Modulation of cytosolic phospholipase A_2 by PPAR activators in human preadipocytes

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Abstract **Cytosolic phospholipase A_2 ($cPLA_2$) is responsible for the release of arachidonic acid, a precursor for eicosanoid biosynthesis, from cellular phospholipids. The objective of this study is to examine the regulation of $cPLA_2$ by peroxisome proliferator-activated receptor (PPAR) activators in preadipocyte SW872 (SW) cells. PPAR belong to the superfamily of nuclear hormone receptors that heterodimerize with the retinoid X receptor. In this study, the presence of both PPARα and PPARγ was confirmed in SW cells by positive identification of their mRNA in the cellular homogenate. Clofibrate, a PPARα activator, caused an enhancement of ionophore A-23187-induced arachidonate release in SW cells. This increase resulted from an enhancement of $cPLA_2$ activity, which was caused by an increase in enzyme protein. Clofibrate at lower concentrations (10–200 μM) produced increases in the mRNA levels of $cPLA_2$ in a dose-response manner. At higher concentrations (>400 μM), clofibrate treatment resulted in the attenuation of the $cPLA_2$ mRNA level and protein expression. We postulate that clofibrate, acting through the PPARα, caused an induction in the transcription of $cPLA_2$ gene, which led to an increase in the $cPLA_2$ protein. The observed increase in arachidonate release in SW cells appeared to be a direct result of the enhanced $cPLA_2$ activity.**—Jiang, Y. J., G. M. Hatch, D. Mymin, T. Dembinski, E. A. Kroeger, and P. C. Choy. **Modulation of cytosolic phospholipase A_2 by PPAR activators in human preadipocytes.** ***J. Lipid Res.*** **2001.** 42: **716–724.**

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Phospholipase A_2 (PLA_2) (EC 3.1.1.4) belongs to a group of enzymes that catalyzes the hydrolysis of the phospholipids at the *sn*-2 position. This group of enzymes plays a central role in a variety of cellular processes, and the products of the reaction are involved in host defense and signal transduction (1, 2). PLA_2 are classified into several major types according to their primary structure, subcellular localization, calcium requirement, and substrate specificity (reviewed in 3–6). In mammalian cells, the 14-kDa secretory PLA_2 (type II) (7) and the 85–110-kDa cytosolic PLA_2 (type IV) (8–10) are the prominent types. The cytosolic PLA_2 ($cPLA_2$) is different from other PLA_2 types in that it is an intracellular enzyme with high molecular weight and has a high degree of specificity for the arachidonoyl group. The arachidonic acid released by the action of PLA_2 may be converted into eicosanoids via the cyclooxygenase (COX) pathway, leading to the synthesis of prostaglandins and thromboxanes (11, 12). In view of its importance in lipid homeostasis, the activity of $cPLA_2$ is regulated via several tiers of control. The post-translational modification is responsible for the rapid activation of $cPLA_2$ in response to a variety of physiological stimuli, including the calcium-dependent translocation to the membrane fraction via the CaLB domain (13, 14). Alternatively, the enzyme activity is enhanced by phosphorylation, a process mediated by mitogen-activated protein (MAP) kinase, as well as by the indirect activation by protein kinase C and G-protein-coupled receptors (15). For prolonged responses, the expression of $cPLA_2$ is regulated at the transcriptional level by mediators such as cytokines and growth factors, including interferon-gamma (16), macrophage colony stimulating factor (17), tumor necrosis factor-alpha (18, 19), epidermal growth factor (20), and glucocorticoid (21). The regulation of $cPLA_2$ activity by peroxisome proliferator-activated receptors (PPAR), however, has not been fully explored.

PPAR belong to a subgroup of the nuclear receptor superfamily of transcription factors. The functional DNA binding of these factors would require the ligand-induced heterodimerization with retinoid X receptor (22). Three different types of PPAR, α, γ, and δ, have been identified. They are encoded from separate genes, have distinct tissue distribution, and carry out different functions. The PPAR are activated by a variety of fatty acids and arachidonic acid metabolites, as well as by hypolipidemic agents such as clofibrate (23). Although the direct effect of PPAR in phospholipid catabolism has not been demonstrated,

Abbreviations: COX, cyclooxygenase; $cPLA_2$, cytosolic phospholipase A_2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PPAR, peroxisome proliferator-activated receptor; SW cells, human preadipocyte SW872 cells.

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the activation of PPARα has been shown to affect lipid metabolism in liver, intestine, and kidney (22, 24). For example, the activation of PPARα causes an enhancement in lipid oxidation, an alteration in lipoprotein metabolism, and an inhibition of vascular inflammation (22, 24, 25). In addition, the activation of PPARγ has been implicated as a mediator for the differentiation of preadipocytes into adipocytes and provides a mechanism for thiazolidinedione to exert its effect during in vivo insulin sensitization (26). Recently, PPARγ has been shown to be associated with the inhibition of human colorectal tumorigenesis mediated by nonsteroid anti-inflammatory drugs (27). Because preadipocytes contain both lipogenesis and lipolysis gene sets, they serve as a good model to study the modulation of $cPLA_2$ expression on PPAR ligand stimulation.

We reported previously that lysophosphatidylcholine caused an elevation of cellular Ca^{2+} and the activation of protein kinase C, which resulted in an increased release of arachidonate in endothelial cells (28) and in H9c2 cells (29). In the current study, the regulation of $cPLA_2$ for the release of arachidonic acid by PPARα was investigated. Clofibrate, an activator of PPARα, was found to up-regulate the expression of $cPLA_2$ and COX-2 in preadipocytes.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), Ham's F12 nutrition mixture (F12), phosphate-buffered saline, and clofibrate were obtained from Sigma Chemical Company (St. Louis, MO). Heat-inactivated newborn calf serum, TRIZOL Reagent, Moloney murine leukemia virus reverse transciptase, *Taq* DNA polymerase, gentamycin, and trypsin were purchased from Life Technologies, Inc. Ribonuclease inhibitor and deoxynucleotides were obtained from Promega Inc. (Madison, WI). Both the $cPLA_2$ polyclonal antibody and the $cPLA_2$ protein standard were generous gifts from the Genetics Institute (Boston, MA). Polyclonal (rabbit) anti-PPARα antibody was purchased from Affinity BioReagents, Inc. (Golden, CO). 4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1) was purchased from Roche Inc. (Quebec, Canada). 5-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (PGJ_2,) was obtained from Cayman Chemical Co. (Ann Arbor, MI). Calcium ionophore A-23187 was obtained from Biomol Inc. (Plymouth Meeting, PA). [5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid (209.6 Ci/mmol), 1-stearoyl-2-[1-^{14}C]arachidonyl-*sn*-glycerol-3-phosphocholine (55 mCi/mmol) were obtained from Amersham Corp. All lipid standards were purchased from Serdary Research Laboratory (Ontario, Canada). Thin-layer chromatography (TLC) plates (silica gel G) were obtained from Fisher Scientific.

Cell culture and radiolabeling

Preadipocyte SW 872 (SW) cells, derived from human liposarcoma, were acquired from American Type Culture Collection. These cells were cultured at 37°C with 5% CO_2 and 95% air in DMEM/F12 nutrition mixture at a ratio of 3:1 and supplemented with 5% heat-inactivated fetal bovine serum (FBS), 50-μg/ml gentamycin, and 20 mM HEPES. Cells were allowed to reach 30% confluence in a monolayer and were then incubated in a medium containing either clofibrate, 5-deoxy-$\Delta^{12,14}$-PGJ_2, or vehicle alone (<0.05% dimethyl sulfoxide, <0.05% ethanol) for the prescribed amount of time. The medium was changed every 48 h until the end of the experiment.

