Biochemical and Biophysical Research Communications 430 (2013) 1322–1328

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ombuin-3-*O*-β-D-glucopyranoside from *Gynostemma pentaphyllum* is a dual agonistic ligand of peroxisome proliferator-activated receptors α and δ/β

Mastura Abd Malek [a,b,1], Minh-Hien Hoang [a,b,1], Yaoyao Jia [a,b], Ji Hae Lee [a,b], Hee Jin Jun [a,b], Dong-Ho Lee [a], Hak Ju Lee [c], Chul Lee [d], Myung Koo Lee [d], Bang Yeon Hwang [d,*], Sung-Joon Lee [a,b,*]

[a] Department of Biotechnology, Graduate School of Biotechnology, Korea University, Seoul 136-713, Republic of Korea
[b] Division of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea
[c] Division of Green Business Management, Department of Forest Resources Utilization, Korean Forest Research Institute, Seoul 130-712, Republic of Korea
[d] College of Pharmacy, Chungbuk National University, Cheongju, Chungbuk 361-763, Republic of Korea

ARTICLE INFO

Article history:
Received 9 November 2012
Available online 13 December 2012

Keywords:
Dual agonist
Lipid metabolism
Ombuin-3-*O*-β-D-glucopyranoside
PPARs

ABSTRACT

We demonstrated that ombuin-3-*O*-β-D-glucopyranoside (ombuine), a flavonoid from *Gynostemma pentaphyllum*, is a dual agonist for peroxisome proliferator-activated receptors (PPARs) α and δ/β. Using surface plasmon resonance (SPR), time-resolved fluorescence resonance energy transfer (FRET) analyses, and reporter gene assays, we showed that ombuine bound directly to PPARα and δ/β but not to PPARγ or liver X receptors (LXRs). Cultured HepG2 hepatocytes stimulated with ombuine significantly reduced intracellular concentrations of triglyceride and cholesterol and downregulated the expression of lipogenic genes, including sterol regulatory element binding protein-1c (SREBP1c) and stearoyl-CoA desaturase-1 (SCD-1), with activation of PPARα and δ/β. Activation of LXRs by ombuine was confirmed by reporter gene assays, however, SPR and cell-based FRET assays showed no direct binding of ombuine to either of the LXRs suggesting LXR activation by ombuine may be operated via PPARα stimulation. Ombuine-stimulated macrophages showed significantly induced transcription of ATP binding cassette cholesterol transporter A1 (ABCA1) and G1 (ABCG1), the key genes in reverse cholesterol transport, which led to reduced cellular cholesterol concentrations. These results suggest that ombuine is a dual PPAR ligand for PPARα and δ/β with the ability to decrease lipid concentrations by reducing lipogenic gene expression in hepatocytes and inducing genes involved in cholesterol efflux in macrophages.

1. Introduction

Hyperlipidemia, a disorder of lipid metabolism, is one of the major precursors of atherosclerosis and other metabolic disorders. Hypertriglyceridemia, a clinical symptom with elevated levels of plasma triglycerides (TGs) and fatty acids (FAs), could lead to the progression of coronary heart disease, insulin resistance, and diabetes type II [1] and is correlated with the prevalence of several metabolic diseases such as obesity and hepatic steatosis [2], which are major causes of mortality in most developing countries. Accordingly, the development and improvement of therapeutic and preventative agents for hypertriglyceridemia in lipid metabolism are current areas of interest worldwide.

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that form obligate heterodimers with retinoid X receptors (RXR) to function as key transcription factors in lipid and glucose metabolism, inflammation, and cell proliferation [3]. Three PPARs subtypes, PPARα, δ/β, and γ, have unique tissue distribution and metabolic functions: PPARα is predominantly expressed in liver and plays a critical role in hepatic fatty acid β-oxidation; PPARγ is expressed at high levels in adipose tissue, regulating glucose and lipid homeostasis and triggering adipocyte differentiation [4]; and PPAR δ/β is expressed ubiquitously and has been reported to regulate thermogenesis and inflammation [5]. PPARs have self-regulation mechanisms with feed-forward characteristics. Additionally, PPARs regulate other nuclear receptors, such as liver X receptors (LXRs) as target genes. LXRs have two isoforms: LXRα is highly expressed in the liver, and LXRβ is ubiquitously expressed. Both isoforms play a critical function in regulating and controlling cholesterol and fatty-acid metabolism [6]. It is well documented that PPAR agonists increase the expression of ATP-binding cassette A1 (ABCA1), thereby enhancing the biogenesis of HDL via a process that involves LXRs, particularly LXRα [7].

* Corresponding authors. Address: Department of Biotechnology, Graduate School of Biotechnology, Korea University, Seoul 136-713, Republic of Korea (S.-J. Lee), +82 2 3290 3653 (S.-J. Lee), fax: +82 043 268 2732 (B.Y. Hwang).
E-mail addresses: byhwang@chungbuk.ac.kr (B.Y. Hwang), junelee@korea.ac.kr (S.-J. Lee).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.12.020

Therefore, identifying potential ligands for PPARs and LXRs as effective and safe therapeutic agents in treating hyperlipidemia is essential.

Gynostemma pentaphyllum (Thunb.) Makino is a perennial creeping herb belonging to the family Cucurbitaceae that is widely distributed in Korea, China, and Japan [8]. *G. pentaphyllum* has been reported to exhibit various biological activities including antitumor [9], hypoglycemic [10], and cholesterol-lowering effects [11]. Flavonoids were considered one of the major components that contribute to the health beneficial properties of *G. pentaphyllum* [12]. We previously screened approximately 900 Korean natural compounds and extracts for PPAR activity using reporter gene activity-based assays (unpublished data) and determined that flavonoid ombuin-3-*O*-β-D-glucopyranoside (ombuine, Fig. 1A) from methanol extracts of the aerial parts of *G. pentaphyllum* had potent PPAR activities. In this study, we investigated the agonistic activity of ombuine on PPARs and its effect on lipid metabolism. We determined that ombuine is a dual ligand for PPARα and δ/β, which affects the regulation of hepatic and macrophage lipid metabolism.

