Molecular effect of fenofibrate on PBMC gene transcription related to lipid metabolism in metabolic syndrome patients

Molecular effects of Fenofibrate in lipid metabolism

Moreno-Indias I[$1,2], Tinahones FJ[$1,2], Clemente-Postigo M[1,2], Castellano-Castillo D[1,2], Fernández-García JC[1,2], Macias-Gonzalez M[1,2], Queipo-Ortuño MI[1,2], Cardona F[1,2].

[1]CIBER Fisiopatología de la Obesidad y Nutrición. CB06/03. Instituto de Salud Carlos III, Madrid, Spain. [2]Unidad Gestión Clínica Endocrinología y Nutrición, Instituto de Investigación Biomédica de Málaga (IBIMA), Universidad de Málaga. Hospitales Universitarios Regional y Virgen de la Victoria de Málaga, Universidad de Málaga, Spain.

[$] These authors have contributed equally to this work.

Corresponding author: María Isabel Queipo Ortuño

Laboratorio Investigación biomédica 1ª planta.

Hospital Clínico Universitario Virgen de la Victoria.

Campus de Teatinos s/n 29010 Malaga, Spain.

Tel: +34 951032647 / 48 Fax: +34 951924651

Email: maribelqo@gmail.com

ABSTRACT

Background: Both fasting and postprandial hypertriglyceridemia (HTG) are considered independent risk factors for atherosclerosis. Treatment of HTG is based on fibrates, which activate the peroxisome proliferator-activated receptor alpha (PPARα). However, the metabolic pathways that activate or inhibit fibrates, and how the postprandial triglyceride levels are modified, have not yet been fully described. Accordingly, we have studied the effects of fenofibrate on both fasting and postprandial triglyceride levels through the expression of several genes regulated by PPARα.

Material and methods: 50 patients were treated with fenofibrate for 3 months and fat overload test before and after treatment. Anthropometric and biochemical variables as well as gene expression in peripheral mononuclear cells were analysed.

Results: After treatment with fenofibrate, we observed a decrease in both basal and postprandial serum triglyceride levels, cholesterol, uric acid and an increase in HDL cholesterol and apolipoprotein AI levels. After treatment there was also a rise in PPARα and RXRα expression and changes in genes regulated by PPARα, both basal and postprandial. Furthermore, in vitro experiments showed that a PPARα agonist changed the expression of genes related with lipid metabolism.

Conclusion: Treatment with fenofibrate reduced fasting and postprandial serum triglyceride levels, possibly through a mechanism related with an increase in the expression of RXRα and PPARα, by activating the pathways involved in the uptake and degradation of triglycerides and increasing the synthesis of apolipoprotein. These results position to PBMC as a good material to easily study fenofibrate actions.

Keywords: fenofibrate, postprandial hypertriglyceridemia, lipid metabolism, metabolic syndrome, PBMC.

INTRODUCTION

Hypertriglyceridemia (HTG) is a component of the metabolic syndrome (MS), which is associated with insulin resistance, hypertension, inflammation and a pro-thrombotic state [1, 2]. High levels of plasma triglycerides are considered an independent risk factor for atherosclerosis and cardiovascular disease [3, 4]. Postprandial triglyceride concentration has been postulated as a better independent risk factor than fasting triglyceride level to predict cardiovascular disease [5-7].

Pharmacological treatment of HTG involves the use of fenofibrate [8]. Fenofibrate acts by reducing plasma triglyceride levels up to 40% below their basal level [9], and increasing the HDL cholesterol level [10]. The lipid-modifying effects of fenofibrate are not completely understood, but it is known effects are mediated by the activation of the nuclear transcription factor peroxisome proliferator-activated receptor-a (PPARα) which in turn modifies other genes involved in lipid metabolism through the heterodimer formed with RXR. [11-15]

Blood is one of the most easily available materials in humans, which makes it ideal for markers and analytical analysis. mRNA expression profile in PBMCs has been demonstrated to be a suitable tool for diagnosing diseases through the study of their mRNA expression profile [16]. Thus, it has been recently described a different gene expression profile between MS and control patients of nuclear receptors genes on PBMC, finding that nuclear receptors are useful as markers of diseases [17]. It has also been demonstrated that the PPARα-agonist WY14,643 is capable of producing changes in the mRNA expression in these cells [18]. This situation would set PBMCs as the perfect tool to study the fenofibrate effects.

