Original Article

Lauric Acid Beneficially Modulates Apolipoprotein Secretion and Enhances Fatty Acid Oxidation via PPARα-dependent Pathways in Cultured Rat Hepatocytes

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Abstract

Background and objectives: Dietary fat type can differentially modulate the fatty acid oxidation and secretion of lipoproteins in hepatocytes. The purpose of the present study was to investigate how the nature of fatty acids present in dietary oils influences fatty acid oxidation as well as secretion of apolipoproteins.

Methods: Primary rat hepatocytes were cultured with major fatty acids present in common cooking oils, which vary in their degree of saturation: *viz.* coconut oil (lauric acid; C12:0); olive oil (oleic acid; C18:1); and sunflower oil (linoleic acid; C18:2). Each fatty acid was used at a dose of 500 µM for 12 hours.

Results: Cells treated with laurate beneficially modulated the secretion of apolipoproteins relative to cells treated with oleate and linoleate ($p < 0.05$). These results correlated with the respective apolipoproteins' mRNA expression. Laurate increased activities and mRNA expression of enzymes involved in β-oxidation (*viz.* carnitine palmitoyl transferase I and acyl CoA oxidase) and also up-regulated the respective transcription factor, peroxisome proliferator-activated receptor alpha (PPARα), when compared to other fatty acids ($p < 0.05$). Studies using the PPARα agonist WY 14643 revealed that lauric acid may act as a natural ligand for PPARα and mediates its effects partly via PPARα-dependent pathways in hepatocytes.

Conclusions: These results clearly indicate that lauric acid, the major fatty acid present in oil extracted from coconut, acts as a natural ligand for PPARα, beneficially regulating secretion of apolipoproteins and enhancing fatty acid oxidation via PPARα signaling pathways in hepatocytes.

Introduction

It has been known for many years that the type of fat in the diet influences blood lipid levels and, consequently, the risk for development of atherosclerosis and related cardiovascular diseases.[1] Epidemiological and clinical studies have consistently demonstrated that human serum lipid concentration may be altered by diet, which is itself mostly influenced by the nature of dietary fatty acids present in it, particularly differences in the chain length, degree of unsaturation, position and stereo-isomeric configuration of the double-bonds as well as the bioactive components present in dietary oils.[2] Although plasma low-density lipoprotein is well established as a predictor of cardiovascular disease, recent observations have revealed that apolipoproteins may in fact be more powerful lipid-related predictors of cardiovascular disease risk.[3,4] Among them, apolipoprotein B is the chief protein component of atherogenic lipoprotein particles and apolipoprotein A1 is the major apolipoprotein constituent of the anti-atherogenic high-density lipoproteins in animal models.[5] Apolipoprotein E also acts as a key regulator of plasma lipid level and plays an important role in cholesterol transport.[6]

In vitro cell culture studies have shown that availability of lipid is a critical factor in the assembly and secretion of lipoproteins.[7] There are reports that β-oxidation of fatty acids, as well as synthesis and assembly of lipoproteins, is coordinated via transcriptional regulation.[8] Experimental evidence has revealed that peroxisome proliferator-activated receptor alpha (PPARα) activation regulates

Keywords: Apolipoprotein A1; Apolipoprotein B; Carnitine palmitoyl transferase-I; Oleic acid; Virgin coconut oil.

Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; SEM, standard error of the mean.

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fatty acid oxidation as well as metabolism of both high-density lipoproteins and apolipoprotein B-containing lipoproteins in rodents and humans.[9,10] Studies have suggested that PPARα connects fatty acid oxidation to synthesis of apolipoproteins; PPAR activation leads to increased hepatic oxidation of fatty acids and less synthesis of triglycerides for very low-density lipoprotein assembly.[11]

Gas chromatography-mass spectrometry analysis of the test oils revealed that there were no significant differences in fatty acid composition among the two types of coconut oil extracted by different modes of extraction (copra oil and virgin coconut oil), which contained mostly short and medium chain fatty acids and were mainly composed of lauric acid (C12:0; ~46%). But, olive oil contains 62% oleic acid (C18:1) and sunflower oil contains higher amounts of linoleic acid (C18:2; 47%).[12] Later on, studies using these test oils found that supplementation of virgin coconut oil extracted from fresh coconut kernel produced a significant effect on lowering blood lipid levels, mostly reflected in their corresponding apolipoprotein levels (a significant decrease in apolipoprotein B and an increase in apolipoprotein A1 levels) compared to monounsaturated fatty acid-rich olive oil and polyunsaturated fatty acid-rich sunflower oil in rats.[13] Moreover, there are reports that a polyunsaturated fatty acid enriched diet enhances high-density lipoprotein turnover, which was found to be associated with higher fractional catabolic rates for both apolipoprotein A1 and apolipoprotein AII relative to saturated and monounsaturated oils.[14,15]

It has also been established that oleic acid exerts a concentration-dependent increase in apolipoprotein B secretion from cultured rat hepatocytes into the medium, relative to cells treated with saturated or polyunsaturated fatty acids.[16] However, the effect of lauric acid on apolipoprotein secretion and fatty acid oxidation is not yet understood. Our recent reports demonstrated that virgin coconut oil supplementation up-regulates PPARα-mediated fatty oxidation pathways *in vivo*, as compared to the monounsaturated fatty acid-rich olive oil and polyunsaturated fatty acid-rich sunflower oil in rats.[17] In view of these findings, detailed studies were carried out to investigate the relative effects of major fatty acids present in these test oils (*viz.* lauric acid (C12:0), oleic acid (C18:1) and linoleic acid (C18:2)) on fatty acid oxidation and apolipoprotein secretion in primary rat hepatocytes. The role of PPARα pathways in fatty acid-mediated apolipoprotein secretion and fatty acid oxidation in hepatocytes were also elucidated.

Methods

Chemicals

TRI Reagent, oligonucleotides, primers for PCR, anti-rabbit IgG, Eagle's minimum essential medium, penicillin, streptomycin, type IV collagenase, lauric acid, oleic acid, linoleic acid, the MTT tetrazolium dye and WY 14643 were purchased from Sigma Aldrich Co. (St Louis, MO, USA). Antibodies against apolipoprotein A1 and apolipoprotein B were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). cDNA synthesis and PCR reactions were carried out using kits purchased from Fermentas, Thermo Fisher Scientific Inc. (Canada). All chemicals used were of the highest analytical grade.

Preparation and maintenance of hepatocytes

Hepatocytes were isolated from normal rats (Sprague-Dawley strain) by collagenase perfusion.[18,19] Hepatocytes (0.65×10^6 cells/mL in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, non-essential amino acids and antibiotics) were seeded into 35 mm petri dishes. After 4 h, the unattached cells were removed and the medium was changed to serum-free medium supplemented with 500 µM fatty acids (laurate, oleate and linoleate) at a fatty acid: bovine serum albumin ratio of 2:1 and incubated for 12 h. Untreated cells received an equal amount of bovine serum albumin equal to that brought with the bovine serum albumin-fatty acid complex.