Subsequent to ligand treatment, cells were maintained in the DMEM/F12 medium (3:1) for the prescribed period of time. After three washes with HEPES-buffered saline (HBS), cells were incubated for 16 h in a DMEM/F12 medium containing 5% FBS, various concentrations of ligand, and 0.88–1.0 μCi/ml [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (209.6 Ci/mmol) (30). HBS contained 140 mM NaCl, 4 mM KCl, 5.5 mM glucose, 10 mM HEPES, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 0.025% (w/v) bovine serum albumin (BSA), with a final pH of 7.4.

Determination of arachidonate release

The arachidonate released from the cells was determined as described previously (28). At the end of the labeling with [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid, cells were rinsed three times with HBS containing 0.025% BSA to remove any nonspecific binding of arachidonic acid to the cell surface. Cells were then challenged with 10 μM calcium ionophore A-23187 for 10 min. The labeled arachidonate released into the medium was determined by scintillation counting. The viability of cells under this treatment was examined by Trypan blue exclusion. Our result indicates that agonist stimulation had no deteriorating effect on the viability of these cells. Additional cell viability assays were performed with the WST-1 assay.

Electrophoresis and Western blot analyses

Samples containing 50 μg of protein were subjected to sodium dodecylsulfate/7.5% polyacrylamide gel electrophoresis (SDS-PAGE) with $cPLA_2$ protein standard (15–20 ng) as a reference. Protein fractions in the gel were transferred to the hybond nitrocellulose membrane. The membrane was incubated with the polyclonal anti-$cPLA_2$ antibodies (1:1,500–2,000) dissolved in tris(hydroxymethyl)aminomethane (Tris)-buffered saline containing 0.1% Tween-20 and 2% skim milk overnight at 4°C. Subsequently, the membrane was washed and incubated with the peroxidase-labeled anti-rabbit secondary antibodies (1:3,000) for 30 min at room temperature. Protein bands in the membrane were visualized by enhanced chemiluminescence. The relative intensities of the bands were analyzed by scanning the film and subsequently determined by Scion Image software.

For the detection of PPARα in SW cells, 30–80 μg protein samples were used. Following the transfer, the membrane containing protein samples was incubated with polyclonal anti-PPARα antibodies (1:500–1,000). The membrane was then washed and incubated with the peroxidase-labeled anti-rabbit secondary antibodies (1:2,000) for 30 min at room temperature.

Determination of phospholipase A_2 activity

After clofibrate (10–400 μM) treatment, SW cells were lysed by sonication in a buffer containing 50 mM Tris-HCl (pH 8.0), 1 mM ethylenediaminetetraacetic acid (EDTA), 10 μM leupetin, 10 μM aprotinin, 20 mM NaF, 10 mM Na_2HPO_4, and 1 mM dithiothreitol. The cell lysate was centrifuged at 100,000 *g* for 1 h. The supernatant was designated as the cytosolic fraction, and the pellet was designated as the membrane fraction. PLA_2 activity in the subcellular fractions was determined by the hydrolysis of 1-stearoyl-2-[1-^{14}C]arachidonyl-*sn*-glycerol-3-phosphocholine to yield the labeled arachidonate. The assay mixture contained 50 mM Tris-HCl (pH 8.0), 3.0 mM $CaCl_2$, 0.9 nmol of 1-stearoyl-2-[1-^{14}C]arachidonyl-*sn*-glycerol-3-phosphocholine (100,000 dpm/assay), and 10-μg protein in a final volume of 100 μl. The mixture was incubated for 30 min at 37°C, and the reaction was terminated by the addition of 1.5 ml of chloroform/methanol (2:1, v/v). Oleic acid was added as a fatty acid carrier. The fatty acid

was extracted and separated by TLC in a solvent system containing hexane/diethyl ether/acetic acid (70:30:1, v/v/v). The fatty acid fraction was visualized by exposure to iodine vapor, and the radioactivity of the arachidonic acid released was determined by liquid scintillation counting.

Isolation of total RNA

Total RNA was isolated using the TRIZOL Reagent according to the manufacturer's instruction. Briefly, cells were lysed with 1 ml of TRIZOL Reagent in each 100-mm dish, extracted with phenol-chloroform, and precipitated with ethanol. The RNA pellet was suspended in autoclaved, double-distilled water (31) and quantified by absorbance at 260 nm, using the 260:280 nm ratio as an index of purity. The integrity of the RNA was confirmed by denaturing agarose gel electrophoresis on the isolated RNA sample.

Polymerase chain reaction primers and reverse transcriptase polymerase chain reactions

The cDNA for $cPLA_2$, COX-1, COX-2, acyl-CoA oxidase (ACO), PPARα, or PPARγ was amplified with a pair of specific primers synthesized by Gibco Life Technology, Inc. The sequence of primers and the length of predicted polymerase chain reaction (PCR) products are listed in **Table 1**.

The first-strand cDNA from 1-μg total RNA was synthesized by employing 150 U of moloney murine leukemia virus reverse transcriptase (RT), 25 pmol of random hexamer primer, 20 U of ribonuclease inhibitor, 1 mM dithiothreitol, and 10 pmol each of the four deoxynucleotides, in a total volume of 15 μl (32). The reaction mixture was incubated at 37°C for 1 h and terminated by boiling the sample at 95°C for 5 min. An aliquot of 1.0–1.2 μl of the resultant cDNA preparation was used directly for each amplification reaction.

PCR was performed in 20-μl reaction mixtures containing 8 pmol of each primer, 8 pmol of each dNTP, and 0.4-U *Taq* DNA polymerase. The mixture was overlaid with 30-μl mineral oil to prevent evaporation and was incubated in a Perkin-Elmer DNA Thermal Cycler under the following conditions. For the PCR of glyceraldehyde 3-phosphate dehydrohenase (GAPDH), PPARα or PPARγ: 25–30 cycles, denaturation, 30 s at 94°C; annealing, 30 s at 55–60°C; extension, 30 s at 72°C. For the PCR of $cPLA_2$, COX-1 or COX-2: 25–32 cycles, denaturation, 60 s at 94°C; annealing, 60 s at 55–60°C; extension, 2 min at 72°C. The amplified RT-PCR product was analyzed by 1.2–1.8% agarose gels electrophoresis in 1× TAE buffer (40 mM Tris acetate, 2 mM sodium EDTA) and visualized by staining with 0.5-μg/ml ethidium bromide. The GAPDH band was used as an internal control.

An increase in the level of a specific mRNA is caused by either an increase in its rate of synthesis, a decrease in its rate of degradation, or a combination of these two processes. Hence, mRNA stability assays were conducted using actinomycin D as an inhibitor for RNA synthesis. SW cells were treated with clofibrate (50–200 μM) for 48 h, and actinomycin D (5 μg/ml) was added after clofibrate treatment. The mRNA levels for $cPLA_2$ and GAPDH were determined by RT-PCR at 4-h intervals following the actinomycin D treatment. No apparent changes in mRNA degradation were observed within a 24-h period, indicating that clofibrate did not cause any change in the degradation of $cPLA_2$ mRNA.

SYBR Green I real-time PCR analysis

Real-time quantitative PCR was performed using a fluorescence temperature cycler (LightCycler; Roche Molecular Biochemicals, Mannheim, Germany). The primers for amplification of $cPLA_2$ cDNA were the same as previously described. Amplification was done according to the manufacturer's general guidelines after optimization of the various reaction parameters. The reaction mixture (20 μl) was placed in a glass capillary and contained 2 μl of LightCycler-DNA Master SYBR Green I mix (Taq DNA polymerase, 10× PCR buffer, deoxynucleotide triphosphate mix, and SYBR Green I dye), 3 mM $MgCl_2$, and 0.4 pmol of each primer. PCR-grade H_2O instead of cDNA was used as a negative control.