2. Materials and methods

2.1. Extraction, isolation, and identification of ombuine from *Gynostemma pentaphyllum*

The dried aerial parts of *G. pentaphyllum* (10 kg) were extracted three times with MeOH at room temperature. Removal of the solvent under vacuum yielded a dark extract (0.8 kg). The extract was suspended in H_2O and then partitioned with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-butanol, successively. The *n*-butanol fraction was separated into six subfractions (GPB1–GPB6) by Diaion HP-20 column chromatography using H_2O and a MeOH gradient. The subfraction GPB3 was eluted on a Sephadex LH-20 chromatography column with CH_2Cl_2 and MeOH (1:1) to yield a flavonoid glycoside fraction (GPB31). Fraction GPB31 was further subjected to octadecylsilane (ODS) column chromatography using a H_2O and MeOH step-gradient system (20–45% MeOH) to obtain ombuin-3-*O*-β-D-glucopyranoside (130 mg). Electronspray ionization mass spectrometry (ESI-MS) and LCQ Fleet mass spectrometry were employed, and ^1H and ^{13}C NMR spectra were recorded using a

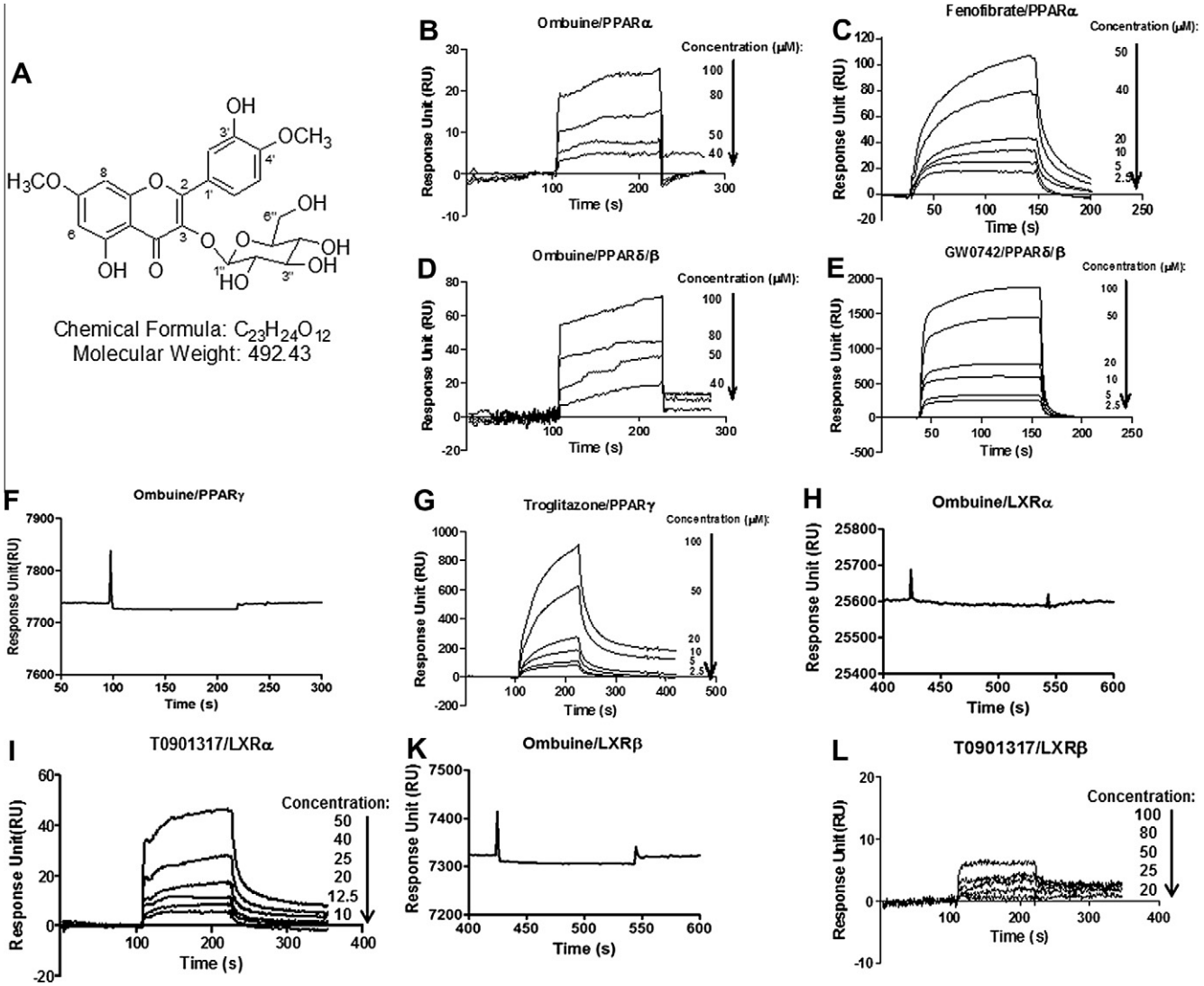

Fig. 1. (A) Chemical structure of ombuin-3-*O*-β-D-glucopyranoside (ombuine) isolated from *G. pentaphyllum*. (B–L) SPR sensograms show the binding affinities at different concentrations of ombuine and positive controls for PPARs and LXRs on immobilized PPAR- and LXR-LBDs on the CM 5 sensor chip. Fenofibrate, GW7042, and troglitazone are PPARα, δ/β, and γ ligands; T0901317 is a ligand for both LXRs.