Preparation of albumin-bound fatty acids

A 10 mM stock solution of each fatty acid was prepared by diluting the free fatty acid in ethanol and precipitating it with the addition of NaOH (0.25M final concentration). Excess ethanol was evaporated under N_2 gas, and the precipitated sodium salt was reconstituted with 0.9% NaCl and stirred at room temperature for 10 m with defatted bovine serum albumin (final concentration of 10% in 0.15M NaCl). Each solution was adjusted to pH 7.4 with NaOH and stored in multiple aliquots at −20 °C protected from light, and tubes were purged with N_2.[20]

Biochemical analysis

The activities of enzymes involved in β-oxidation in hepatocytes (*viz.* carnitine palmitoyl transferase I and acyl CoA oxidase) were assayed.[21,22] The MTT tetrazolium dye assay was performed according to the method described by Mosmann,[23] which is directly correlated to cell viability; cell viability was calculated as a percentage from the viability of the control (untreated) cells.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay was carried out according to the procedure of Lin,[24] and the protein concentration was estimated by the method of Lowry *et al*.[25] using Folin phenol reagent with bovine serum albumin as the standard.

Isolation of total genomic RNA

Total genomic RNA was isolated from the heart using TRI Reagent by the method described by Chomczynski and Mackey.[26]

cDNA synthesis and semi-quantitative reverse transcription-PCR

cDNA synthesis and reverse transcription-PCR reactions were performed in a thermocycler (Eppendorf AG, Hamburg, Germany) as per manufacturer's instructions using a kit purchased from Fermentas. The primers used were: apolipoprotein A1 (forward) 5′-GAAATGGAAAGAGGATGTGGAG-3′ and (reverse) 5′-GT TCAAGGTAGGGTTGCTCTTG-3′; apolipoprotein B (forward) 5′-TAGAGGATCCCTGAGCAGGCTTCCTCAGCAG-3′ and (reverse) 5′-TTTAAAGCTTCAATGATTCTATCAATAATCTG-3′; carnitine palmitoyl transferase I (forward) 5′-GGA GAC AGA CAC CAT CCA ACA TA-3′ and (reverse) 5′-AGG TGA TGG ACT TGT CAA ACC-3′; acyl CoA oxidase (forward) 5′-CTT TCT TGC TTG CCT TCC TTC TCC3′ and (reverse) 5′-GCC GTT TCA CCG CCT CGT A-3′; PPARα (forward) 5′-TCACACAATGCAATC-CGTTT-3′ and (reverse) 5′-GGCCTTGACCTTGTTCATGT-3′;

Table 1. Effect of exogenous fatty acids on apolipoprotein secretion

	Group I	Group II	Group III	Group IV	*p*-value
Apolipoprotein A1*	0.15±0.01[a]	0.25±0.01[b]	0.15±0.03[a]	0.20±0.08[d]	<0.05
Apolipoprotein B*	0.29±0.01[a]	0.40±0.01[b]	1.25±0.05[c]	0.80±0.05[d]	<0.05

Values are mean ± SEM of three separate experiments in triplicate. Values not sharing a common superscript differ significantly at p <0.05. Group I-control; Group II-500 µM laurate; Group III-500 µM oleate; Group IV-500 µM linoleate. *OD U/mg protein. Abbreviation: SEM, standard error of the mean.

and glyceraldehyde-3-phosphate dehydrogenase (forward) 5′-CCT TCA TTG ACC TCA ACT AC-3′ and (reverse) 5′-GGA AGG CCA TGC CAG TGA GC-3′. The PCR reaction mixture contained cDNA, PCR master mix and appropriate primers. The reaction mixtures were incubated at 94 °C for 3 m for initial denaturation and cycled 35 times at 94°C for 30 s (template denaturation), primer annealing for 30 s at 72°C for 2 m. After a final extension at 72°C for 10 m, the PCR products were electrophoresed on 1.5% agarose gel and visualized by staining with ethidium bromide. The gels were subjected to densitometric scanning (Bio-Rad Gel Doc, Hercules, CA, USA) to determine the density of each band and then normalized against an internal control, glyceraldehyde-3-phosphate dehydrogenase, using QuantityOne imaging software.

Statistical analysis

Data are presented as means with their standard errors. Statistical analysis was performed by one-way analysis of variance followed by Duncan's multiple range tests using the SPSS/PC (version 17.0; SPSS) software package program. A p-value <0.05 was considered significant.

Results

Effect on apolipoprotein secretion

In order to study how the nature of fatty acid influence on the secretion of apolipoproteins, hepatocytes were incubated with different fatty acids, including lauric acid (C12:0), oleic acid (C18:1) and linoleic acid (C18:2), each at a dose of 500 µM for 12 h of exposure. At the end of the experimental period, the medium and cell layer were harvested. The medium was centrifuged for 10,000 *g* for 15 m and was collected for apolipoprotein concentration by enzyme-linked immunosorbent assay. In the presence of laurate, there was a significant (p <0.05) decrease in the apolipoprotein B secretion relative to oleate- and linoleate-treated cells. While treatment with oleate significantly (p <0.05) increased the apolipoprotein B secretion compared to cells treated with other fatty acids.

Hepatocytes treated with linoleate decreased the apolipoprotein B secretion compared to oleate treatment (Table 1). Among the different fatty acids, oleate showed maximum effect on apolipoprotein B secretion (1.25 OD U/mg protein), followed by linoleate (0.80 OD U/mg protein), laurate (0.40 OD U/mg protein) and untreated cells (0.29 U/mg protein). In contrast, cells treated with laurate significantly (p <0.05) increased the secretion of apolipoprotein A1 compared to cells treated with other fatty acids. While cells treated with oleate significantly (p <0.05) reduced the apolipoprotein A1 secretion relative to laurate and linoleate treatment. Cells treated with linoleate significantly (p <0.05) increased the apolipoprotein A1 secretion compared to cells treated with oleic acid (Table 1).

Among the different fatty acids, the saturated fatty acid laurate (0.25 OD U/mg protein) showed maximum effect on apolipoprotein A1 secretion, followed by linoleate (0.20 OD U/mg protein) and oleate (0.15 OD U/mg protein), suggesting that the nature of fatty acids plays an important role in the synthesis and secretion of apolipoproteins in hepatocytes.

Effect on mRNA expression of apolipoproteins in hepatocytes

The results are summarized in Figure 1. In the presence of laurate, there was an up-regulation in the mRNA expression of apolipoprotein A1 in hepatocytes compared to cells treated with other fatty acids. Treatment with oleate decreased mRNA expression of apolipoprotein A1 relative to cells treated with laurate and linoleate. While treatment with linoleate increased apoA1 expression compared to oleate treatment. However, hepatocytes incubated with laurate showed a significant down-regulation in the mRNA expression of apolipoprotein B compared to incubation with the other fatty acids. Treatment with oleate showed a significant increase in apolipoprotein B mRNA expression compared to hepatocytes incubated with laurate and linoleate.

Effect on fatty acid oxidation in hepatocytes

For studying the effect of fatty acids present in dietary oils on fatty acid oxidation, the activities of major enzymes involved in fatty acid oxidation in hepatocytes (*viz.* carnitine palmitoyl transferase I and acyl CoA oxidase) were studied. The results indicate that hepatocytes treated with laurate have a significant increase in the activities of carnitine palmitoyl transferase I and acyl CoA oxidase compared to cells treated with other fatty acids (Table 2). The activities of these enzymes were found to be decreased in cells treated with oleate, relative to laurate and linoleate. Relative to oleate-treated cells, linoleate treatment led to increased activities of enzymes in hepatocytes.