Although in last years new molecular approaches have helped to solve some of the mechanism of the fenofibrate responses [19], it is necessary further study due to the great variation observed in the fenofibrate effects [3]. Therefore, given the difficulty of studying these mechanisms *in vivo* in humans, we propose the use of the PBMC cells, a tissue easy to

access, to measure the effects of the treatment with fenofibrate in subjects with metabolic syndrome undergoing a fat overload test, as a model of acute hypertriglyceridemia. Moreover, we also test the effect of fenofibrate in a hepatocyte cell line, HGEP2, as *in vitro* model.

MATERIAL AND METHODS

Subjects and study design

The current study was undertaken in 50 subjects (84% men and 16% woman) with MS (as defined by the Adult Treatment Panel III criteria) [20], age (45.25±10.27) and HTG, who had been treated with 160 mg/day of fenofibrate (Secalip®) for 3 months. Patients were excluded from the study if they had cardiovascular disease, diabetes, arthritis, acute inflammatory disease, infectious disease, renal disease, were receiving treatment for hyperlipidemia or were receiving drugs that could alter the lipid profile or the metabolic parameters at the time of inclusion in the study. All the participants gave written informed consent and the study was reviewed and approved by the Ethics and Research Committee of Virgen de la Victoria Clinical University Hospital, Malaga, Spain. At basal time, a fat overload test was performed; then, after a 3-month fenofibrate treatment period, the fat overload test was repeated. A validated fat overload test was put into practice as in a previous study by our group [21]. Briefly, after fasting for 12 hours, all the patients received a fat overload test based on a preparation of 100 mL containing 50 g fat, of which 30% are saturated, 49% are monounsaturated, and 21% are polyunsaturated. This preparation contains less than 1 g of lauric acid, less than 1 g of myristic acid, 4.8 g of palmitic acid, 1.4 g of stearic acid, 27.7 g of oleic acid, 9.6 g of linoleic acid, 1.4 g of behenic acid and 0.5 g of lignoceric acid (patent Nº P201030776). Only water was permitted during the process, and no physical exercise was undertaken. All the

participants followed the same diet on the day prior to the fat overload test, according to the guidelines [22].

Biochemical analyses

Blood samples were obtained from the antecubital vein and placed in vacutainer tubes (BD vacutainer™, London, UK) at baseline and 3 hours after the high-fat meal [23]. The serum was separated by centrifugation for 10 min at 4000 rpm and immediately frozen at -80ºC until analysis. Serum glucose, uric acid, cholesterol, triglycerides and HDL cholesterol were measured in a Dimension autoanalyzer (Dade Behring Inc., Deerfield, IL) by enzymatic methods (Randox Laboratories Ltd., UK). Insulin was quantified by radioimmunoassay supplied by BioSource International, Camarillo, S.A. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting insulin and glucose with the following equation: HOMA-IR=fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5 [24]. Moreover, PBMC were isolated from anticoagulant-treated blood by Ficoll standard density gradient centrifugation and maintained at −80°C until their analysis. These cells were chosen due to it is a model easily available without discomfort for patients. Moreover, as it has been described before, PBMCs are a suitable *in vivo* cell model to study the effects of PPAR agonists and a useful tool to observe the effects of these agonists in humans [25] and that PPARα produces a differential expression in genes measured in PBMCs [26].

In vitro experiment with HEPG2

Human culture cell line HEPG2 were selected for the in vitro experiment due to liver is the main action place of the therapies affecting the lipid metabolism. Human culture cell line HEPG2 seeded into 6-well plates (200,000 cells/well) and grown overnight in phenol red-free Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% charcoal-stripped fetal bovine serum. At this time, the cells were incubated with or without DNA constructs.

The reporter gene constructs were made as follows: four copies of the human CPT1 gene DR1-type response element [DR1-RE] (core sequence 5′-GTAGGGAAAAGGTCA.-3′ individually fused with the thymidine kinase (tk) minimal promoter driving the firefly luciferase reporter gene).