The amplification program included the initial denaturation step at 95°C for 30 s. It was followed by 55 cycles of denaturation at 95°C for 0 s, annealing at 55°C for 10 s, and extension at 72°C for 20 s. The temperature transition rate was 20°C/s. Fluorescence was determined at the end of each extension step. After amplification, a melting curve was acquired by heating the product at 20°C/s to 95°C, cooling it at 20°C/s to 60°C, keeping it at 60°C for 30 s, and then slowly heating it at 0.2°C/s to 97°C. Fluorescence was determined throughout the slow heating phase. Melting curves were used to determine the specific PCR products, which were further confirmed using conventional gel electrophoresis. Absolute copy numbers of the target transcripts per ng of transcribed total RNA were determined with cloned plasmid DNA for $cPLA_2$.

Sequence analysis of cDNA

The identity of each PCR product generated from RT-PCR was verified and confirmed by direct DNA sequencing. The gel

TABLE 1. Polymerase chain reaction primers

Name	Sequence	Start Site	Product Length (base pairs)
GAPDH (U)	5′-ACC CAC TCC TCC ACC TTTG-3′		176
GAPDH (L)	5′-CTC TTG TGC TCT TGC TGG G-3′		
$cPLA_2$ (U)	5′-AAA GAA CAC TAT AGG GAG AG-3′	609′– 628′	502
$cPLA_2$ (L)	5′-AAA GAG GTA AAG GGC ATT GT-3′	1072′–1091′	
COX-1 (U)	5′-TGC CCA GCT CCT GGC CCG CCG CTT-3′	174′–198′	306
COX-1 (L)	5′-GTG CAT CAA CAC AGG CGC CTC TTC-3′	456′–480′	
COX-2 (U)	5′-TTC AAA TGA GAT TGT GGG AAA ATT GCT-3′	2613′–2640′	307
COX-2 (L)	5′-AGA TCA TCT CTG CCT GAG TAT CTT-3′	3746′–3770′	
PPARα (U)	5′-AAG TGC CTT TCT GTC GGG ATG-3′	601′–622′	433
PPARα (L)	5′-CGT TCA GGT CCA AGT TTG CG-3′	1014′–1034′	
PPARγ (U)	5′-CAG AAA TGC CTT GCA GTG GG-3′	276′–286′	289
PPARγ (L)	5′-GGG GGT GAT GTG TTT GAA CTT G-3′	581′–602′	
ACO (U)	5′-TTA CAC ACA TCC TGG ACG GCA G-3′	108′–130′	762
ACO (L)	5′-GCT TGT TAC TCA GCG GTT TCA CG-3′	874′–897′	

Abbreviation: ACO, acyl-CoA oxidase.

fraction containing the target DNA, including COX-1, COX-2, $cPLA_2$, PPARα, or PPARγ, was excised and submitted for sequencing analysis. The sequence analysis was performed using the Perkin-Elmer Applied Biosystems ABI 310 Genetic Analyzer (Institute of Cell Biology, University of Manitoba, Winnipeg, Canada). The sequences obtained were used for homologous search by the Basic Local Alignment Search Tool (BLAST; NIH, Bethesda, MD). The purified $cPLA_2$ fragment was subcloned into One Shot TOPO10 competent cells (Invitrogen Corp., Carlsbad, CA), and the $cPLA_2$ plasmid was used to generate a standard curve for real-time PCR analysis.

Statistical analysis

The data were analyzed with a two-tailed independent Student's *t*-test. In all cases, the level of significance was defined as $P < 0.05$. Results are presented as the mean ± standard deviation.

RESULTS

The effect of clofibrate on A-23187-induced arachidonic acid release in SW cells

SW cells were cultured in DMEM/F12 (3:1) medium containing 5% FBS and 50–200 μM clofibrate for either 8 or 32 h. [^{3}H]arachidonate (0.88 μCi/ml) was added to the incubating mixture, and the cells were incubated for another 16 h. Under such experimental conditions, SW cells were exposed to clofibrate for a total of 24 or 48 h. Initial experiments revealed that incubation with arachidonate for more than 16 h would cause the breakdown of the labeled arachidonic acid and/or result in chain elongation of arachidonate into adrenate (33) and that the formation of adrenate would inhibit the agonist-induced release of arachidonate from phospholipids (33). Under the current experimental conditions, about 33% of the labeled arachidonic acid was taken up by the cells, and the majority of the label was associated with phosphatidylcholine. No morphological change in the shape or size of cells was apparent during the course of treatment. In a control study, the preincubation of clofibrate for 0, 8, or 32 h did not significantly alter the total arachidonic acid uptake. Subsequent to labeling, the cells were challenged with calcium ionophore A-23187 (10 μM) (34) for 10 min, and the arachidonate released into the medium was determined. An increase in arachidonate release (40%) was observed at 24 h of incubation with 200 μM clofibrate, whereas a higher increase (50%) was obtained at 48 h of incubation. In a dose-response study, a 34% increase in arachidonate release was observed when cells were treated with 50 μM of clofibrate for 48 h. Maximum increase in arachidonate release (50%) was obtained when the cells were treated with 200 μM of clofibrate under the same conditions (**Table 2**). Our results indicate that clofibrate treatment caused the enhancement of arachidonic acid release in human preadipocytes.

TABLE 2. The effect of clofibrate on A-23187-induced arachidonic acid release in SW 872 preadipocytes

Clofibrate Treatment	Arachidonate Release
μM	*dpm/dish × 10^{-4}*
0	7.22 ± 0.392
50	9.64 ± 0.941[a]
200	10.8 ± 0.413[a]

Cells were incubated with clofibrate for 32 h prior to the labeling with 0.88-μCi/ml [^{3}H]arachidonic acid for another 16 h. Subsequent to labeling, cells were rinsed and challenged with 10 μM A-23187 for 10 min. The amount of labeled arachidonate released into the medium was determined. Results are expressed as the mean ± standard deviation from three independent sets of experiments, each carried out in triplicate. [a] $P < 0.05$ when compared with control.

The effect of clofibrate on cPLA$_2$ activity

Because clofibrate caused an increase in arachidonic acid release, it would be of interest to examine whether the increase was associated with a change in $cPLA_2$ activity. Hence, the activity of the enzyme was determined after 24 and 48 h of clofibrate (200 μM) treatment. The $cPLA_2$ activity was found to increase in the cell homogenate after cells were treated with clofibrate for 24 h (110%) and reached a maximum (240%) at 48 h of incubation. The activity of the phospholipase A_2 was not significantly changed when assayed in the presence of 1 mM of dithiothreitol, suggesting that there was not a significant amount of secretory phospholipase A_2 in the homogenate. Despite the increase in $cPLA_2$ activity induced by clofibrate, the ratio of enzyme activity remained constant between the cytosolic and membrane fractions, suggesting that no significant translocation of enzyme occurred during clofibrate treatment. In a dose-response study, cells were incubated with 50–400 μM of clofibrate for 48 h (**Fig. 1**). Maximum activation of the $cPLA_2$ activity was obtained at 200 μM clofibrate, but the enzyme activity was attenuated from its maximal value when cells were incubated with 400 μM clofibrate.