Bruker DRX 500 NMR spectrometer, respectively. The structure of ombuin-3-*O*-β-D-glucopyranoside was confirmed by comparison of its physicochemical and spectroscopic data with those reported previously [13]. Purification of ombuin-3-*O*-β-D-glucopyranoside was identified by HPLC. HPLC analysis was performed on a Waters system (515 pumps with a 2996 photodiode array detector) and an YMC J'sphere ODS-H80 column (4 μm, 150 × 20 mm ID), using the mixed solvent system MeCN–H_2O (20:80 to 100:0, 0–30 min) at a flow rate of 1.0 ml/min.

Ombuin-3-*O*-β-D-glucopyranoside: yellow amorphous powder; ESI-MS *m/z*: 515 $[M + Na]^+$; ^1H NMR (500 MHz, DMSO-d_6) δ: 12.6 (5-OH), 7.73 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′), 7.60 (1H, d, *J* = 2.0 Hz, H-2′), 7.05 (1H, d, *J* = 2.0 Hz, H-5′), 6.73 (1H, d, *J* = 2.5 Hz, H-6), 6.38 (1H, d, *J* = 2.5 Hz, H-8), 5.52 (1H, d, *J* = 7.5 Hz, H-1″), 3.86 (6H, s, –OCH_3), 3.08–3.61 (6H, m, H-2″, H-3″, H-4″, H-5″, H-6″); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 177.7 (C-4), 165.2 (C-7), 160.9 (C-5), 156.4 (C-9), 156.2 (C-2), 150.2 (C-4′), 145.9 (C-3′), 133.8 (C-3), 122.6 (C-6′), 121.5 (C-1′), 115.8 (C-2′), 111.4 (C-5′), 105.1 (C-10), 100.7 (C-10″), 97.9 (C-6), 92.3 (C-8), 77.7 (C-3″), 76.5 (C-5″), 74.1 (C-2″), 69.9 (C-4″), 60.9 (C-6″), 56.1 (–OCH_3), 55.7 (–OCH_3) (Supplementary Fig. 1). HPLC-DAD chromatograms and UV spectra showed the characteristic spectra of ombuine, with bands at 254.8–353.5 nm (Supplementary Fig. 2). According to ESI-MS, ^1H NMR, ^{13}C NMR and HPLC-DAD data, we showed that ombuine is of good purity (98.6%) and no cross-contaminations was obtained.

2.2. Cell culture and experiments

HepG2 and RAW 264.7 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing L-glutamine supplemented with 1% penicillin/streptomycin (PEST) and 10% heat-inactivated fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 and 95% O_2 at 37 °C. Human monocytic THP-1 cells were maintained in RPMI-1640 medium supplemented with 10% FBS, 0.05 mM β-mercapethanol, and 1% PEST then were differentiated into macrophages by phorbor 12-myristate 13-acetate (50 μg/mL) for 72 h. At confluency, cells were plated in 6-well plates at a density of 5×10^5 cells/well and incubated for 48 h in serum-free medium containing ombuine (5 and 10 μM), fenofibrate (10 μM), T0901317 (1 μM), or 1% dimethyl sulfoxide (DMSO) as vehicle control. Experiments were carried out in triplicate.

2.3. Protein purification

The ligand-binding domain (LBD) of hPPARα (amino acid (aa) 280–468), hPPARδ-LBD (aa 254–441), hPPARγ-LBD (aa 317–505), hLXRα-LBD (aa 200–445), and hLXRβ-LBD (aa 213–461) were cloned into expression vector pET32 a-c(+) (Novagen, Madison, WI, USA) to induce protein expression. Proteins were purified using a HiTrap™ Chelating HP Column (GE Healthcare, Milwaukee, WI, USA) according to the manufacturer's instructions.

2.4. SPR analysis of direct interaction between LBD proteins and ombuine

The binding affinities of ombuine toward PPARα-LBD, PPARδ-LBD, PPARγ-LBD, LXRα-LBD, and LXRβ-LBD were analyzed using an SPR-based Biacore 2000 instrument (GE Healthcare, Uppsala, Sweden) as described previously [14].

2.5. Time resolved-fluorescence resonance energy transfer (TR-FRET) coactivator recruitment assay

The potential active effects of ombuine on three subtypes of PPAR (α, δ/β, and γ) and two subtypes of LXR (α and β) were performed with commercial kit from Invitrogen (Madison, WI, USA) according to the manufacturer's instructions. To determine the concentration required to produce a 50% effect (EC_{50}), the data were fitted to a sigmoidal dose-response curve (varying slope) using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA).

2.6. Reporter gene assays

The transfection and reporter gene assay were performed with HEK 293 cells, as described previously [14,15].

2.7. Oil Red O staining

HepG2 cells were fixed in 10% formalin for 10 min at room temperature. The formalin was then discarded and replaced with fresh formalin for at least 1 h. Cells were washed with PBS twice and incubated with 60% isopropanol for 5 min. The solvents were aspirated and cells were dried under the hood. Oil Red O working solution (Sigma) was added and incubated for 12 h. Images were acquired using an inverted microscope (Eclipse T*i-s*, Nikon).

2.8. Cellular triglyceride and cholesterol concentrations

Cellular lipids were extracted as described previously [15]. The cellular concentrations of TG were quantified enzymatically with a Cobas C111 analyzer (Roche Diagnostic Systems Inc., Indianapolis, IN, USA). Cholesterol levels were measured using an Amplex Red Cholesterol Assay Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Protein quantification was measured using the Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA) for normalization.

2.9. Fluorescence-activated cell sorting (FACS)-based fatty-acid uptake

HepG2 cells were cultured in 6-well plates for 24 h and treated with ombuine (5 or 10 μM), fenofibrate (10 μM), or 1% DMSO. Fluorescent-labeled lipid [C1-BODIPY 500/512 C_{12}; 4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoic acid: 2 μM in Hank's buffered salt solution (HBSS) with 0.1% BSA] was added to each well for 1 min at 37 °C. The assay was terminated by adding ice-cold HBSS containing 0.2% BSA. Cells were resuspended in FACS buffer and analyzed on a FACScalibur (BD Biosciences, San Jose, CA, USA) by determining FL-1 fluorescence. The data were analyzed using Cell Quest Pro software (BD Biosciences, San Jose, CA, USA).