Cell transfection was performed using liposomes containing plasmid DNA which were formed by incubating 1 μg of an expression vector for wild type PPARα and RXRα (pSG5), as described [27], and 1 μg of reporter plasmid CPT1 with 10 μg of N-[1-(2,3-Dioleoyloxy)]-N,N,N-trimethylammonium propane (DOTAP) from Roche Applied Science (Basel, Switzerland) for 15 min at room temperature in a total volume of 100 ml. After dilution with 900 μL phenol red-free DMEM, the liposomes were added to the cells. Phenol red-free DMEM supplemented with 500 μL 15% charcoal-stripped fetal bovine serum was added 4 h after transfection [27].

In all cases, the wells were treated for 16 h with solvent (DMSO), with 10^{-6} M of a potent and highly selective PPARa agonist (GW7647 by Tocris Bioscience, Bristol, UK) [28] and 10^{-4} M of the different specific compounds indicated. The cells were lysed 16 h after onset of stimulation using the lysis buffer (Roche Applied Science, Basel, Switzerland). The constant light signal luciferase reporter gene assay was performed as recommended by the supplier. Stimulation of normalized luciferase activity was calculated in comparison with solvent-induced cells that did not overexpress protein.

RNA isolation and real-time quantitative PCR

Total RNA from PBMC and culture cell line HEPG2 was obtained using Trizol reagent (Gibco BRL Life Technologies,) according to the manufacturer's instructions. The purity of the RNA was determined by the 260/280 absorbance ratio on Nanodrop. The integrity of total purified RNA was checked by denaturing agarose gel electrophoresis and ethidium bromide staining.

For first-strand cDNA synthesis, a constant amount of 1 µg of total RNA was reverse transcribed using random hexamers as primers and Transcriptor Reverse Transcriptase (Roche, Mannheim, Germany). Gene expression was assessed by real-time PCR using an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Darmstadt, Germany) with TaqMan technology. The reaction was performed, following the manufacturer's protocol, in a final volume of 25 µl. The cycle program consisted of an initial denaturing of 10 min at 95 °C, then 40 cycles of 15 sec denaturing phase at 95 °C, and 1 min annealing and extension phase at 60°C. The commercially available and prevalidated TaqMan primer/probe sets used for human samples were as follows: β-Actin (4352935E, Ref seq. NM_001101.2) and PPIA (4326316E, RefSeq. NM_021130.3) used as endogenous control for the target gene in each reaction, SREBP1c (Hs01088691_m1, Ref seq. NM_004176.3 and NM_001005291.1), RXRa (Hs01067634_m1, Ref seq. NM_002957.4), CPT1a (Hs00912671_m1, Ref seq: NM_001031847.2 and NM_001876.3), ACOX1 (Hs01074241_m1 Ref seq: NM_001185039.1, NM_004035.6 and NM_007292.5), TNFa (Hs00174128_m1 Ref seq: NM_000594.3), PPAR-a, (Hs00231882 Ref seq: NM_001001928.2 and NM_005036.4), MTP (Human MTTP Hs00165177_m1 Ref seq: NM_000253.2) Apo A-V (Hs00983449_g1, RefSeq: NM_001166598.1 NM_052968.4). A threshold cycle (Ct value) was obtained for each gene amplification curve and the ΔCt value was first calculated by subtracting the Ct value for endogenous control cDNA from the Ct value for each sample and transcript. Fold changes compared with the endogenous control were then determined by calculating 2-ΔCt; expression results are expressed as the expression ratio relative to β-actin gene expression for humans, according to the manufacturer's guidelines. All samples were quantified in triplicate and positive and negative controls were included in all the reactions.

Statistical analysis

Analysis of the postprandial effects and fenofibrate treatment on the biological variables was done with the Wilcoxon test. Study groups were compared by the Kruskal-Wallis test in transfection assays and the Mann-Whitney U test to compare the gene expression in HEPG2 with or without the agonist. Spearman correlation analyses were done to study the associations between variables. In all cases the rejection level for a null hypothesis was an alpha=0.05 for two tails. Calculations were performed with SPSS software (version 19.0; SPSS Iberica, Madrid, Spain).