Effect on mRNA expression of fatty acid oxidation enzymes and PPARα in hepatocytes

A significant up-regulation in the mRNA expression of carnitine palmitoyl transferase I, acyl CoA oxidase and its transcription factor, PPARα, were observed in laurate-treated cells, relative to cells treated with other fatty acids. Cells treated with linoleate also showed a significant increase in the mRNA expression of carnitine palmitoyl transferase I, acyl CoA oxidase and PPARα, relative to oleate-treated cells (Fig. 2).

Effects of exogenous fatty acids and PPARα agonist on cell viability

MTT assay was used to measure the cell viability; results showed that fatty acid- and PPAR-α agonist-treated cells had the same vi-

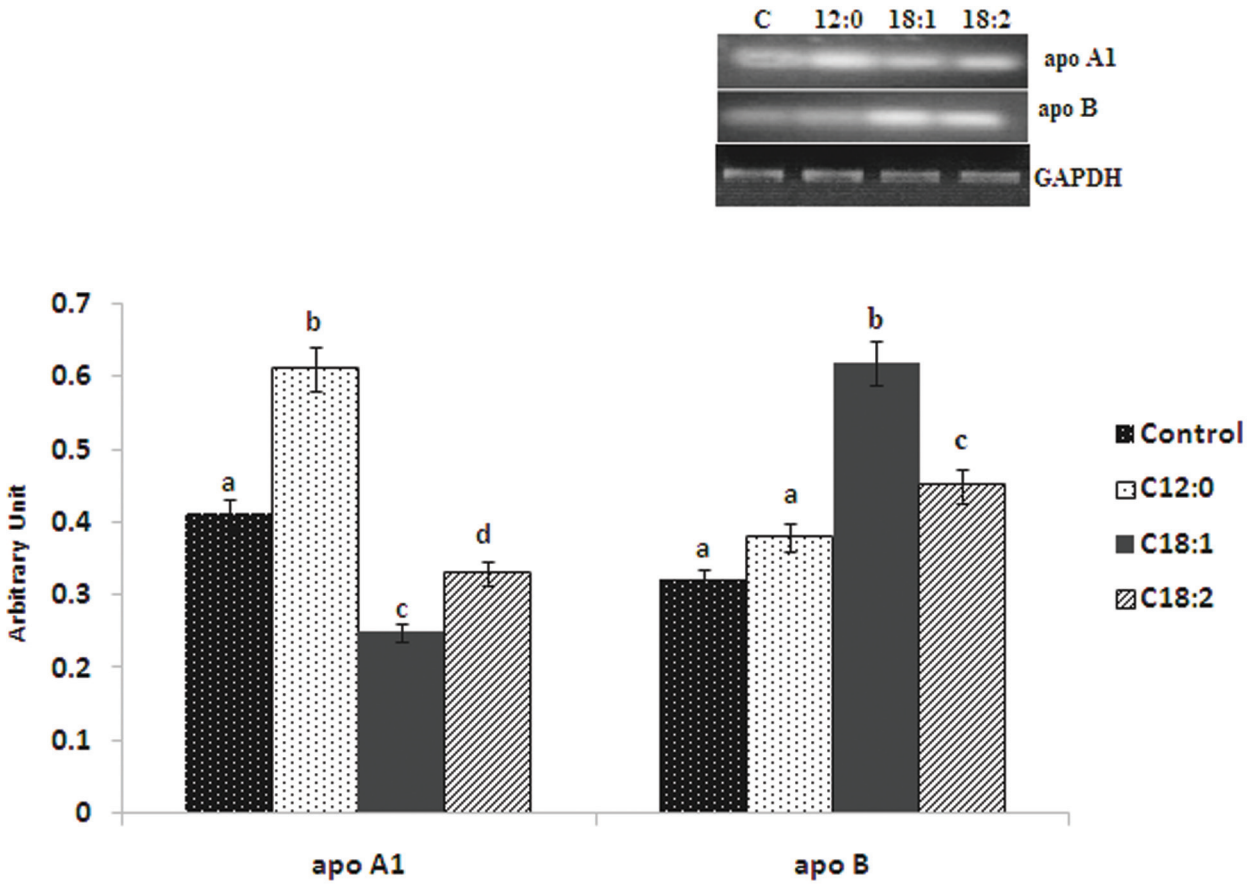

Fig. 1. Effect of exogenous fatty acids on the mRNA expression of apolipoproteins in hepatocytes. Values are mean ± SEM of three separate experiments in triplicate. Values not sharing a common superscript differ significantly at p <0.05. Group I-control; Group II-500 µM laurate; Group III-500 µM oleate; Group IV-500 µM linoleate. Abbreviation: SEM, standard error of the mean.

ability as control cells during the experimental period (Fig. 3).

Effect of PPARα ligands on fatty acid-mediated apolipoprotein secretion

To study further the involvement of PPARα signaling pathways on the fatty acid-mediated apolipoprotein secretion, isolated hepatocytes were cultured with different fatty acids (at 500 µM) in the presence or absence of the PPARα agonist WY 14643 (10 µM) for 12 h. The presence of the PPARα agonist WY 14643 decreased the amount of apolipoprotein B secreted into the medium, relative to cells treated with fatty acids. Laurate treatment also led to a significant decrease in apolipoprotein B secretion relative to cells treated with oleate and linoleate alone; moreover, laurate coincubation did not inhibit the effect of PPARα agonist on apolipoprotein B secretion relative to cells cultured in combination with PPARα agonist along with other fatty acids.

However, cells treated with oleate increased the apolipoprotein B secretion into the medium and inhibited the effect of PPARα agonist on coincubation, relative to laurate or linoleate co-treatment with PPARα agonist. Linoleate treatment also showed a significant increase in apolipoprotein B secretion relative to cells treated with PPARα agonist and laurate. But, the effect of PPARα agonist on apolipoprotein B secretion was not remarkably inhibited by linoleate cotreatment relative to lauric acid cotreatment with PPARα agonist. The inhibitory effect of PPARα agonist on apolipoprotein B secretion was maximum in cells when cotreated with lauric acid followed by linoleate and oleate (Fig. 4). These results may suggest that this effect of fatty acids on apolipoprotein B secretion is partly mediated via a PPARα-dependent pathway in hepatocytes.

Treatment with the PPARα agonist WY 14643 led to a significant increase in the apolipoprotein A1 secretion, relative to other fatty acids. Laurate treatment also showed an increased apolipo-

Table 2. Effect of exogenous fatty acids on the activities of fatty acid oxidation enzymes in hepatocytes

	Group I	Group II	Group III	Group IV	*p*-value
Carnitine palmitoyl transferase I*	0.16±0.01^a	0.45±0.04^b	0.19±0.02^c	0.25±0.03^d	<0.05
Acyl CoA oxidase*	0.15±0.01^a	0.39±0.04^b	0.17±0.02^c	0.23±0.03^d	<0.05

Values are mean ± SEM of three separate experiments in triplicate. Values not sharing a common superscript differ significantly at p <0.05. Group I-control; Group II-500 µM laurate; Group III-500 µM oleate; Group IV- 500 µM linoleate. *U/mg protein. Abbreviation: SEM, standard error of the mean.