2.10. Determination of mRNA expression

Total RNA was extracted from THP-1-derived macrophages, HepG2 and RAW 264.7 cells using an RNAiso Plus reagent (Takara, Japan) as instructed by the manufacturer. Realtime qPCR was performed with Bio-Rad iQ SYBR® Green Supermix reagent and the Bio-Rad iQ5 Cycler System. The primers were described in Supplementary Table 1. Expression levels were normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with the normalized expression (CT) method according to the manufacturer's guidelines [15].

2.11. Statistical analysis

Values are expressed as mean ± standard error (SE). The number of repeats were $n = 3$, unless mentioned otherwise. Student's *t*-test was performed for comparisons between two groups. Differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Ombuine directly interacts with PPARα and PPARδ/β-LBD

To investigate the direct interaction of ombuine with PPARs and LXRs, we performed ligand binding assays using Biacore SPR experiments and cell free-FRET coactivator recruitment assays with purified LBDs of three PPAR and two LXR subtypes (Fig. 1B–L). The results showed that ombuine bound directly to the PPARα and PPARδ/β-LBD, with dissociation constants of 98.9 nM and 10.3 nM, respectively (Fig. 1B and D). When compared with dissociation constants of fenofibrate and GW0742 (300 nM and 210 nM, respectively), these results suggest direct and robust binding of ombuine to PPARα and PPARδ/β-LBD (Fig. 1C and E). However, no significant binding of ombuine to the PPARγ-LBD or LXR-LBDs was detected (Fig. 1F, H and K). These results demonstrate that ombuine is a direct ligand for PPARα and δ/β but not for PPARγ or LXRs.

Next, we examined the ligand binding of ombuine, PPARs, and LXRs in cell-free FRET assays (Fig. 2). This assay determined whether the ligand binding to PPAR or LXR-LBD protein induces coactivator recruitment capability. Ombuine induced the recruitment of PGC1α and C33 coactivator peptide to the PPARα-LBD and PPARδ/β-LBD (EC_{50} = 113 nM and 15.2 nM, respectively; (Fig. 2A and B). In contrast, ombuine failed to recruit neither PPARγ nor LXR-specific coactivator peptides whereas troglitazone and T0901317 showed strong agonist activity in PPARγ and LXRα and LXRβ (Fig. 2C–E), respectively. These data are in line with SPR results and confirm that ombuine is an agonistic ligand for PPARα and δ/β.

We next performed luciferase reporter assays to determine the transactivation activity of ombuine on three subtypes of PPAR (α, δ, and γ) and two subtypes of LXR (α and β). Ombuine treatment significantly induced the transcriptional activity of PPARα and δ/β and two subtypes of LXRs in a dose-dependent manner but not for PPARγ (Fig. 2F). The data showed that ombuine exerted higher activation on PPARα (+120%) and PPARδ/β (+150%) at 10 μM ($P < 0.05$) compared with LXRα (+87%) and LXRβ (+54%) at 10 μM ($P < 0.05$). The transactivation of LXRs by ombuine in the luciferase assay may have occurred indirectly through the activation of PPARs. It has been shown that LXRs are target genes for PPARα. Ogata et al. reported that the activation of PPAR ligands increased the transcription of luciferase reporter driven by the LXR element in fibroblasts and that the effect was completely diminished in the absence of PPARα in fibroblasts from wild-type and PPARα (−/−) mice [16].

3.2. Effect of ombuine on cellular lipid concentrations and the expression of targeted genes in lipid metabolism

Ombuine significantly reduced the intracellular cholesterol concentrations in THP-1-derived macrophage (−9% at 10 μM, $P < 0.05$), RAW 264.7 (−43% at 10 μM, $P < 0.01$) and HepG2 cells (−12% at 10 μM, $P < 0.05$) compared with controls (Fig. 3A). A significant reduction of cholesterol ester levels in THP-1-derived macrophages (−15% at 10 μM, $P < 0.05$, Supplementary Table 2) was also observed. These results suggest that ombuine mediates potent cellular cholesterol efflux in macrophages and hypolipidemic activities in hepatocytes. In addition, ombuine stimulation in HepG2 cells reduced intracellular TG concentration significantly, by 10% ($P < 0.05$). Indeed, the effect was comparable to that of fenofibrate (−15%, $P < 0.05$, Fig. 3A). Oil Red O staining results showed similar trend (Fig. 3B).

