg) isolated from treated cells was separated by electrophoresis, transferred to nylon membranes, and then hybridized with an ACO or FABP cDNA probe. Signals were quantitated by image analysis. Each value was normalized by β-actin (as the internal control). Values are the means ± SD of triplicate experiments. Values not sharing the same letter are significantly different from one another by one-way ANOVA and Duncan’s multiple range test (p < 0.05).
H4IIEC3 Cells Treated with Bitter Gourd EAEs

To test whether the bitter gourd EA extract can act on natural PPARα, H4IIEC3 cells were treated with this extract for 72 h. The activity of ACO in treated cells was about 1.8-fold (p < 0.05) that of vehicle-treated cells, and did not significantly differ from that of cells treated with Wy-14643 or clofibrate (fig. 4a). The expression of mRNA of ACO and FABP in treated cells determined by Northern blot and image analysis is shown in figure 4b, c. Similar to cells treated with 50 μM Wy-14643, cells treated with 100 or 150 μg/ml of bitter gourd EA extract showed significantly higher mRNA expression of ACO and FABP than cells treated with vehicle only. The result indicated that bitter gourd EA extract could act on a natural PPARα signaling pathway.

Discussion

Ligand binding of PPARs can induce the expression of an array of genes that in turn can change the metabolism of lipids and/or the utilization of glucose. A number of therapeutic agents, namely fibrate hypolipidemic drugs and thiazolidinedione antidiabetic drugs, are ligands of PPARs and the pharmacological effects were shown to be mediated by these nuclear receptors [7, 36, 43]. The transactivation assay is used to test the functional potency of a compound to activate PPAR. The assay measures the expression of a reporter gene that is driven by a promoter responsive to the specific binding of the transcription factor, PPAR. As PPARs are ligand-dependent transcription factors, molecules binding to PPARs activate expression of the reporter gene. To avoid complicating the interpretation of results by endogenous receptor activation, a chimeric receptor in which the PPARα LBD is fused to the yeast transcription factor GAL4 DNA-binding domain and a reporter construct containing four copies of the GAL4 DNA-binding site UAS which drives expression of secreted placental alkaline phosphatase were used in this study. The approach is similar to that used for screening compounds in pharmaceutical research and development [13]. Compared to the transient transfection approach, it is more convenient to use clones of cells stably expressing the transfected receptor and reporter for compound screening. However, it usually takes a longer time to obtain such a clone, and very often the clone may suffer from instability and loss of sensitivity due to passages of cells. In this study, this can be seen from variations in the folds of activation of the positive control, Wy-14643.

The EA, rather than the water, extract of food materials was chosen for testing in this study, since the ligand-binding structure of PPARα was confirmed to prefer hydrophobic molecules. Among various food materials tested, bitter gourd was found to activate PPARα to an extent comparable to Wy-14643. Bitter gourd is an important cultivated food crop in Asian countries and is recognized as being of medicinal value in Indian and Chinese traditional medicine. Indeed, the juice or whole powder of bitter gourd fruit has been reported to have various medicinal properties, including anticarcinogenic [22], hypoglycemic [14, 18, 28, 42], and hypotriglyceridemic [17] effects. These effects are closely related to the role of PPAR in regulating lipid and glucose homeostasis, cellular differentiation and apoptosis, and cancer development as well as in controlling the inflammatory response [7, 19]. Our transactivation assay further demonstrated that all four varieties of bitter gourd commonly seen in local Taiwanese markets produce high activation of PPARα. The activity is higher in whole fruit than in flesh or seeds and can be extracted by relatively nonpolar organic solvents, such as EA and n-hexane, as would be expected by the hydrophobic nature of the active compound.