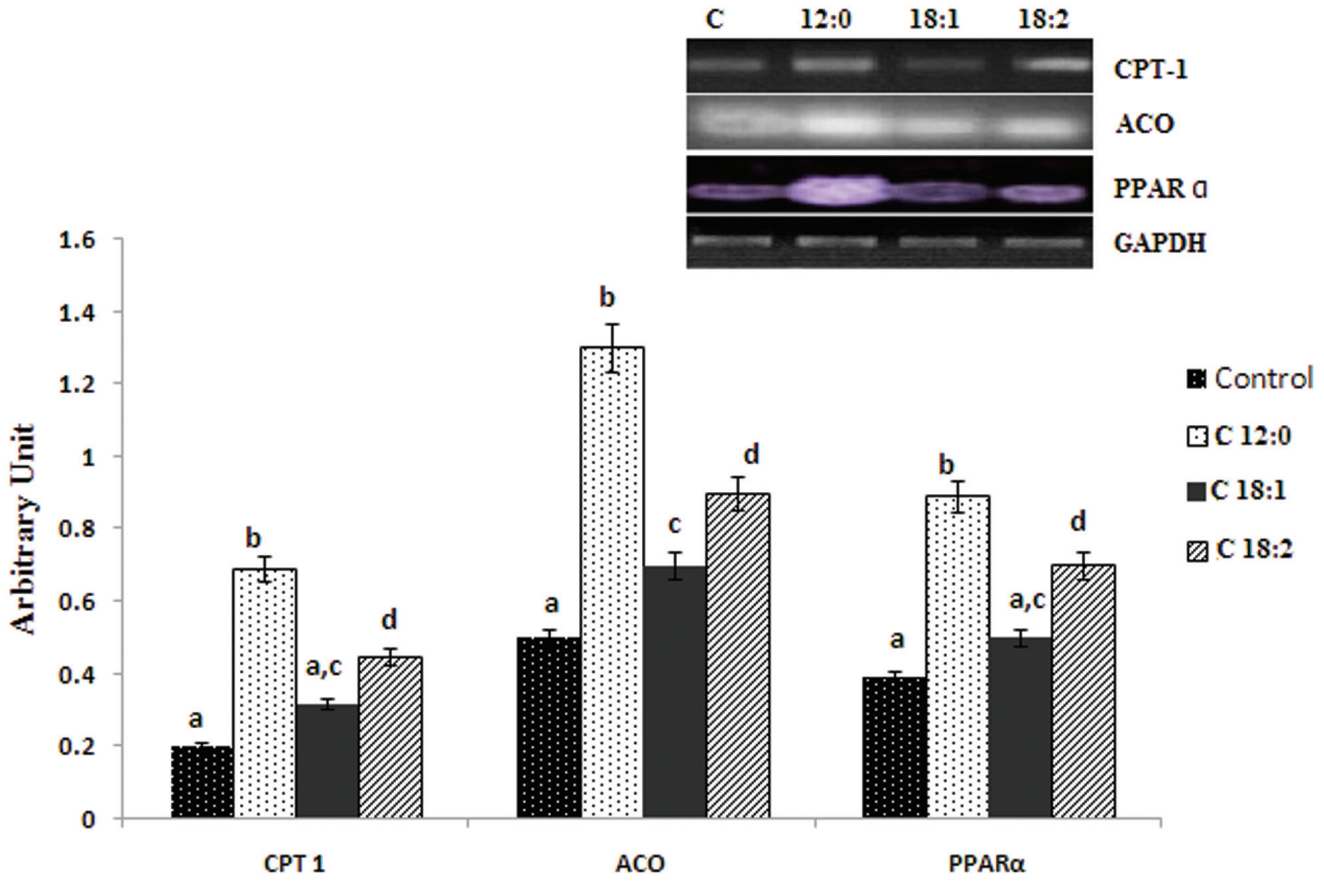

Fig. 2. Effect of exogenous fatty acids on the mRNA expression of fatty acid oxidation enzymes and PPARα in hepatocytes. Values are mean ± SEM of three separate experiments in triplicate. Values not sharing a common superscript differ significantly at p <0.05. Group I-control; Group II-500 µM laurate; Group III-500 µM oleate; Group IV-500 µM linoleate. Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; SEM, standard error of the mean.

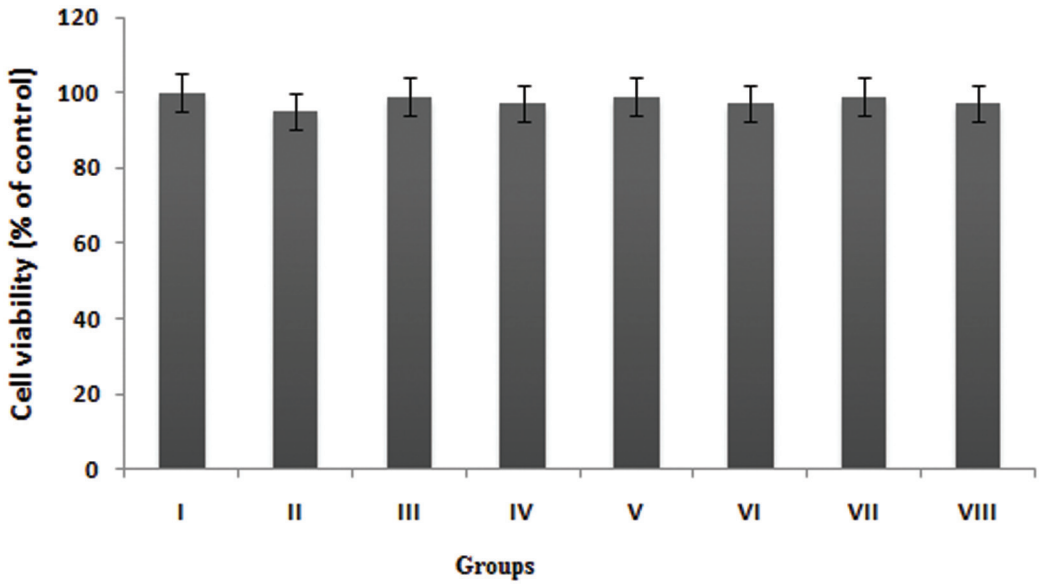

Fig. 3. Effects of exogenous fatty acids and WY 14643 on cell viability. Values are means ± SEM of three independent experiments. I-control; II-WY 14643 (10 µM); III-laurate (500 µM); IV-laurate (500 µM) + WY 14643(10 µM); V-oleate (500 µM); VI-oleate (500 µM) + WY 14643 (10 µM); VII-linoleate (500 µM); VIII-linoleate (500 µM) + (10 µM) WY 14643. Abbreviation: SEM, standard error of the mean.