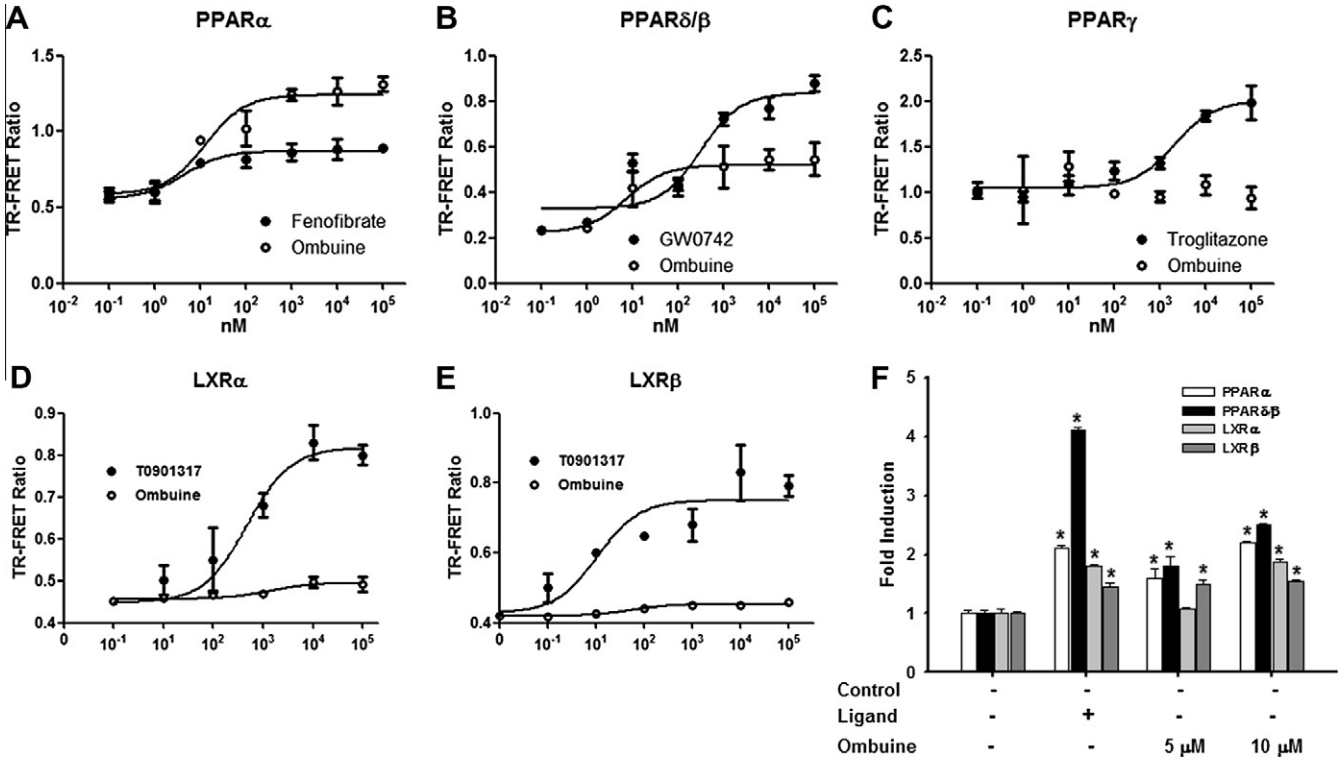

Fig. 2. Ombuine is a dual PPAR ligand for PPARα and δ/β. (A–E) Cell-free TR-FRET assays. Ombuine activates PPARα and δ/β-LBDs thus recruits corresponding coactivator peptides. (F) Luciferase reporter gene assay. HEK 293 cells were transfected with expression plasmids for receptors and a pSV-b-galactosidase together with a reporter plasmid (pCMV-3xPPRE-Luc for PPARs and pGL4.35[luc2P/9XGAL4UAS/Hygro] for LXRs). The cells were exposed to ombuine (5 and 10 μM), receptor-specific ligands (10 μM fenofibric acid for PPARα, 10 μM GW7042 for PPARδ/β, 10 μM troglitazone for PPARγ, and 1 μM T0901317 for LXRs), or vehicle control (1% DMSO) for 24 h before assaying luciferase activity. Data shown represents mean ± SE ($n = 3$); *$P < 0.05$ compared with controls (no treatment).