The effect of clofibrate on the protein level of cPLA$_2$

The enhancement of the $cPLA_2$ activity might be caused by an increase in the enzyme protein level. Hence, the level of $cPLA_2$ protein in SW cells after clofibrate treatment was determined by Western blot analysis. The change

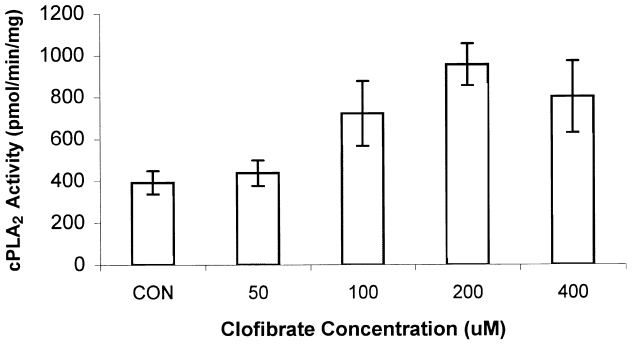

Fig. 1. The effect of clofibrate on $cPLA_2$ activity in human preadipocytes. SW cells were incubated in Dulbecco's modified Eagle's medium/Ham's F12 nutrition mixture (DMEM/F12) (3:1) containing 5% fetal bovine serum with increasing concentrations of clofibrate for 48 h. Cells were lysed, and cPLA2 activity was assayed in the total homogenate. Results are means ± standard deviation of three separate sets of experiments. * $P < 0.05$. CON: vehicle control.

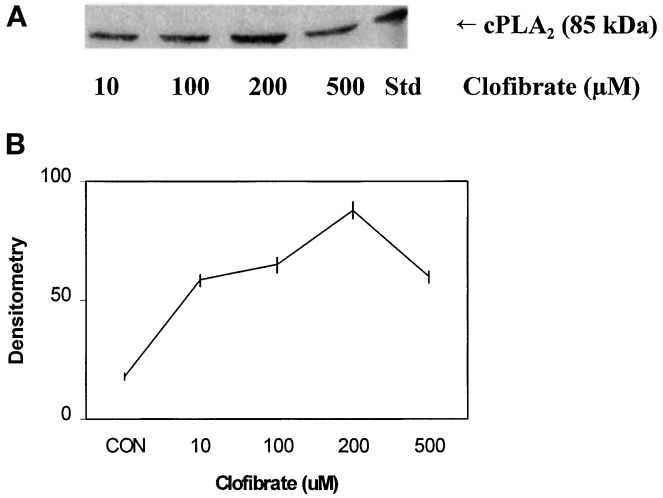

Fig. 2. The effect of clofibrate on the protein level of $cPLA_2$. SW cells were incubated in DMEM/F12 (3:1) with increasing concentrations of clofibrate as described in Fig. 1. Western blot analyses of $cPLA_2$ protein were performed using a rabbit polyclonal $cPLA_2$ antibodies. A: A representative blot is depicted. Std: $cPLA_2$ standard. B: Densitometry plot of A.

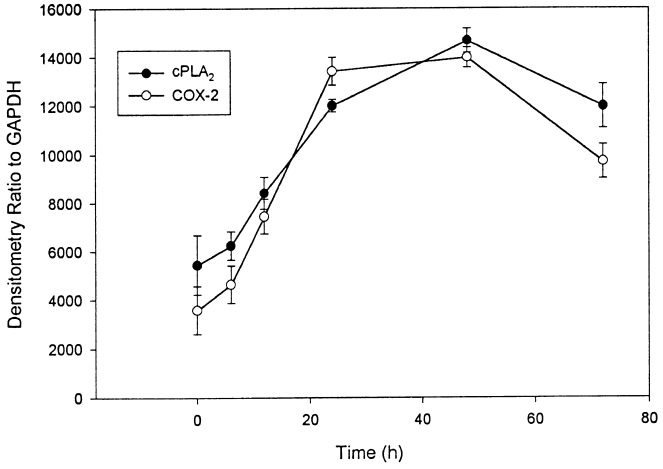

Fig. 3. Induction of $cPLA_2$ and COX-2 mRNA by clofibrate over time. Preconfluent SW cells were incubated up to 72 h in DMEM/F12 medium containing 200 μM clofibrate. The total RNA was isolated, and reverse transcriptase polymerase chain reaction (RT-PCR) on the $cPLA_2$ and COX-2 mRNA was performed. Results are means ± standard deviation of three separate experiments.

in enzyme protein corresponded to the change in enzyme activity at 24 and 48 h of clofibrate treatment. Incubation of cells with increasing concentrations of clofibrate (up to 200 μM) for 48 h resulted in progressive increases in $cPLA_2$ protein levels (**Fig. 2**). Similar to the enzyme activity study, the enzyme protein level was attenuated from its maximal value when cells were incubated with 500 μM clofibrate.

Up-regulation of $cPLA_2$ and COX-2 mRNA by clofibrate

The role of clofibrate in the expression of $cPLA_2$ mRNA was examined. Cells were treated with 200 μM clofibrate for 0–72 h, the mRNA for $cPLA_2$ and GAPDH were amplified by RT-PCR, and the PCR products were analyzed by agarose gel electrophoresis. The relative intensity of each band in the agarose gel was quantified by scanning and then normalized by the intensity of GAPDH band. As depicted in **Fig. 3**, the maximum $cPLA_2$ mRNA level was observed at 48 h of incubation. In a dose-response study, cells were incubated with 0–1000 μM clofibrate for 48 h, and the $cPLA_2$ mRNA level was determined. The $cPLA_2$ mRNA level was elevated by a broad range of clofibrate concentrations (100–1000 μM), with the maximal effect obtained at 200 μM (**Fig. 4**). The $cPLA_2$ mRNA level was attenuated from its maximal value when the cells were incubated with 500 or 1,000 μM clofibrate.

To analyze the increase of $cPLA_2$ mRNA level by clofibrate treatment in a more quantitative manner, the SYBR Green I real-time PCR was performed. The $cPLA_2$ plasmid was serially diluted, and real-time data were plotted with template copy numbers versus threshold cycle values. The linear relationship was obtained when the amplification was plotted against the initial template concentration, and all samples tested were located within the linear range. The results obtained from the real-time PCR study for $cPLA_2$ expression were similar to those obtained from the PCR study described in the preceding section (data not shown).

To confirm that the action of clofibrate was mediated via PPARα, the mRNA level of a known PPARα target gene, ACO, was also studied. As predicted, clofibrate (10–500 μM) caused the increase in the gene expression of ACO in SW cells (Fig. 4D). The effect of clofibrate on ACO, however, was less prominent than that obtained from $cPLA_2$ or COX-2. The effect of other PPARα ligands, such as WY14643 on the $cPLA_2$ mRNA level, was also studied. As depicted in **Fig. 5**, the $cPLA_2$ mRNA level was elevated when cells were incubated with 10 μM of WY14643 for 48 h.

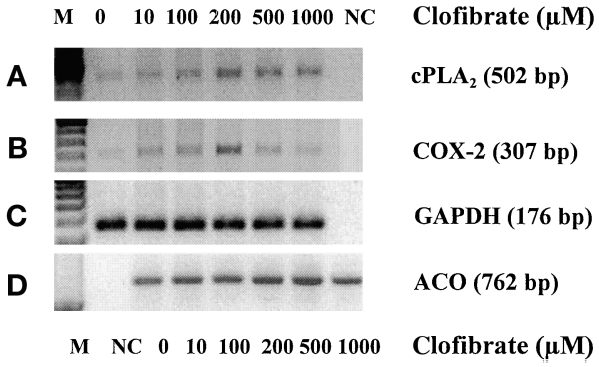

Fig. 4. The effect of clofibrate on $cPLA_2$, COX-2, and acyl-CoA oxidase (ACO) mRNA levels. SW cells were incubated in DMEM/F12 with increasing concentrations of clofibrate (10–1,000 μM) for 48 h. Total RNA was isolated, and RT-PCR on $cPLA_2$, COX-2, and ACO mRNA was performed. Results are means ± standard deviation of three separate experiments. A typical gel analysis is depicted. M: molecular marker. NC: negative control.