RESULTS

In the current study, we have analyzed the fenofibrate effect on the master genes of lipid metabolism in PBMC from metabolic syndrome patients, demonstrating that PBMC are a good candidate to study the effects of the fenofibrate *in vivo*. Moreover, we have used an *in-vitro* model in order to simulate the same conditions and a better understand of the results.

Baseline vs fenofibrate treatment for 3 months

After a 3-month fenofibrate treatment, we observed a significant decrease in the serum levels of triglycerides (32.4%) accompanied by an increase in the levels of HDL-cholesterol (11.0%) and apolipoprotein AI (3.9%). Moreover, a decrease in the uric acid (24.6%) and cholesterol (8.9%) were also observed (Table 1). At the postprandial state, a significant decrease was observed in the level of triglycerides and an increase in the HDL-cholesterol and apolipoprotein AI levels after treatment (Table 1). Moreover, no changes were found in the body weight of these patients after treatment.

Gene expression analysis was performed in PBMC before and after the fenofibrate treatment. The PPARα, RXRα and IL8 gene mRNA expression levels in PBMC showed a significant increase after the three months of treatment with fenofibrate (Table 2). CPT1a, ACOX1, SREBP1c and TNFa did not show significant changes with fenofibrate treatment. Moreover,

no expression of Apo A5 or microsomal triglyceride transfer protein (MTP) was detected in PBMCs (data not shown).

Postprandial gene expression

Table 2 shows postprandial PBMC gene expression levels with respect to fenofibrate treatment. Before fenofibrate treatment, we observed a significant increase in the levels of PPARα at the postprandial state. However, after the fenofibrate treatment, this postprandial increase in PPARα gene expression did not reach significance. With respect to the other genes, a significant increase in the levels of ACOX and CPT1a expressions after fenofibrate treatment were detected with respect to baseline. No significant differences were observed in any of the other genes studied (RXR-α, SREBP1c, IL-8 and TNFα) in the postprandial state (Table 2). We also found a significant positive correlation between the expression levels of PPARα and RXRα in patients with postprandial hypertriglyceridemia after fenofibrate treatment ($r=0.513$, $p<0.05$).

In vitro studies

We studied an *in vitro* model of the activity of the heterodimer PPARα-RXRα CPT1-DR1-activated and observed that the addition of oleic acid (the main fatty acid in the formula of the fat overload test) significantly increased the PPARα response element-luciferase activity in CPT1a, as the synthetic agonist for PPARα (GW7647) did, which is the best agonist described in the literature because of its powerful and selectiveness (Figure 1). Therefore, we can see that both oleic acid and agonist produces an increase in the mRNA expression of PPARα target genes in HEPG2 cells without construct. Thus, Figure 2 shows the gene expression fold change in HEPG2 cells, after the submission of a PPARα agonist: PPARα, Apo A5, ACOX and CPT1a gene expression were significantly increased, while MTP expression was decreased. These data are presented with respect to vehicle in each gene.

DISCUSSION

In the present study we demonstrate *in vivo,* for the first time using PBMC cells, that fenofibrate induces the expression of several lipid metabolism-related genes, especially master gene lipid metabolism such as PPARα, corroborating these data with an *in vitro* experiment. This gene expression induction could partly explain the underlying mechanism of action of fenofibrate on serum lipid parameters in patients with MS, positioning PBMC as good candidates to study these effects.