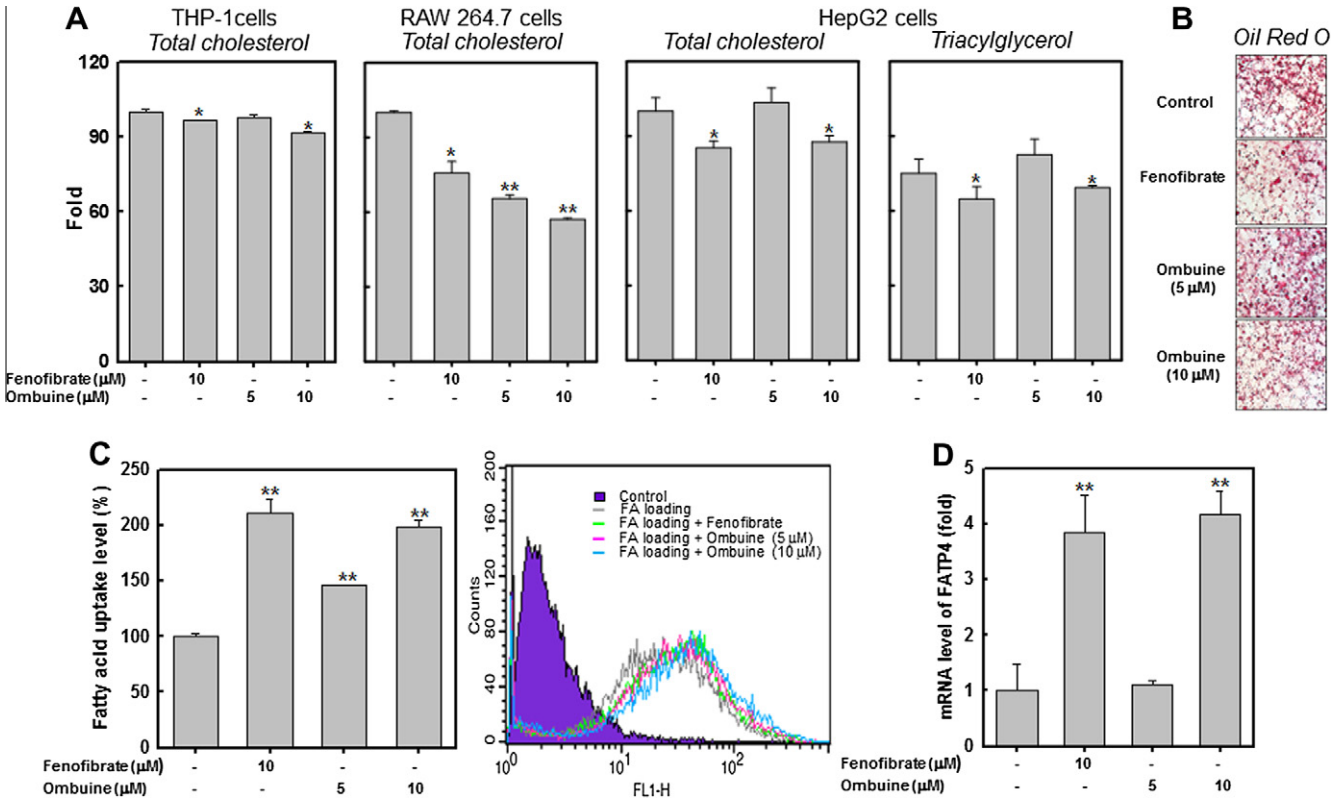

Fig. 3. Ombuine reduces cellular lipid accumulations. (A) Cellular cholesterol concentrations in THP-1-derived macrophages, RAW 264.7 cells, and cellular cholesterol and TG concentrations in HepG2 cells. (B) Oil Red O staining in HepG2 cells. (C) FACS-based cellular fatty acid uptake in with BODIPY-labeled fatty acids. (D) The gene expression of FATP4. HepG2 cells were stimulated with ombuine for 48 h before RNA extraction. Levels of mRNAs were assessed by qRT-PCR and normalized to the GAPDH mRNA levels. Data represent the mean ± SE ($n = 3$); $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with controls (no treatment).

We performed quantitative qRT-PCR to examine the transcription levels of genes involved in reverse cholesterol transport (RCT) in macrophages and hepatic lipogenesis in HepG2 cells. Incubation with ombuine for 48 h significantly induced mRNA expression of LXRα and ABCA1 (2.6- and 3.0-fold at 10 μM, $P < 0.05$, respectively) in THP-1-derived macrophages. ABCG1 expression showed no significant difference between the two concentrations (Fig. 4). Activation of PPARα and δ/β by their agonists has been reported to induce the production of endogenous ligands for LXR, which contribute to stimulation of LXRα expression, thereby inducing ABCA1 gene expression followed by enhanced cholesterol efflux [16]. LXR activation in macrophages induced the expression of genes encoding ABCA1 and ABCG1, which facilitate cholesterol efflux from macrophages to produce plasma high-density lipoproteins (HDL) [6]. In our study, the expression levels of ABCA1 and ABCG1 in RAW 264.7 increased significantly after treatment with ombuine (3.8- and 1.8-fold at 10 μM, $P < 0.05$, respectively, Supplementary Fig. 3). These results indicate that ombuine mediates RCT by inducing ABCA1 and ABCG1 expression.

SREBP1c is a major lipogenic transcription factor highly expressed in hepatic and adipose tissues that stimulates various lipogenic genes in *de novo* FA biosynthesis by inducing the expression of FA synthase (FAS), SCD-1, and acetyl-CoA carboxylase (ACC) [17]. SCD-1 is a rate-limiting enzyme for the biosynthesis of mono-unsaturated FA from saturated FA to form TG during FA esterification [18]. Ai et al. reported that the activation of LXRα directly regulated SREBP-1c expression. It concurrently stimulated the expression of FAS, SCD-1, and ACC, thereby contributing to excessive TG accumulation in hepatocytes and causing steatohepatitis [19]. Results from quantitative PCR shows that the expression of SREBP-1c and SCD-1 were downregulated after treatment with ombuine (10 μM) (Fig. 4), consistent with the reduction of cellular TG by 7% in HepG2 cells (Fig. 2D). The expression of FAS was not affected by ombuine. These observations are similar to those of a previous *in vivo* study, which suggested that genes involved in lipid metabolism might be selectively regulated, eventually leading to the suppression of hepatic lipogenesis [20]. Furthermore, it has been reported that insulin-induced gene (Insig)-2a, the key regulator of SREBP-1c activity, is up-regulated by activation of PPARα [21]. Expression of PPARα (2-fold, 10 μM, $P < 0.01$) and PPARδ/β (1.5-fold, 10 μM) were induced in a dose-dependent manner upon stimulation with ombuine for 48 h. Activation of PPARα led to increased mRNA level of Insig-2a after ombuine simulation (Fig. 4). As a result, the mRNA level of SREBP-1 was reduced and associated with decreased mRNA concentrations of its target gene SCD-1, implicated in FA and TAG synthesis. Taken together, the above observations suggest that ombuine activates PPARα and PPARδ/β activity and may induce RCT in macrophage cells without activating FA synthesis caused through LXR activation, an adverse effect of T0901317 [22].

3.3. Hepatic fatty-acid uptake by ombuine

We analyzed the uptake of hepatic FA with BODIPY-labeled FAs in HepG2 cells with FACS. Based on the average fluorescence intensity, FA uptake was induced in the treated HepG2 cells in a dose-dependent manner, with 2.2-fold increase at an ombuine of 10 μM (Fig. 3C; $P < 0.01$). Furthermore, we examined the effects of ombuine on target genes of PPARα and δ/β that correlate to FA uptake, FA β-oxidation, and TG hydrolysis by quantifying key gene expressions including FATP4, carnitine palmitoyltransferase-1 (CPT-1) and apolipoprotein C3 (APOC3). Ombuine (10 μM)