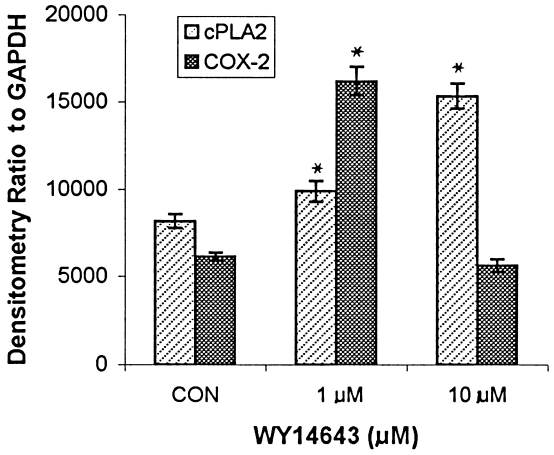

Fig. 5. The effect of WY14643 on the $cPLA_2$ and COX-2 mRNA levels. Preconfluent SW cells were incubated for 48 h in DMEM/F12 medium containing 1–10 μM WY14643. RT-PCR was performed as described in Materials and Methods. Results are means ± standard deviation of three independent experiments. * $P < 0.05$.

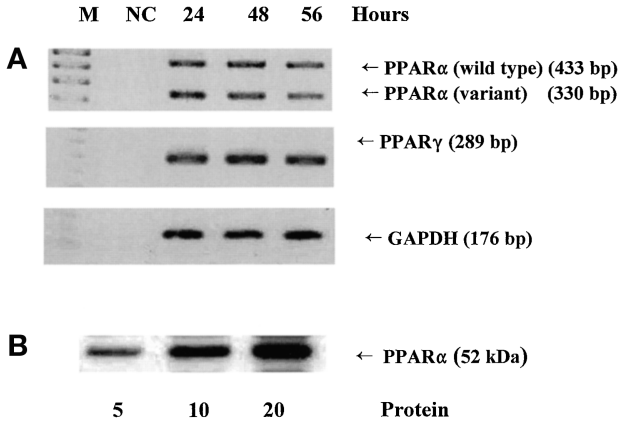

Fig. 6. Expression of mRNA and protein for PPAR in cultured human preadipocytes. A: The total RNA was isolated from cultured SW cells, and the mRNAs for PPARα and PPARγ were analyzed by RT-PCR. A typical gel analysis is depicted. The expression of PPARα mRNA with a wild-type fragment (433 bp), a variant fragment (330 bp), and that of PPARγ mRNA with a 289-bp fragment are shown. M: molecular marker. NC: negative control. B: Western blot analysis of PPARα expression. SW cells were grown in DEME/F12 containing 5% FBS. The expression of PPARα in the whole cell lysate was examined by using polyclonal anti-PPARα antibodies (1:500 dilution).

COX-1 and -2 are integral membrane proteins. COX-1 is responsible mainly for the biosynthesis of prostaglandins for homeostatic or "housekeeping" regulation (35), whereas COX-2 produces prostaglandins that act through nucleoplasmic targets in association with cell differentiation and replication through the nuclear receptor. Fibrates have been shown to regulate the gene expression of COX-2 via its interaction with PPAR (36). A functional coupling and differential regulation of $cPLA_2$ and COX-2 in inflammation has recently been demonstrated (36). In addition, $cPLA_2$ is functionally coupled with COX-2 to involve in both the immediate and delayed PG-biosynthetic responses (37, 38). Hence, the effect of clofibrate on the COX gene expression in SW cells was also examined. As predicted, clofibrate exhibited an effect on COX-2 similar to that observed in $cPLA_2$. Specifically, clofibrate elicited a ~2.0- to 3.8-fold increase in the COX-2 mRNA level when compared with the control (Fig. 4). The effect of clofibrate on COX-2 was found to be concentration dependent, and the maximum effect was obtained at 200 μM. The enhancement of the COX-2 mRNA was also found to be attenuated from the maximal level when 500 μM of clofibrate was used. WY14643, another PPARα ligand, showed a similar effect on COX-2 gene expression when treating cells at the 1–10 μM range (Fig. 5). Taken together, these results demonstrate that clofibrate has the ability to up-regulate mRNA levels of both COX-2 and $cPLA_2$, suggesting that there is a coordinated regulation of both enzymes upon stimulation in SW cells. No change in COX-1 mRNA level was observed in SW cells treated with clofibrate (data not shown), which is consistent with the postulation that COX-1 functions as a housekeeping enzyme.

In another set of experiments, cells were treated with PGJ_2, which is a known PPARγ ligand. Cells were incubated with PGJ_2 (1–10 μM) for 48 h, and the $cPLA_2$ activity, enzyme protein, and $cPLA_2$ mRNA level were determined. The treatment of cells with 1–3 μM PGJ_2 did not elicit any significant effect on $cPLA_2$ activity, changes in enzyme protein, or mRNA level (data not shown). When cells were treated with 5 μM of PGJ_2 under the same conditions, only 50% of the cells survived after the incubated period. Incubation with 10 μM of PGJ_2 caused the complete death of all cells within 24 h.

The expression of PPARα and PPARγ in cultured SW cells

The action of fibrates on the regulation of transcription of lipid metabolic enzymes is mediated via the PPAR. Specifically, the interaction between clofibrate and PPARα has been shown to regulate the transcription of a variety of lipid metabolic enzymes including ACO, lipoprotein lipase, and HMG-CoA synthetase. To show that PPARα is a participant in the control of transcription of $cPLA_2$ in SW cells, the total RNA was isolated from the cells, and the mRNA for PPARα, PPARγ, and GAPDH was amplified by RT-PCR. As depicted in **Fig. 6A**, mRNA for both PPARα and PPARγ were identified in SW cells. Interestingly, both the wild-type fragment of PPARα (433 bp) and its naturally occurring variant fragment (330 bp) were detected. The existence of PPARα was confirmed by Western blot analysis using specific polyclonal anti-PPARα antibodies (Fig. 6B).

Sequencing of PCR products

To verify the identity of the PCR products in this study, the double-stranded DNA obtained from RT-PCR was subjected to sequence analysis. Each DNA fragment obtained, including $cPLA_2$, COX-1, COX-2, PPARα, or PPARγ, was separated, extracted, purified, and sequenced. The results from the sequence analysis were compared with published sequences for $cPLA_2$, COX-1, COX-2, PPARγ, and PPARα

TABLE 3. Sequence comparison and homology

Name	Gene Bank Accession Number	Identity	Reference
		%	
$cPLA_2$	M68874	97	Sharp et al., 1991 (8)
	M72393	97	Clark et al., 1991 (13)
COX-2	NM 000963.1	95	Jones et al., 1993 (58)
	M90100	95	Hla et al., 1992 (59)
COX-1	M59979	100	Funk et al., 1991 (60)
	S36219	100	Diaz et al., 1992 (61)
PPARα	S74349	98	Mukherjee et al., 1994 (62)
(330 bp variant)	Z79997	98	Whiteley, 1998[a]
PPARγ	U79012 (HSU63415)	99	Mukherjee et al., 1997 (63)
	U63415 (HSU 79012)	99	Elbrecht et al., 1996 (64)

[a] This sequence was submitted directly to the GeneBank.

variant. The amplified cDNA fragments from SW cells were found to have a high degree of identity (>95%) with the published sequences (**Table 3** and related references). Unfortunately, the sequence of the DNA sample for the PPARα wild type (433 bp) could not be obtained. This failure might be caused by a small amount of the variant form presented as contamination in the wild-type fragment after agarose gel separation.