As expected, we observed that the use of fenofibrate is able to decrease the postprandial HTG. Our *in vivo* results using the easily accessed PBMCs show that there is an increase in the expression of PPARα in HTG patients after three months of treatment with fenofibrate. Previous studies suggest that the effects of this activation of PPARα are related to an increase in fatty acid beta-oxidation in enterocytes and a decrease in the postprandial serum triglyceride levels [29, 30], results that we have confirmed in this study using PBMCs and HPEG2 cells. This decrease in triglyceride levels could primarily be through two mechanisms: an increase in triglyceride withdrawal in peripheral tissues and an over-activation of the beta-oxidation. Fenofibrates modulate plasma lipoprotein levels by inducing lipolysis and increasing the hepatic fatty acid uptake and reducing triglyceride synthesis in the liver [30]. In this manner, we investigated in HEPG2 cells how the treatment with a potent agonist of PPARα raised the gene expression of PPARα, ACOX, CPTI and ApoA5 (genes involved in lipid metabolism). Apolipoprotein AV (APOA5) has been reported to stimulate lipolytic LPL activity [31], while CPT1 is an initiating step in the mitochondrial oxidation of long-chain fatty acids [32], and ACOX controls mitochondrial beta-oxidation, which could modulate HTG. We also observed a decrease in MTP gene expression in HEPG2 with the PPARα agonist. MTP is a molecular chaperone that catalyzes the rate-limiting step in hepatic

VLDL-triglyceride production [33]. Thus, the effect of fenofibrate treatment on the reduction in triglycerides could be explained by these mechanisms.

In the current study, besides the increase in PPARα, we observed a significant increase in RXRα and IL-8 gene expression in PBMCs after fenofibrate treatment. These gene increases could be linked with the increase in the CPT1 expression observed in HEPG2 cells, producing a decrease in lipid levels. We also showed an increase in serum levels of ApoAI and HDL cholesterol and a decrease in serum levels of total cholesterol after fenofibrate treatment. This may indicate a possible activation in the reverse route of cholesterol transport, and/or increase in LDL receptors [34] or hepatic ApoAI production as a response to the rise in PPARα (Apo AI is a target gene of PPARα) [35].

After fenofibrate treatment we found a significant increase in postprandial CPT1a and ACOX1 expressions in PBMCs. Moreover, we confirmed these results with an *in vitro* experiment using a luciferase gene reporter merged to a CPT1a-DR1 site. This experiment showed that both fenofibrate and oleic acid (the main fatty acid present in the fat overload test used) could increase PPARα activity and transcription of its target genes. Furthermore, we confirmed that in these cells fenofibrate raised the native PPARα targets, such as CPT1a, ACOX and ApoA5, as well as the expression of the PPARα gene itself, whereas MTP gene expression was down-regulated (Figure 2). These results may help to explain the hypotriglyceridemic effects of fenofibrate through its effects on the liver as main target.

On the other hand, activation of PPARα has also been reported to participate in inflammation and vascular function, which can affect the atherosclerotic processes [36]. PPARα could inhibit the expression relative to the inflammation process [36] although other studies showed that fenofibrate had no effect on inflammatory markers [37]. However, in our study we could not confirm this effect on the inflammatory markers measured; although we did find a significant increase in the IL8 gene expression at baseline after fenofibrate treatment.

Although the greatest strength of this study is its novelty in the approach using the PBMC cells approach to study fenofibrate effects, there are several limitations to this study. First of all, we could not detect an up-regulation of the ApoA5 and MTP genes in PBMCs, while in in vitro experiments were detected. PBMCs probably do not have the capacity to express these genes due to we did not find any expression and, until our knowledge, no references reported the expression in the literature. However, the rest of the measured genes have followed similar trends in both experiments. Secondly, our results were generated from gene expression data, and would need replication and a higher battery of lipid metabolism genes. However, these results may open a new approach to further investigate the still non-understood mechanism of the fenofibrate drugs in the fight against the HTG.

Conclusion

To the best of our knowledge, this is the first study to describe the molecular effects of treatment with fenofibrate in patients with hypertriglyceridemia *in vivo* using PBMC cells. We found that fenofibrate is able to modify the expression of key genes involved in lipid metabolism as *in vivo* as in vitro, and consequently produce effects on basal and postprandial metabolism. Thus, we conclude that PBMCs are a good and available material to study *in vivo* the effects of fenofibrate on lipid metabolism.