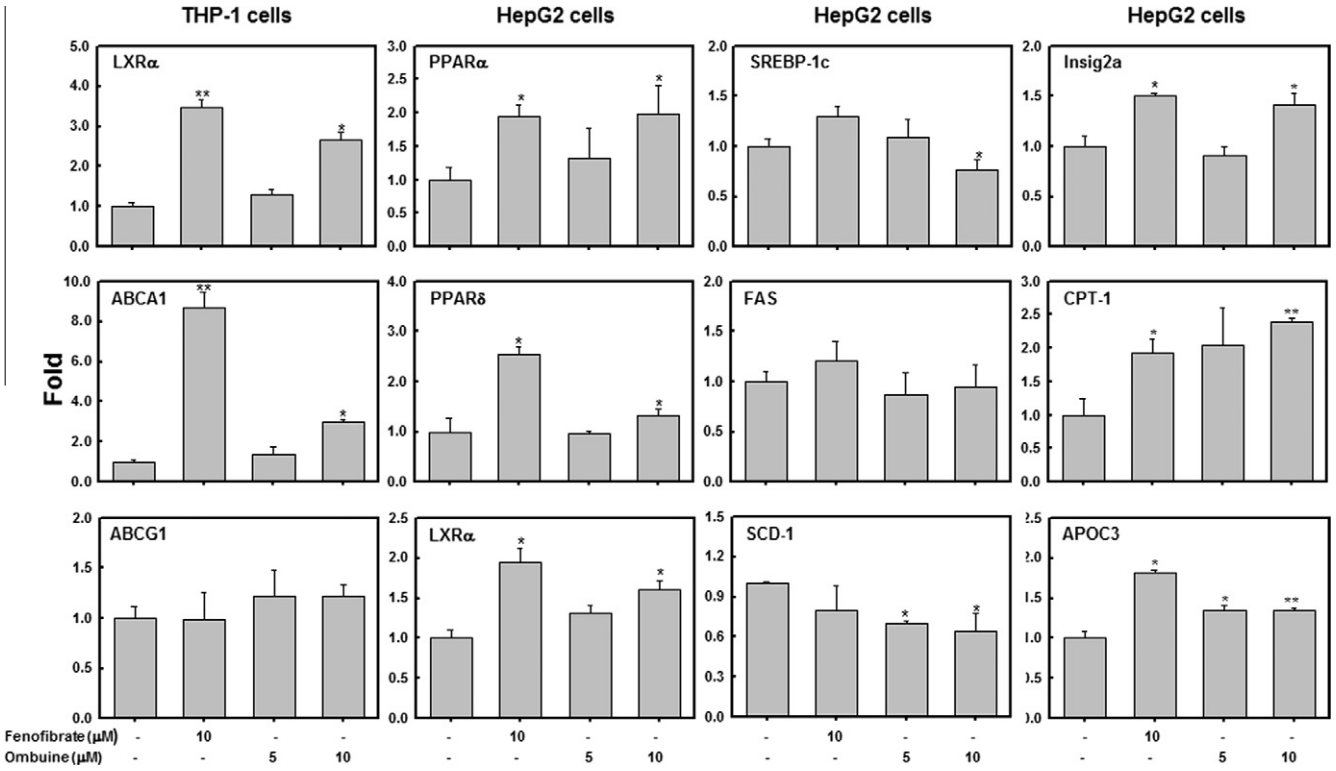

Fig. 4. The expression of PPARα and δ/β target genes in cultured macrophages and hepatocytes. Expression levels of lipid metabolism genes were assessed by qRT-PCR in cells stimulated with fernofibrate or ombuine. Levels of mRNAs were assessed by qRT-PCR and normalized to the GAPDH mRNA levels. Data represent the mean ± SE ($n = 3$); $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with controls (no treatment).

significantly ($P < 0.05$) upregulated the expression of FATP4 and CPT-1 by 4.1- and 2.4-fold, respectively. FATP4, one of the main fatty-acid transporters in liver, is upregulated by PPARα, and CPT-1 is an enzyme that regulates β-oxidation of long-chain FAs in mitochondria, is upregulated by both of PPARα and δ/β [23]. Other studies have shown that PPARα activation leads to the inhibition of APOC3, an inhibitor of lipoprotein lipase (LPL) activity, leading to the reduction of serum TGs [24]. However, in our study, ombuine increased the expression of APOC3 in HepG2 by 1.4-fold even though a significant reduction of TG levels was observed.

In conclusion, ombuine is a dual ligand for PPARα and δ/β which regulates lipid metabolism in multiple cell types. Treatment with ombuine led to an increase in lipid uptake and promotes gene expression in reverse cholesterol transport, fatty acid synthesis, fatty acid uptake and fatty acid β-oxidation. As a result, ombuine reduced lipid levels in macrophages and hepatocyte cells. The number of patients with hyperlipidemia and hepatic steatosis is increasing in developed societies; ombuin-3-*O*-β-D-glucopyranoside may be useful in the treatment of these individuals.

Acknowledgments

This study was supported by the Korean Forest Service (Forest Science & Technology Project No. S120909L130110), the Technology Development Program for Fisheries of the Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (iPET, F20926409H220000110), and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20100028180). The SPR instrument was provided by the Korea Basic Science Institute. We thank Hea-Won Kim for technical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.12.020.

References

[1] J.D. Brunzell, J.E. Hokanson, Dyslipidemia of central obesity and insulin resistance, Diabetes Care 22 (Suppl. 3) (1999) C10–C13.
[2] D. Steinberg, Atherogenesis in perspective: hypercholesterolemia and inflammation and inflammation as partners in crime, Nat. Med. 8 (2002) 1211–1217.
[3] J.K. Saul, Nuclear receptor regulation of hepatic function, J. Hepatol. 36 (2002) 832–850.
[4] K. Schoonjans, B. Staels, J. Auwerx, The peroxisome proliferator activated receptors (PPARs) and their effect on lipid metabolism and adipocyte differentiation, Biochim. Biophys. Acta 1302 (1996) 93–109.
[5] G.D. Barish, V.A. Narkar, R.M. Evans, PPAR delta: a dagger in the heart of the metabolic syndrome, J. Clin. Invest. 116 (3) (2006) 590–597.
[6] J.J. Repa, D.J. Mangelsdorf, The role of orphan nuclear receptors in the regulation of cholesterol homeostasis, Annu. Rev. Cell Dev. Biol. 16 (2000) 459–481.
[7] G. Chinetti, S. Lestavel, V. Bocher, A.T. Remaley, B. Neve, I.P. Torra, E. Teissier, A. Minnich, M. Jaye, N. Duverger, H.B. Brewer, J.C. Fruchart, V. Clavey, B. Staels, PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway, Nat. Med. 7 (1) (2001) 53–58.
[8] V. Razmovski-Naumovsk, T.H. Huang, V.H. Tran, G.Q. Li, C.C. Duke, B.D. Roufogalis, Chemistry and Pharmacology of *Gynostemma pentaphyllum*, Phytochem. Rev. 4 (2005) 197–219.
[9] T.C. Cheng, J.F. Lu, J.S. Wang, L.J. Lin, H.I. Kuo, B.H. Chen, Antiproliferation effect and apoptosis mechanism of prostate cancer cell PC-3 by flavonoids and saponins prepared from *Gynostemma pentaphyllum*, J. Agric. Food Chem. 59 (2011) 11319–11329.
[10] J. Yeo, Y.J. Kang, S.M. Jeon, U.J. Jung, M.K. Lee, H. Song, M.S. Choi, Potential hypoglycemic effect of an ethanol extract of *Gynostemma pentaphyllum* in C57BL/KsJ-db/db mice, J. Med. Food. 11 (2008) 709–716.
[11] A. Norberg, N.K. Hoa, E. Liepinsh, D. Van Phan, N.D. Thuan, H. Jornvall, R. Sillard, C.G. Ostenson, A novel insulin-releasing substance, phanoside, from the plant *Gynostemma pentaphyllum*, J. Biol. Chem. 279 (2004) 41361–41367.