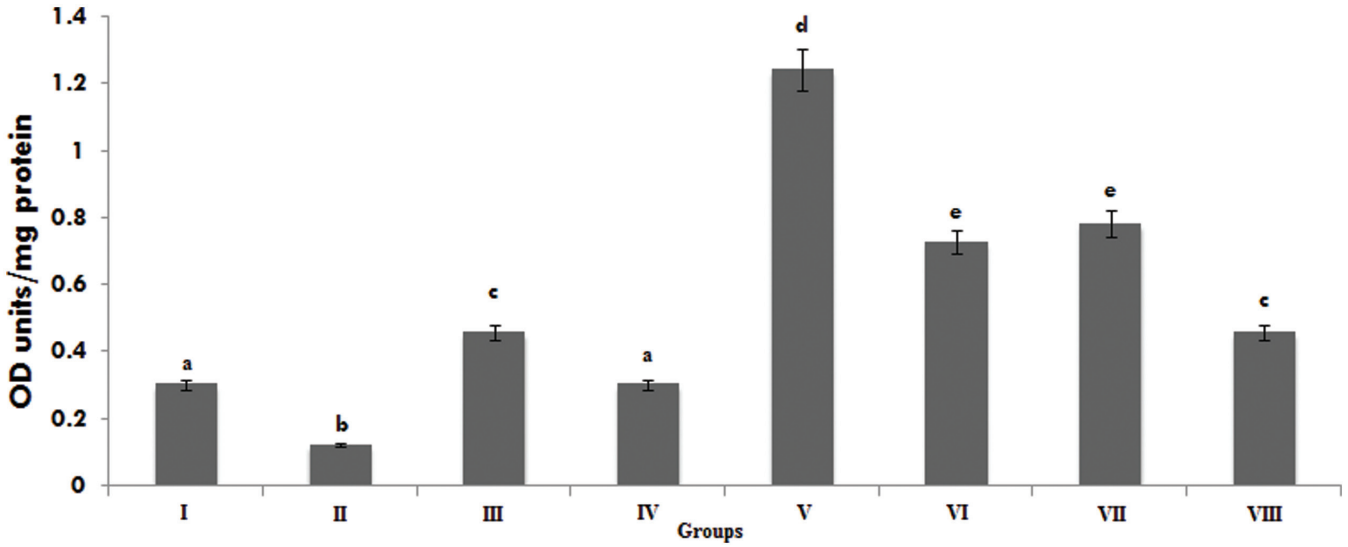

Fig. 4. Effect of PPARα ligands on fatty acid-mediated apolipoprotein B secretion. Values are mean ± SEM of three separate experiments in triplicate. Values not sharing a common superscript differ significantly at p <0.05. I-control; II-WY 14643 (10 μM); III-laurate (500 μM); IV-laurate (500 μM) + WY 14643 (10 μM); V-oleate (500 μM); VI-oleate (500 μM) + WY 14643 (10 μM); VII-linoleate (500 μM); VIII-linoleate (500 μM) + (10 μM) WY 14643. Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; SEM, standard error of the mean.

protein A1 secretion, relative to those treated with oleate and linoleate alone. Co-incubation of laurate with agonist did not inhibit the effect of PPARα agonist on apolipoprotein A1 secretion. Hepatocytes treated with oleate showed a significant decrease in apolipoprotein A1 secretion into the medium and inhibited the effect of PPARα agonist on apolipoprotein A1 secretion. Even though linoleate treatment enhanced the apolipoprotein A1 secretion into the medium, not much significant difference was observed compared to the oleate treatment. Coincubation of linoleate did not inhibit the effect of PPARα agonist on apolipoprotein A1 secretion relative to oleate cotreatment (Fig. 5). Among the three different fatty acid-treated groups, the maximum increase in apolipoprotein A1 secretion was observed in cells treated with laurate, followed by linoleate and oleate. Studies using PPARα agonist confirmed that the effect of fatty acids on apolipoprotein secretion by hepatocytes is mediated at least in part via a PPARα pathway.

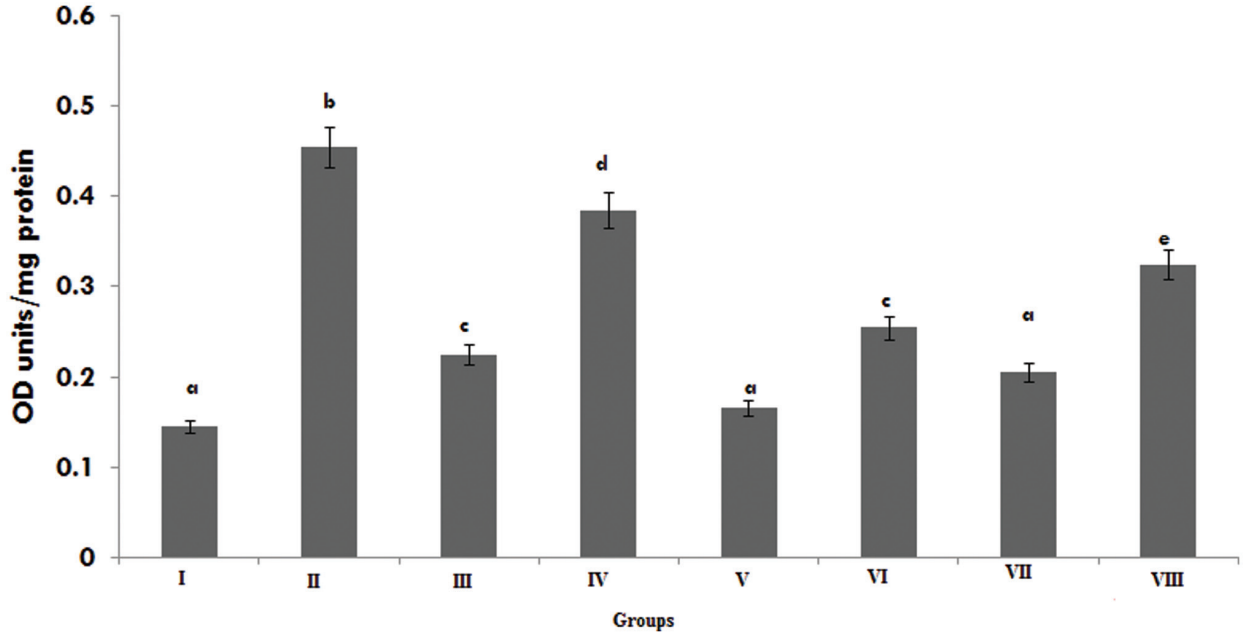

Fig. 5. Effect of PPARα ligands on fatty acid-mediated apolipoprotein A1 secretion. Values are mean ± SEM of three separate experiments in triplicate. Values not sharing a common superscript differ significantly at p <0.05. I-control; II-WY 14643 (10 μM); III-laurate (500 μM); IV- laurate (500 μM) + WY 14643 (10 μM); V-oleate (500 μM); VI-oleate (500 μM) + WY 14643 (10 μM); VII-linoleate (500 μM); VIII-linoleate (500 μM) + (10 μM) WY 14643. Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; SEM, standard error of the mean.