DISCUSSION

Arachidonic acid is an important precursor for ecoisanoid synthesis. Eicosanoids, which include prostaglandins, leukotrienes, and thromboxanes, have been implicated in biological processes as diverse as signal transduction (39, 40), the regulation of cell growth, and the maintenance of vascular integrity (41, and references therein). Under normal physiological conditions, the vast majority of the arachidonic acid is stored in esterified form in phospholipids and can be released by the action of PLA_2. Ionophores are widely used to stimulate PLA_2 for the release of arachidonate in experimental studies (42) because the use of other adrenergic agents may produce many effects innate to the physiological properties of these agents. Among the different types of PLA_2 found in the mammalian cell, $cPLA_2$ is the intracellular enzyme that directs the release of arachidonic acid under stimulation (43). The activation of $cPLA_2$ can be regulated by many mechanisms at both the transcriptional and post-translational levels. The enzyme is regulated at the transcriptional level by cytokines and growth factors (17, 20), which is regarded as a prolonged process for the activation of the enzyme during tissue development and differentiation (16, 19). Alternatively, the post-translational regulation would provide the cell with a rapid process for the activation of $cPLA_2$. Several mechanisms for the post-translational regulation of the enzyme have been identified, including the Ca^{2+}-dependent translocation of the enzyme-to-membrane and phosphorylation-dependent activation of the enzyme mediated by MAP kinase and protein kinase C (44). An additional mechanism involved in $cPLA_2$ activation is mediated via the G-proteins, which regulate the MAP kinase cascade (14). We have reported previously that lyso-PC is a potent activator of the $cPLA_2$ activity in both human endothelial cells and rat cardiac myoblastic H9c2 cells (28, 29). In these studies, we have shown that the lyso-PC exerted its effect through mechanisms of post-transcriptional regulation. These observations led us to explore the regulation of this enzyme at the transcriptional level.

The nuclear hormone receptor PPARα is a member of the thyroid/vitamin D/retinoid superfamily of transcription factors that require heterodimerization with the retinoid X receptor for optimal DNA binding (45). Whereas the activation of $cPLA_2$ by PPARα activators has not been previously reported, the ligand/activator of other hormone receptors in this superfamily, such as the analog or precursor of vitamin D, have been shown to activate $cPLA_2$ or COX-2 (46, 47). Clofibrate is a classical peroxisome proliferator and PPARα activator (22, 23), and it has been shown to stimulate the transcription of the lipoprotein lipase gene via peroxisome proliferator response element in the cultured murine 3T3 preadipocytes (24). As the first generation of lipid-lowering drugs, clofibrate has been approved for therapeutic use in the USA for the last three decades. Treatment with clofibrate results in a substantial decrease in plasma triglyceride-rich lipoproteins and an increase in the high density lipoprotein (HDL) (48, 49). These alterations are correlated with decreased levels of apolipoprotein-B (apoB), apoC-III, and apoE in triglyceride-rich lipoproteins and increased levels of apoA-I and apoA-II in HDL. The effect of fibrate on human apoA-I has been shown to be mediated by the transcription factor PPARα, which interacts with a positive PPAR-response element at its promoter region (50).

The human liposarcoma cell line SW872 has been shown to express and secrete high levels of cholesteryl ester transfer protein and therefore has been effectively used for studies on lipid metabolism (51, 52). We have shown that this cell line has the ability to express high levels of PPARα mRNA and protein and therefore is subjected to modulation by PPARα ligands. In view of the key importance of $cPLA_2$ in the release of arachidonate, it is of inter-

est to elucidate the modulation of $cPLA_2$ expression at the transcriptional level upon PPAR ligand stimulation in these cells.

Long-term stimulation of SW cells by insulin (1 μM) caused the transformation of these cells into adipocytic phenotypes (53). Prolonged stimulation of 3T3-L1 preadipocytes with fibrates (5–7 days) caused the conversion into adipocytes via the activation of PPAR (54, 55). In this study, treatment of SW cells with clofibrate or PGJ_2 was limited to 48 h to avoid any cell transformation. Transformation into adipocytes would be detected by the presence of fat globules, which would be verified by red oil O staining (53, 54). No adipocyte formation was observed throughout the course of clofibrate treatment (data not shown). It is interesting that PGJ_2 at low concentrations did not produce any effect on the gene expression of $cPLA_2$ or COX-2. The potency of PGJ_2 to SW cells, however, was clearly demonstrated because treatment with 10 μM PGJ_2 caused 100% cell death within 24 h.

Although the direct involvement of the PPAR pathway in the activation of $cPLA_2$ has not been demonstrated in this study, there is ample evidence that the action of clofibrates is mediated via the PPARα (22, 23). Clofibrates have been shown to regulate the gene expression of COX-2 via its interaction with PPAR (37), and a functional coupling and differential regulation of $cPLA_2$ and COX-2 in inflammation has recently been demonstrated (38). Although PPARα ligands have the ability to induce the ACO gene in rodents, their ability to induce the ACO gene in human cells has not been established (56). The relatively weak induction of ACO mRNA in SW cells by clofibrate treatment is in agreement with this notion. Nevertheless, the presence of PPAR in SW cells has been firmly established because these cells have the ability to express both the mRNA encoding PPARα and the receptor protein itself. Interestingly, both PPARα and PPARγ are expressed in the SW cells. This is not surprising, because this cell line is derived from the human liposarcoma and contains specific gene sets, which are responsible for lipogenesis and lipolysis upon stimulation. The presence of the wild-type fragment and the naturally occurring variant of PPARα mRNA indicate that the SW cell has the ability to synthesize the full length PPARα receptor as well as the truncated PPARα receptor (57). The function of the PPARα variant in preadipocytes, however, remains undefined.