ACO and FABP are PPARα target genes since the PPAR-specific binding sequence (PPRE) has been detected in the promoter region of these genes. Administration of a PPARα ligand will lead to the increased expression of such genes, which in turn will affect metabolism. This regulation is known as the PPARα signaling pathway. The increased ACO activity and mRNA, as well as FABP mRNA, expression in H4IIEC3 cells treated with bitter gourd EA extract observed in this study indicate that bitter gourd EA extract may upregulate target genes of PPARα in this cell line. As chimeric receptors were used in our transactivation assay, the results observed in H4IIEC3 cells demonstrate that bitter gourd EA extract works on natural PPARα and triggers the signaling pathway. It can thus be speculated that eating an appropriate amount of bitter gourd might have health benefits such as hypolipidemic effects through the PPARα signaling pathway. It was reported that feeding a diet containing 0.5 or 1% freeze-dried bitter gourd powder significantly reduced liver triglycerides and cholesterol concentrations in rats [17]. We are currently conducting an in vivo animal experiment, and the results show that feeding bitter gourd EA extract upregulates the expression of some PPARα target genes.

The most noteworthy health benefit of bitter gourd is the hypoglycemic potential demonstrated in normal [42] and diabetic [2, 6, 34, 37, 39] rats as well as in human...
subjects with type II diabetes mellitus [23]. Using a rat model with high-fat-diet-induced obesity and hyperglycemia, it was [6] found that bitter gourd significantly reduced fasting plasma concentrations of glucose and insulin, and visceral fat pad weight, and improved oral glucose tolerance. The mechanism for the hypoglycemic effect of bitter gourd is still unknown, although it has been shown to inhibit glucose absorption [27], promote hepatic glucose utilization [37], possess an insulin-like polypeptide [20], and even to increase the insulin-positive cell number in the pancreas [1]. Excitingly, we observed that the bitter gourd EA extract could also activate PPARγ, and the fold of activation was comparable to that of 0.5 μM BRL-49653. It is well known that the antidiabetic drug, thiazolidinedione, is a PPARγ ligand. It would be of great value to identify the compound responsible for PPARγ activation and test its antidiabetic potential.

Numerous biologically active components have been identified in bitter gourd. For example, seed extracts contain MAP-30, an anti-HIV and antitumor protein [24], momorcharins [32] and momoridins [21], which inactivate ribosomes, and momoridin Ic [25] and oleanolic acid glycoside [26], both of which can alter gastrointestinal transit time and blood glucose. In an attempt to search for the chemical constituent responsible for the hypoglycemic effect, a series of triterpene glycosides and oleanane-type triterpene saponins have been identified from Japanese bitter gourd fresh fruit, yet their biological effects have not been reported [30].

The lipid of bitter gourd seeds contains more than 50% of a conjugated linolenic acid (CLN), α-eleostearic acid [9 cis (c), 11 trans (t), 13 t-18:3] [10, 40]. A small but distinct amount of a different CLN, 9t, 11t, 13c-18:3 [40], was also found in the flesh of bitter gourd. As CLN has structural similarity with known PPAR activators, such as CLA, it can be speculated to be 1 of the chemical constituents that contribute to PPAR activation in bitter gourd. On the other hand, regardless of the big difference in the CLN content between the seed and flesh of bitter melon, we observed no such big difference in folds of activation between seed and flesh extracts. Moreover, maximal folds of activation toward PPAR by common or uncommon fatty acids are generally much lower than those of drugs used as positive controls (Wy-14643 for PPARα and BRL-49653 for PPARγ) in the transactivation assay. Hence, bitter gourd may still contain chemical constituent(s) other than CLN that contribute to the high PPAR activation seen in this study.

In spite of its bitter taste that is unacceptable to some individuals, bitter gourd is a very common vegetable which has been consumed in oriental societies for hundreds of years. Data reported in this study indicate that this food material contains activators for both PPARα and PPARγ. Irrespective of the fact that the molecular species responsible for PPARα and PPARγ activation are still unknown, these data provide an evidence base indicating that it is worth designing clinical trials to test the potential health benefits of bitter gourd as part of the daily diet for improving hyperglycemic and hyperlipidemic conditions.

In conclusion, we used a transactivation assay to demonstrate that the EA extract of bitter gourd activated PPARα and PPARγ to an extent comparable to Wy-14643 and BRL-49653, respectively. In H4IIEC3 cells, the bitter gourd EA extract increased ACO activity and the expressions of ACO and FABP mRNA, indicating that it can also act on a natural PPARα signaling pathway. It is worth further investigating the use of this common oriental vegetable to attain PPARα- and PPARγ-mediated health benefits, such as improving hyperlipidemic and hyperglycemic conditions.

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References

Bitter Gourd Activates PPARα