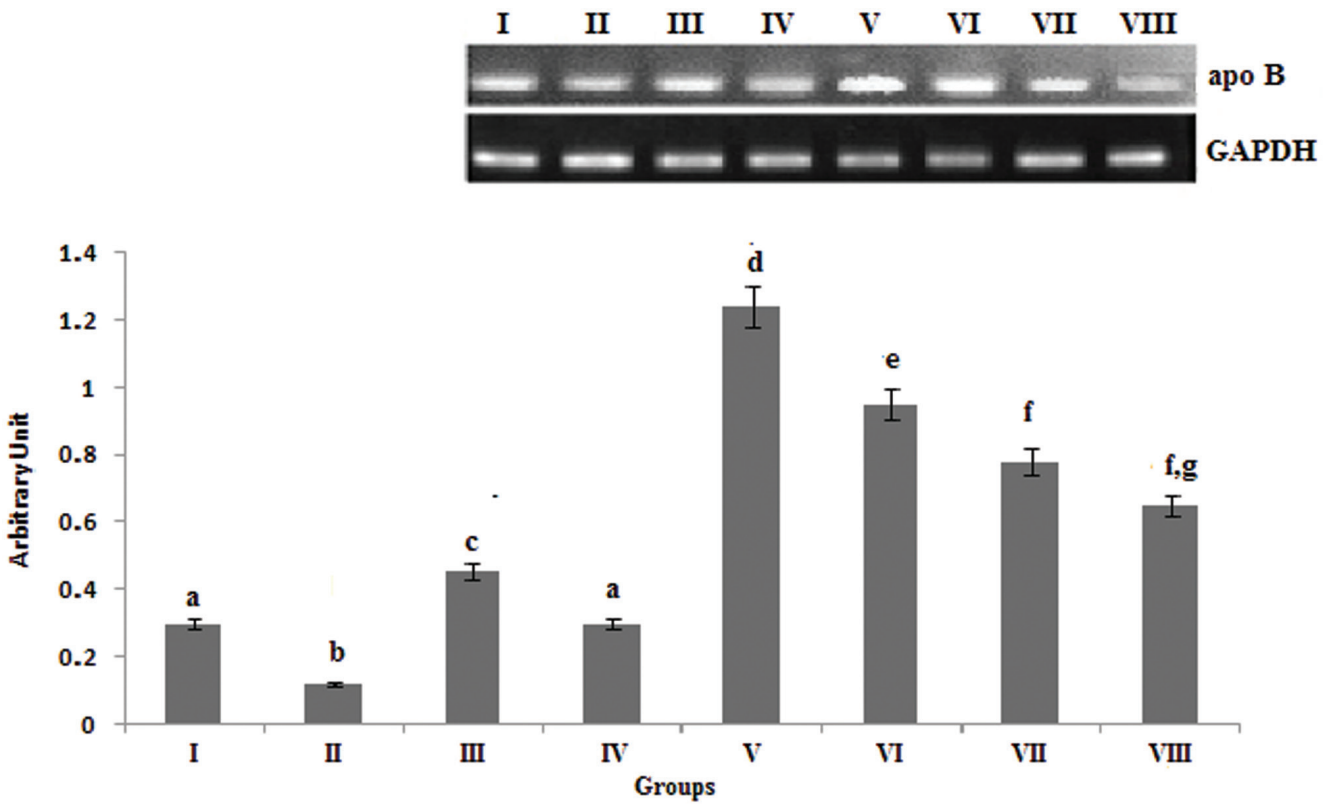

Fig. 6. Effect of PPARα ligands on fatty acid-mediated mRNA expression of apolipoprotein B. Values are mean ± SEM of three separate experiments in triplicate. Values not sharing a common superscript differ significantly at p <0.05. I-control; II-WY 14643 (10 μM); III-laurate (500 μM); IV-laurate (500 μM) + WY 14643 (10 μM); V-oleate (500 μM); VI-oleate (500 μM) + WY 14643 (10 μM); VII-linoleate (500 μM); VIII-linoleate (500 μM) + (10 μM) WY 14643. Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; SEM, standard error of the mean.

Effect of PPARα ligands on fatty acid-mediated mRNA expression of apolipoprotein

Hepatocytes treated with PPARα agonist decreased the mRNA expression of apolipoprotein B compared to other groups. Similarly, cells treated with laurate decreased the mRNA expression of apolipoprotein B relative to cells treated with other fatty acids; moreover, cotreatment of laurate did not inhibit the effect of PPARα agonist on apolipoprotein B mRNA expression compared to cells treated with a combination of agonist with other fatty acids. Relative to other fatty acids, however, the oleate treatment led to an up-regulation in mRNA expression of apolipoprotein B and also inhibited the effect of PPARα on apolipoprotein B mRNA expression on coincubation. Cells treated with linoleate also up-regulated the apolipoprotein B mRNA expression compared to cells treated with laurate. Relative to oleate co-treated cells, the linoleate cotreatment did not produce much inhibition on apolipoprotein B mRNA expression by PPARα agonist in hepatocytes (Fig. 6).

Treatment with PPARα agonist showed a significant increase in the mRNA expression of apolipoprotein A1 in hepatocytes compared to other groups. Laurate treatment also showed an increased mRNA expression of apolipoprotein A1 relative to cells treated with oleate and linoleate. Coincubation of PPARα agonist with laurate did not inhibit the effect of agonist. Meanwhile, oleate treatment showed a significant decrease in apolipoprotein A1 mRNA expression compared to laurate treated cells. Furthermore, oleate cotreatment inhibited the effect of agonist on apolipoprotein A1 expression (Fig. 7). Hepatocytes incubated with linoleate showed decreased mRNA expression of apolipoprotein A1 compared to those treated with laurate. Compared to oleate cotreated cells, coincubation of PPARα agonist with linoleate caused less inhibition of the apolipoprotein A1 mRNA expression by PPARα agonist. These results further confirm that fatty acid-induced apolipoprotein synthesis is transcriptionally regulated by PPARα in hepatocytes.

Effect of PPARα ligands on mRNA expression of carnitine palmitoyl transferase I and acyl CoA oxidase in hepatocytes

In the presence of PPARα agonist WY 14643 there was an up-regulation in the mRNA expression of carnitine palmitoyl transferase I and acyl CoA oxidase in hepatocytes compared to other fatty acids (Fig. 8). Treatment with laurate also showed a significant increase in the expression of these genes and cotreatment with laurate did not inhibit the effect of agonist on mRNA expression relative to cells treated with oleate and linoleate. Meanwhile, the mRNA expression of carnitine palmitoyl transferase I and acyl CoA oxidase was decreased by oleate treatment and inhibited the effect of agonist on the gene expressions (carnitine palmitoyl transferase I and acyl CoA oxidase) in hepatocytes during coincubation. Hepatocytes treated with linoleate, however, showed increased expression of carnitine palmitoyl transferase I and acyl CoA oxidase compared to those treated with oleate. Furthermore, linoleate cotreatment did not inhibit the effect of PPARα agonist