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REFERENCES

1. Vance, D. E. 1991. Phospholipid metabolism and cell signaling in eukaryotes. *In* Biochemistry of Lipids, Lipoproteins and Membranes. D. E. Vance and J. E. Vance, editors. Elsevier Science Publishers, Amsterdam. 205–240.
2. Dennis, E. A., S. G. Rhee, M. M. Billah, and Y. A. Hannun. 1991. Role of phospholipase in generating lipid second messengers in signal transduction. *FASEB J.* **5:** 2068–2077.
3. Leslie, C. C. 1997. Properties and regulation of cytosolic phospholipase A_2. *J. Biol. Chem.* **272:** 16709–16712.
4. Balsinde, J., and E. A. Dennis. 1997. Function and inhibition of intracellular calcium-independent phospholipase A_2. *J. Biol. Chem.* **272:** 16069–16072.
5. Dennis, E. A. 1994. Diversity of group types, regulation, and function of phospholipase A_2. *J. Biol. Chem.* **269:** 13057–13060.
6. Clark, J. D., A. R. Schievella, E. A. Nalefski, and L. L. Lin. 1995. Cytosolic phospholipase A_2. *J. Lipid Mediat. Cell Signal.* **12:** 83–117.
7. Kudo, I., M. Murakami, S. Hara, and K. Inoue. 1993. Mammalian non-pancreatic phospholipase A_2. *Biochim. Biophys. Acta.* **1170:** 217–231.
8. Sharp, J. D., D. L. White, X. G. Chiou, T. Goodson, G. C. Gamboa, D. McClure, S. Bugett, J. Hoskins, P. L. Skatrud, J. R. Sportman, G. W. Becker, L. H. Kang, E. F. Roberts, and R. M. Kramer. 1991. Molecular cloning and expression of human Ca^{2+}-sensitive cytosolic phospholipase A_2. *J. Biol. Chem.* **266:** 14850–14853.
9. Dennis, E. A. 1997. The growing phospholipase A_2 superfamily of signal transduction enzymes. *Trends Biochem. Sci.* **22:** 1–2.
10. Kramer, R. M., and J. D. Sharp.1997. Structure, function and regulation of Ca^{2+}-sensitive cytosolic phospholipase A_2 ($cPLA_2$). *FEBS Lett.* **410:** 49–53.
11. Needleman, P., J. Turk, B. A. Jakschik, A. R. Morrison, and J. B. Lefkowith. 1986. Arachidonic acid metabolism. *Ann. Rev. Biochem.* **55:** 69–102.
12. Smith, W. L., P. Borgeat, and F. A. Fitzpatrick. 1991. The eicosanoids: cyclooxygenase, lipoxygenase, and epoxygenase pathways. *In* Biochemistry of Lipids, Lipoproteins and Membranes. D. E. Vance and J. E. Vance, editors. Elsevier Science Publishers, Amsterdam. 297–325.
13. Clark, J. D., L. L. Lin, R. W. Kriz, C. S. Ramesha, L. A. Sultzman, A. Y. Lin, N. Milona, and J. L. Knolf. 1991. A novel arachidonic acid-selective cytosolic PLA_2 contains a Ca^{2+}-dependent translocation domains with homology to PKC and GAP. *Cell.* **65:** 1043–1051.
14. Winitz, S., S. K. Gupta, N. X. Qian, L. E. Heasley, R. A. Nemenoff, and G. L. Johnson. 1994. Expression of a mutant $Gi_2\alpha$ subunit inhibits ATP and thrombin stimulation of cytosoic phospholpase A_2-mediated arachidonic acid release independent of Ca^{2+} and mitogen-activated protein kinase regulation. *J. Biol. Chem.* **269:** 1889–1895.
15. Clark, J. D., A. R. Schievella, E. A. Nalefski, and L. L. Lin. 1995. Cytosolic phospholipase A_2. *J. Lipid Mediat. Cell Signal.* **12:** 83–117.
16. Wu, T., S. L. Levine, M. G. Lawrence, C. Logun, C. W. Angus, and J. H. Shelhamer. 1994. Interferon gamma induces the synthesis and activation of cytosolic phospholipase A_2. *J. Clin. Invest.* **93:** 571–577.
17. Nakamura, T., L. L. Lin, S. Kharbanda, J. Knopf, and D. Kufe.1992. Macrophage stimulating factor activates phosphatidylcholine hydrolysis by cytosolic phospholipase A_2. *EMBO J.* **11:** 4917–4922.
18. Hoeck, W. G., C. S. Ramesha, D. J. Chang, N. Fan, and R. A. Heller. 1993. Cytosolic phospholipase A_2 activity and gene expression are stimulated by tumor necrosis factor: dexamethasone blocks the induced synthesis. *Proc. Natl. Acad. Sci. USA.* **90:** 4475–4479.
19. Hernandez, M., Y. Bayon, M. Sanchez-Crespo, and M. L. Nieto. 1999. Signaling mechanisms involved in the activation of arachidonic acid metabolism in human astrocytoma cells by tumor necrosis factor-alpha. *J. Neurochem.* **73:** 1641–1649.
20. Maxwell, A. P., H. J. Goldberg, A. H. N. Tay, Z. G. Li, G. S. Arbus, and K. L. Skorecki. 1993. Epidermal growth factor and phorbol myristate acetate increase expression of the mRNA for cytosolic phospholipase A_2 in glomerular mesangial cells. *Biochem. J.* **295:** 763–766.
21. Newton, R., L. M. Kuitert, D. M. Slater, I. M. Adcock, and P. J. Narnes. 1997. Cytokine induction of cytosolic phospholipase A_2 and cyclooxygenase-2 mRNA is suppressed by glucocorticoid in human epithelial cell. *Life Sci.* **60:** 67–78.
22. Schoonjans, K., B. Staels, and J. Auwerx. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* **37:** 907–925.