[12] T.H. Huang, V.H. Tran, B.D. Roufogalis, Y. Li, Gypenoside XLIX, a naturally occurring PPAR-alpha activator, inhibits cytokine-induced vascular cell adhesion molecule-1 expression and activity in human endothelial cells, Eur. J. Pharmacol. 565 (2007) 158–165.
[13] J. Si, D. Chen, Q. Chang, L. Shen, Z. Zhu, Isolation and identification of flavonoids from *Gynostemma yixingense*, Acta Botan. Sin. 36 (1994) 239–243.
[14] Y. Jia, J.Y. Kim, H.J. Jun, S.J. Kim, J.H. Lee, M.H. Hoang, K.Y. Hwang, S.J. Um, H.I. Chang, S.J. Lee, The natural carotenoid astaxanthin, a PPAR-α agonist and PPAR-γ antagonist, reduces hepatic lipid accumulation by rewiring the transcriptome in lipid-loaded hepatocytes, Mol. Nutr. Food Res. 56 (2012) 878–888.
[15] M.H. Hoang, Y. Jia, H.J. Jun, J.H. Lee, D.H. Lee, B.Y. Hwang, W.J. Kim, H.J. Lee, S.J. Lee, Ethyl 2,4,6-trihydroxybenzoate is an agonistic ligand for liver X receptor that induces cholesterol efflux from macrophages without affecting lipid accumulation in HepG2 cells, Bioorg. Med. Chem. Lett. 22 (2012) 4094–4099.
[16] M. Ogata, M. Tsujita, M.A. Hossaina, N. Akita, F.J. Gonzalez, B. Staels, S. Suzuki, T. Fukutomi, G. Kimura, S. Yokoyama, On the mechanism for PPAR agonists to enhance ABCA1 gene expression, Atherosclerosis 205 (2009) 413–419.
[17] A. Grefhorst, B.M. Elzinga, P.J. Voshol, T. Plösch, T. Kok, V.W. Bloks, F.H. van der Sluijs, L.M. Havekes, J.A. Romijn, H.J. Verkade, F. Kuipers, Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low density lipoprotein particles, J. Biol. Chem. 27 (37) (2002) 34182–34190.
[18] A. Peter, C. Weigert, H. Staiger, F. Machicao, F. Schick, J. Machann, N. Stefan, C. Thamer, H.U. Haring, E. Schleicher, Individual stearoyl-CoA desaturase 1 expression modulates endoplasmic reticulum stress and inflammation in human myotubes and is associated with skeletal muscle lipid storage and insulin sensitivity *in vivo*, Diabetes 58 (2009) 1757–1765.
[19] Z.-L. Ai, C.-H. Zhu, M. Min, J. Wang, C.-H. Lan, L.-L. Fan, W.-J. Sun, D.-F. Chen, The role of hepatic liver X receptor and sterol regulatory element binding protein-1c-mediated lipid disorder in the pathogenesis of non-alcoholic steatohepatitis in rats, J. Int. Med. Res. 39 (2011) 1219–1229.
[20] H. Choi, H. Eo, K. Park, M. Jin, E-J. Park, S-H. Kim, J.E. Park, S. Kim, A water-soluble extract from *Cucurbita moschata* shows anti-obesity effects by controlling lipid metabolism in a high fat diet-induced obesity mouse model, Biochem. Biophys. Res. Commun. 359 (2007) 419–425.
[21] T. Yoshikawa, T. Ide, H. Shimano, N. Yahagi, M.A. Kudo, T. Matsuzaka, S. Yatoh, T. Kitamine, H. Okazaki, Y. Tamura, et al., Cross-talk between peroxisome proliferator-activated receptor (PPAR) α and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. I. PPARs suppress sterol regulatory element binding protein-1c promoter through inhibition of LXR signaling, Mol. Endocrinol. 17 (2003) 1240–1254.
[22] L.R. Brunham, J.K. Kruit, T.D. Pape, J.S. Parks, F. Kuipers, M.R. Hayden, Tissue-specific induction of intestinal ABCA1 expression with a liver X receptor agonist raises plasma HDL cholesterol levels, Circ. Res. 99 (2006) 672–674.
[23] D.H. van Raalte, M. Li, P.H. Pritchard, K.M. Wasan, Peroxisome proliferator-activated receptor (PPAR) α: a pharmacological target with a promising future, Pharma. Res. 21 (2004) 1531–1538.
[24] R. Hertz, J. Bishara-Shieban, J. Bar-Tana, Mode of action of peroxisome proliferators as hypolipidemic drugs. Suppression of apolipoprotein C-III, J. Biol. Chem. 270 (1995) 13470–13475.