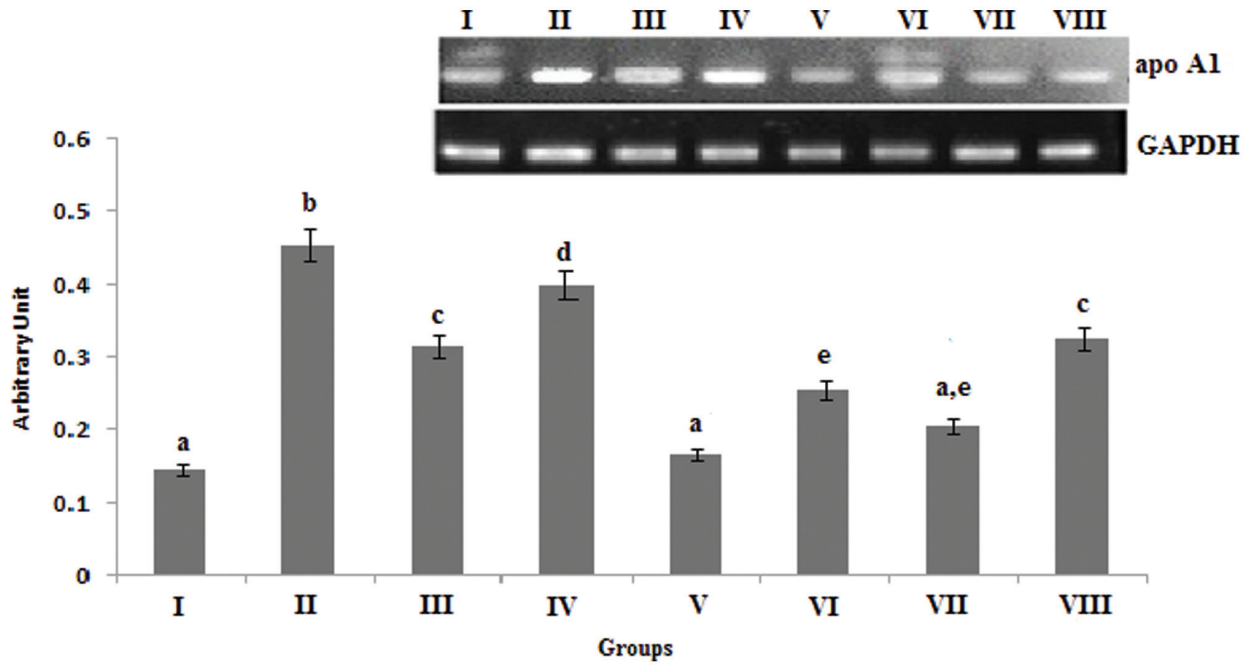

Fig. 7. Effect of PPARα ligands on fatty acid-mediated mRNA expression of apolipoprotein A1. Values are mean ± SEM of three separate experiments in triplicate. Values not sharing a common superscript differ significantly at p <0.05. I-control; II-WY 14643 (10 μM); III-laurate (500 μM); IV-laurate (500 μM) + WY 14643 (10μM); V-oleate (500 μM); VI-oleate (500 μM) + WY 14643 (10 μM); VII-linoleate (500 μM); VIII-linoleate (500 μM) + (10 μM) WY 14643. Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; SEM, standard error of the mean.

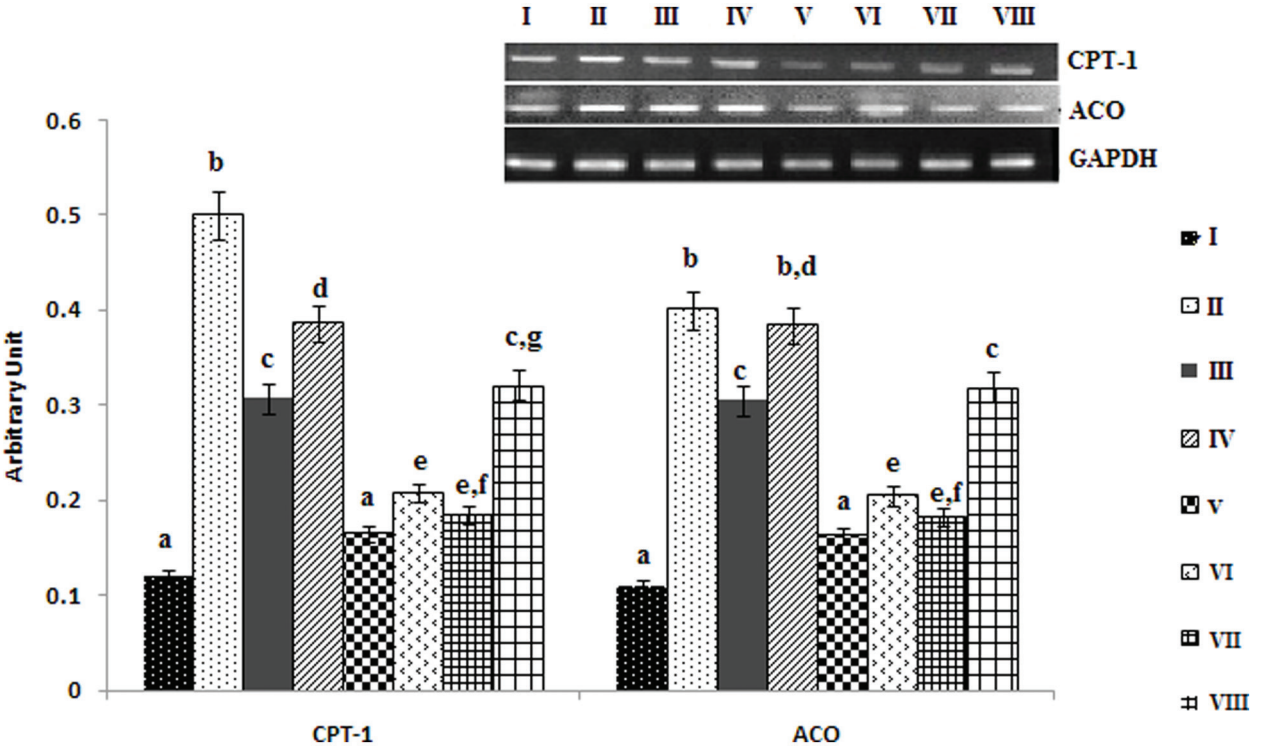

Fig. 8. Effect of PPARα ligands on fatty acid mediated mRNA expression of carnitine palmitoyl transferase I and acyl CoA oxidase in hepatocytes. Values are mean ± SEM of three separate experiments in triplicate. Values not sharing a common superscript differ significantly at p <0.05. I-control; II-WY 14643 (10 μM); III-laurate (500 μM); IV-laurate (500 μM) + WY 14643 (10 μM); V-oleate (500 μM); VI-oleate (500 μM) + WY 14643 (10 μM); VII-linoleate (500 μM); VIII-linoleate (500 μM) + (10 μM) WY 14643. Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; SEM, standard error of the mean.

relative to agonist and oleate cotreated cells. These results indicate that oxidation of fatty acids in hepatocytes is also mediated via PPARα-dependent pathways.

Discussion

Results obtained indicate that the nature of fatty acid has a role in influencing apolipoprotein secretion from and fatty acid oxidation in hepatocytes. Among the three different fatty acids investigated, laurate showed a significant reduction in apolipoprotein B secretion relative to oleate and linoleate treatment. Yet, increased apolipoprotein A1 secretion was observed in the case of laurate, followed by linoleate and oleate. These changes in apolipoprotein secretion may be due to the fatty acid-mediated differential regulation of apolipoprotein expression at the mRNA level. It is clear from our results that laurate treatment up-regulated the mRNA expression of apolipoprotein A1 in hepatocytes relative to cells treated with oleate and linoleate. In contrast, there was a down-regulation in apolipoprotein B mRNA expression in hepatocytes when treated with laurate, which correlated with the decreased apolipoprotein B secretion into the media.

These observations clearly revealed that the apolipoprotein secretion by the hepatocytes directly correlated with the corresponding mRNA expressions in hepatocytes. Previous reports on the differential effect of fatty acids on apolipoprotein B secretion revealed that oleic acid increases apolipoprotein B secretion by 239%, followed by linoleic acid (164%) and lauric acid 32%.[27] Our observations were also consistent with these reports. Cells treated with oleate showed maximum apolipoprotein B secretion compared to laurate and linoleate treatment. There are reports that oleic acid exerts a concentration-dependent increase in apolipoprotein B secretion into the medium, followed by saturated and polyunsaturated fatty acids. This might be due to the fact that oleic acid can influence the synthesis and secretion of apolipoprotein B in hepatocytes, probably by affecting cholesterol/cholesteryl ester formation, which may be a critical component in the secretion of apolipoprotein B as lipoproteins.[16] In addition, oleic acid can increase the secretion of apolipoprotein B from HepG2 cells by stimulating its translocation across the endoplasmic reticulum and by preventing its degradation by a ubiquitin-dependent proteosome pathway.[28,29] These might be the reasons for increased apolipoprotein B secretion in hepatocytes treated with oleate compared to other groups.