23. Forman, B. M., J. Chen, and R. M. Evans. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. *Proc. Natl. Acad. Sci. USA.* **94:** 4312–4217.
24. Schoonjans, K., J. Peinado-Onsurbe, A. M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb, B. Staels, and J. Auwerx. 1996. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.* **15:** 5336–5348.
25. Staels, B., J. Dallongeville, J. Auwerx, K. Schoojans, E. Leitersdorf, and J. C. Fruchart. 1998. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation.* **98:** 2088–2093.
26. Berger, J., M. D. Leibowitz, T. W. Doebber, A. Elbrecht, B. Zhang, G. Zhou, C. Biswas, C. A. Cullinan, N. S. Hayes, Y. Li, M. Tanen, J. Ventre, M. S. Wu, G. D. Berger, R. Mosley, R. Marquis, C. Santini, S. P. Sahoo, R. L. Tolman, R. G. Smith, and D. E. Moller. 1999. Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPAR-delta ligands produce distinct biological effects. *J. Biol. Chem.* **274:** 6718–6725.
27. He, T. C., T. A. Chan, B. Vogelstein, and K. W. Kinzler. 1999. PPAR-delta is an APC-regulated target of nonsteroidal anti-inflammatory drugs. *Cell.* **99:** 335–345.
28. Wong, J. T., K. Tran, G. N. Pierce, A. C. Chan, O. K., and P. C. Choy. 1998. Lysophosphatidycholine stimulates the release of arachidonic acid in human endothelial cells. *J. Biol. Chem.* **273:** 6830–6836.
29. Golfman, L. S., N. J. Haughey, J. T. Wong, J. Y. Jiang, D. Lee, J. D. Geiger, and P. C. Choy. 1999. Lysophosphatidylcholine indues arachidonic acid release and calcium overload in cardiac myoblastic H9c2 cells. *J. Lipid Res.* **40:** 1818–1826.
30. Tran, K., and A. Chan. 1988. Effect of vitamin E enrichment on arachidonic acid release and cellular phospholipids in cultured human endothelial cells. *Biochim. Biophys. Acta.* **963:** 468–475.
31. Huang, Y. H., P. Leblanc, V. Apostolou, B. Stewart, and R. B. Moreland. 1995. Comparison of Mili-Q PF Plus water and DEPC-treated water in the preparation and analysis of RNA. *Biotechniques.* **19:** 656–661.
32. Leygue, E., P. H. Watson, and L. C. Murphy. 1996. Estrogen receptor variants in normal human mammary tissue. *J. Natl. Cancer. Inst.* **88:** 274–290.
33. Rosenthal, M. D., and J. R. Hill. 1986. Elongation of arachidonic and eicosapentaenoic acids limits their availability for thrombin-stimulated release from the glycerolipids of vascular endothelial cells. *Biochim. Biophys. Acta.* **875:** 382–391.
34. Street, I. P., H. K. Lin, F. Laliberte, F. Ghomashichi, Z. Wang, H. Perrier, N. M. Trembly, Z. Huang, P. K. Weech, and M. H. Gelb. 1993. Slow- and tight-binding inhibitors of the 85-kDa human phospholipase A_2. *Biochemistry.* **32:** 5935–5940.
35. Dubois, R. N., S. B. Abramson, L. Crofford, R. A. Gupta, L. S. Simon, L. B. A. Van De Putte, and P. E. Lipsky. 1998. Cyclooxygenase in biology and disease. *FASEB J.* **12:** 1063–1073.
36. Scott, K. F., K. L. Bryant, and M. J. Bidgood. 1999. Functional coupling and differential regulation of the phospholipase A_2-cyclooxygenase pathways in inflammation. *J. Leukoc. Biol.* **66:** 535–541.
37. Ledwith, B. J., C. J. Pauley, L. K. Wagner, C. L. Rokos, D. W. Alberts, and S. Manam. 1997. Induction of cyclooxygenase-2 expression by peroxisome proliferators and non-tetradecanoylphorbol 12, 13-myristate-type tumor promoters in immortalized mouse liver cells. *J. Biol. Chem.* **272:** 3707–3714.
38. Murakami, M., T. Kambe, S. Shimbara, and I. Kudo. 1999. Functional coupling between various phospholipase A_2s and cyclooxygenases in immediate and delayed prostanoid biosynthetic pathways. *J. Biol. Chem.* **274:** 3103–3115.
39. Habenicht, A. J. R., H. A. Dresel, M. Goerig, J. A. Weber, M. Stoehr, J. A. Glomset, R. Ross, and G. Schettler. 1986. Low density lipoprotein receptor-dependent prostaglandin synthesis in Swiss 3T3 cells stimulated by platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA.* **83:** 1344–1348.
40. Kiesel, L., A. F. Przylipiak, A. J. R. Habenicht, M. S. Przylipiak, and B. Runnebaum. 1991. Production of leukotrienes in gonadotropin-releasing hormone-stimulated pituitary cells: potential role in luteinizing hormone release. *Proc. Natl. Acad. Sci. USA.* **88:** 8801–8805.
41. Janssen-Timmen, U., I. Tomic, E. Specht, U. Beileche, and A. J. R. Habenicht. 1994. The arachidonic acid cascade, eicosanoids and signal transduction. *Ann. N. Y. Acad. Sci.* **733:** 325–334.
42. Tran, K., J. T. Wong, E. Lee, A. C. Chan, and P. C. Choy. 1996. Vitamin E potentiates arachidonate release and phospholipase A_2 activity in rat heart myoblastic cells. *Biochem. J.* **319:** 385–391.
43. Kramer, R. M. 1994. Signal-Activated Phospholipases. M. Liscovitch, editor. R.G. Landes, Co., Austin.13–30.
44. Lin, L. L., M. Wartmann, A. Y. Lin, J. L. Knopf, A. Seth, and R. G. Davis. 1993. $cPLA_2$ is phosphorylated and activated by MAP kinase. *Cell.* **72:** 269–278.
45. Mangelsdorf, D. J., and R. M. Evans. 1995. The RXR heterodimers and orphan receptor. *Cell.* **83:** 841–850.
46. Pirianov, G., C. Danielsson, C. Carlberg, S. Y. James, and K. W. Colston. 1999. Potentiation by vitamin D analogs of TNF alpha and ceramide-induced apoptosis in MCF-7 cells is associated with activation of cytosolic phospholipase A_2. *Cell Death Differ.* **6:** 890–901.
47. Kanekura, T., S. L. Lauledernkind, K. Kirtkara, S. Goorha, and L. R. Ballou. 1998. Cholecalciferol induces protaglandin E_2 biosynthesis and transglutaminase activity in human keratinocytes. *J. Invest. Dermatol.* **111:** 634–639.
48. Davignon, J. 1994. Fibrates: a review of important issues and recent findings. *Can. J. Cardiol.* **10:** 61B–71B.
49. Sirtori, C. R., and G. Franceschini. 1988. Effects of fibrates on serum lipids and atherosclerosis. *Pharmacol. Ther.* **37:** 167–191.
50. Staels, B., and J. Auwerx. 1998. Regulation of apo A-I gene expression by fibrates. *Atherosclerosis.* **137:** S19–S23.
51. Richardson, M. A., D. T. Berg, P. A. Johnston, D. McClurre, and B. W. Grinnell. 1996. Human liposarcoma cell line SW872, secretes cholesteeryl ester transfer protein in response to cholesterol. *J. Lipid. Res.* **37:** 1162–1166.
52. Gauthier, B., M. Robb, F. Gaudet, G. S. Ginsburg, and R. McPherson. 1999. Characterization of a cholesterol response element (CRE) in the promoter of the cholesteryl ester transfer protein gene: functional role of the transcription factors SREBP-1a, -2, and YY1. *J. Lipid Res.* **40:** 1284–1293.
53. Nuttall, M. E., A. J. Patton, D. L. Olivera, D. P. Nadeau, and M. Gowen. 1998. Human trabecular bone cells are able to express both osteoblastic and adipocytic phenotype: implications of osteopenic disorders. *J. Bone Miner. Res.* **13:** 371–382.
54. Brandes R., R. Arad, and Bar-Tana. 1995. Inducers of adipose conversion activate transcription promoted by a peroxisome proliferators response element in 3T3–L1 cells. *Biochem. Pharmacol.* **50:** 1949–1951.
55. Tontonoz, P., E. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid activated transcription factor. *Cell.* **79:** 1147–1156.
56. Rodriguez, C., N. Veronique, A. Cabrero, C. J. Ciudad, and J. C. Laguna. 2000. Differences in the formation of PPARα-RXR/acoPPRE complexes between responsive and nonresponsive species upon fibrate administration. *Mol. Pharmacol.* **58:** 185–193.
57. Gervois, P., I. P. Torra, G. Chinetti, T. Grotzinger, G. Dubois, J. C. Fruchart, J. Fruchart Najib, E. Leitersdorf, and B. Staels. 1999. A truncated human peroxisome proliferator activated receptor alpha splice variant with dominant negative activity. *Mol. Endocrinol.* **13**: 1535–1539.
58. Jones, D. A., D. P. Carlton, T. M. McIntyre, G. A. Zimmerman, and S. M. Prescott. 1993. Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokine. *J. Biol. Chem.* **268:** 9049–9054.
59. Hla, T., and K. Neilson. 1992. Human cyclooxygenase-2 cDNA. *Proc. Natl. Acad. Sci. USA.* **89:** 7384–7388.
60. Funk, C. D., L. B. Funk, M. E. Kennedy, A. S. Pong, and G. A. Fitzgerald. 1991. Human platelet/erythroleukemia cell prostaglandin G/H synthase: cDNA cloning, expression and gene chromosomal assignment. *FASEB J.* **5:** 2304–2312.
61. Diaz, A., A. M. Reginato, and S. A. Jimenez. 1992. Aternative splicing of human prostaglandin G/H synthase mRNA and evidence of differential regulation of the resulting transcripts by transforming growth factor beta1, interleukin-1 beta and tumour necrosis factor alpha. *J. Biol. Chem.* **267:** 10816–10822.
62. Mukherjee, R., L. Jow, D. Noonan, and D. P. McDonnell. 1994. Human and rat peroxisome proliferator-activated receptors demonstrate similar tissue distribution but different responsiveness to PPAR activators. *J. Steroid Biochem. Mol. Biol.* **51:** 157–166.
63. Mukherjee, R., L. Jow, G. E. Creoston, and J. R. Paterniti, Jr. 1997. Identification, characterization, and tissue distribution of peroxisome proliferator-activated receptor isoforms PPARγ1 versus PPARγ2 and activation with retinoid X receptor agonist and antagonist. *J. Biol. Chem.* **272:** 8071–8076.
64. Elbrecht, A., Y. Chen, C. A. Cullinan, N. Hayes, M. D. Leibowitz, D. E. Moller, and J. Berger. 1996. Molecular cloning, expression and characterization of human peroxisome. proliferator-activated receptor gamma1 and gamma2. *Biochem. Biophys. Res. Commun.* **224:** 431–437.