It is well established that all lipoproteins share a common structure, consisting of a neutral lipid core of triacylglycerol and cholesterol esters surrounded by a surface monolayer of phospholipids, un-esterified cholesterol and specific proteins called apolipoproteins. The assembly and secretion of lipoproteins are complex processes that occur in the lumen of the Golgi apparatus.[30] These processes mainly involve the synthesis of apolipoproteins in the rough endoplasmic reticulum and the synthesis of lipids in the smooth endoplasmic reticulum, followed by their assembly. It has been reported that the nature of fatty acids differentially influences the synthesis and secretion of lipoproteins in the hepatocytes.[27]

Moreover, dietary fat type can differentially modulate the activity and expression of fatty acid oxidation enzymes.[31] To examine how those fatty acids present in dietary oils can influence fatty acid oxidation, hepatocytes were maintained in a medium containing the major fatty acids present in common dietary oils, which vary in their degrees of saturation. Cell viability assays revealed that the exogenous fatty acid-treated cells had the same viability as control cells. Results showed that laurate treatment increases the activity of enzymes involved in β-oxidation of fatty acids (*viz.* carnitine palmitoyl transferase I and acyl CoA oxidase in hepatocytes) compared to other fatty acid-treated groups. Further investigations revealed that laurate up-regulates the mRNA expression of carnitine palmitoyl transferase I and acyl CoA oxidase in hepatocytes, which might be the reasons for increased activity of these enzymes in hepatocytes. A noticeable reduction in carnitine palmitoyl transferase I activity was previously shown to be associated with decreased β-oxidation of fatty acid in hepatocytes.[32]

There is evidence that dietary supplementation of medium-chain fatty acids suppresses the fat deposition through enhanced fatty acid oxidation in animals and humans.[33] Studies have shown that short and medium chain fatty acids present in dietary oils are mostly absorbed through the hepatic portal vein and become oxidized rapidly by both mitochondrial and peroxisomal pathways, and that they are less effectively incorporated into tissue lipids than the long chain fatty acids.[34,35] The metabolic fate of lauric acid in cultured rat hepatocytes have shown that lauric acid was rapidly taken up by the cells and its incorporation into cellular lipids was low.[36] In addition, *in vivo* laurate supplementation increases fatty acid oxidation at a higher rate in liver.[37] Hepatocytes exposed to oleate showed the maximum down-regulation in mRNA expression and activity of fatty acid oxidation enzymes, followed by linoleate. Previous reports have also suggested that linoleate has higher affinity for the mitochondrial fatty acid oxidation system than oleate in isolated rat liver mitochondria.[38]

Furthermore, it is clear that the β-oxidation of fatty acids as well as the synthesis and assembly of lipoproteins are under transcriptional regulation of PPARα.[8,39] PPARα is a transcription factor belonging to the nuclear receptor superfamily, is activated by natural ligands, such as the fatty acids, and plays an important role in fatty acid oxidation.[40] It is reported that the genes encoding β-oxidation enzymes are transcriptionally regulated by PPARα.[41] An up-regulation in the PPARα expression was observed in hepatocytes treated with laurate, relative to cells treated with oleate and linoleate. Meanwhile, treatment with oleate decreased PPARα expression in hepatocytes compared to other fatty acids.

PPARs are a unique set of fatty acid-regulated transcription factors controlling lipid metabolism.[42] To be transcriptionally active, PPARs need to heterodimerize with the retinoid-X receptor. PPAR/retinoid-X receptor heterodimers bind to DNA-specific sequences called peroxisome proliferator-response elements, consisting of an imperfect direct repeat of the consensus binding site for nuclear hormone receptors. To initiate transcriptional activity, the PPAR/retinoid-X receptor complex has to be activated by ligand binding. Natural ligands of PPARα include a variety of fatty acids as well as numerous fatty acid derivatives and compounds showing structural resemblance to fatty acids, including acyl-CoAs, oxidized fatty acids, eicosanoids, endocannabinoids, and phytanic acid.[43,44] In addition, there are reports that medium chain fatty acids are the natural PPAR ligands and that supplementation of coconut oil up-regulates mRNA expression of PPARα.[45,46] Our recent report also suggested that virgin coconut oil supplementation increases mRNA expression of PPARα and its target genes involved in fatty acid oxidation, as compared with rats fed olive oil and sunflower oil.[17] Upon ligand binding, the conformation of the receptor is altered, promoting high affinity interactions with coactivators that remodel chromatin structure and activate the transcriptional machinery.[29]

Many aspects of hepatic lipid metabolism are under control of PPARα. Regulation of lipid metabolism is mainly coordinated by liver, which actively metabolizes fatty acids as fuel and continuously produces very low-density lipoprotein particles to provide a constant supply of fatty acids to peripheral tissues. Clinical studies in humans have provided ample evidence that fibrate drugs effectively lower fasting plasma triglycerides and raise plasma high-

density lipoprotein.[47] At the molecular level, fibrates act as synthetic agonist for PPARα, indicating an important role of PPARα in the control of lipoprotein metabolism. PPARα activation lowers plasma triglycerides in part by reducing very low-density lipoprotein production.[48] There are reports that PPARα deficiency increases the secretion and serum levels of apolipoprotein B-containing lipoproteins.[8] Moreover, PPARα activation increases plasma high-density lipoprotein via the induction of hepatic apolipoprotein A1 and apolipoprotein AII expression in humans.[49]

In the last part of the study, involvement of PPARα on fatty acid-mediated apolipoprotein secretion and fatty acid oxidation were studied. For this, hepatocytes were treated with different types of fatty acids in the presence or absence of WY 14643, a highly potent synthetic PPARα agonist. Results indicate that treatment with laurate decreases the mRNA expression and secretion of apolipoprotein B in hepatocytes and that co-treatment did not inhibit the effect of PPARα agonist on apolipoprotein B secretion. In addition, hepatocytes treated with laurate showed up-regulated mRNA expression and secretion of apolipoprotein A1, as well as increased fatty acid oxidation; the laurate treatment did not inhibit the effect of PPARα agonist on fatty acid oxidation. There are reports that medium chain fatty acids are natural ligands for PPAR,[45] and PPARα activation up-regulates fatty acid oxidation-related genes, such as acyl CoA oxidase and carnitine palmitoyl transferase I, which suppresses postprandial lipidemia and lipid accumulation in enterocytes.[50,51] Our study clearly proved that lauric acid may act as ligand for PPARα, which mediates its effects partly via the PPARα pathway.

In conclusion, the present study clearly indicates that lauric acid, the major fatty acid present in oil extracted from coconut, acts as a natural ligand for PPARα, beneficially regulating secretion of apolipoproteins and enhancing fatty acid oxidation via the PPARα signaling pathways in hepatocytes.

Future research prospective

Lauric acid, may act as a natural ligand for PPARα and has a beneficial role in modulating apolipoprotein secretion as well as fatty acid oxidation. Since regulating the synthesis of endogenous apolipoprotein secretion would be an attractive therapeutic target for reducing the atherogenicity, lauric acid itself or lauric acid enriched dietary oils can be recommended as healthy dietary interventions for reducing the risk factors for atherosclerosis.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Designing research (SA, TR), performing research (SA), analyzing data (SA, TR), writing paper (SA), and proofreading paper (TR).

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