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Contributions

The authors have no multiplicity of interest to disclose. IMI contributed to the study conception and design, analysis and interpretation of data, drafting, reviewing, and critically revising the manuscript. FJT contributed to the study conception and design, analysis and interpretation of data, reviewed the article and critically revised it for important intellectual content. MCP performed PBMCs expression experiments. DCC performed PBMCs expression experiments, interpretation of data, drafting, reviewing, and critically revising the manuscript. JCFG contributed with the study conception and design, interpretation of data and revision of the article. MMG contributed performing in vitro experiment with HEPG2. MIQO to the study conception and design, analysis and interpretation of data, reviewed the article and critically revised it for important intellectual content. FC contributed to the study conception and design, analysis and interpretation of data, reviewed the article and critically revised it for important intellectual content and gave final approval of the version to be published.

References

1 Chahil, T.J. & Ginsberg, H.N. (2006) Diabetic dyslipidemia. *Endocrinol Metab Clin North Am* **35**, 491-510, vii-viii.

2 Eckel, R.H., Alberti, K., Grundy, S.M. & Zimmet, P.Z. (2005) The metabolic syndrome. *The Lancet* **375**, 181-183.

3 Sarwar, N., Sandhu, M.S., Ricketts, S.L., Butterworth, A.S., Di Angelantonio, E., Boekholdt, S.M., Ouwehand, W., Watkins, H., Samani, N.J., Saleheen, D., Lawlor, D., Reilly, M.P., Hingorani, A.D., Talmud, P.J. & Danesh, J. (2010) Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. *Lancet* **375**, 1634-1639.

4 Wong, K. & Ryan, R.O. (2007) Characterization of apolipoprotein A-V structure and mode of plasma triacylglycerol regulation. *Curr Opin Lipidol* **18**, 319-324.

5 Delgado-Lista, J., Perez-Jimenez, F., Ruano, J., Perez-Martinez, P., Fuentes, F., Criado-Garcia, J., Parnell, L.D., Garcia-Rios, A., Ordovas, J.M. & Lopez-Miranda, J. (2010) Effects of variations in the APOA1/C3/A4/A5 gene cluster on different parameters of postprandial lipid metabolism in healthy young men. *J Lipid Res* **51**, 63-73.

6 Wierzbicki, A.S. (2006) Fibrates after the FIELD study: Some answers, more questions. *Diab Vasc Dis Res* **3**, 166-171.

7 Pirillo, A., Norata, G.D. & Catapano, A.L. (2014) Postprandial lipemia as a cardiometabolic risk factor. *Curr Med Res Opin* **30**, 1489-1503.

8 Koh, K.K., Quon, M.J., Lim, S., Lee, Y., Sakuma, I., Lee, Y.H., Han, S.H. & Shin, E.K. (2011) Effects of fenofibrate therapy on circulating adipocytokines in patients with primary hypertriglyceridemia. *Atherosclerosis* **214**, 144-147.

9 Farnier, M. (2008) Update on the clinical utility of fenofibrate in mixed dyslipidemias: mechanisms of action and rational prescribing. *Vasc Health Risk Manag* **4**, 991-1000.

10 Hegele, R.A. & Pollex, R.L. (2007) Apolipoprotein A-V genetic variation and plasma lipoprotein response to fibrates. *Arterioscler Thromb Vasc Biol* **27**, 1224-1227.

11 Araki, H., Tamada, Y., Imoto, S., Dunmore, B., Sanders, D., Humphrey, S., Nagasaki, M., Doi, A., Nakanishi, Y., Yasuda, K., Tomiyasu, Y., Tashiro, K., Print, C., Charnock-Jones, D.S., Kuhara, S. & Miyano, S. (2009) Analysis of PPARalpha-dependent and PPARalpha-independent transcript regulation following fenofibrate treatment of human endothelial cells. *Angiogenesis* **12**, 221-229.

12 Hegele, R.A., Ginsberg, H.N., Chapman, M.J., Nordestgaard, B.G., Kuivenhoven, J.A., Averna, M., Boren, J., Bruckert, E., Catapano, A.L., Descamps, O.S., Hovingh, G.K., Humphries, S.E., Kovanen, P.T., Masana, L., Pajukanta, P., Parhofer, K.G., Raal, F.J., Ray, K.K., Santos, R.D., Stalenhoef, A.F., Stroes, E., Taskinen, M.R., Tybjaerg-Hansen, A., Watts, G.F. & Wiklund, O. (2014) The polygenic nature of hypertriglyceridaemia: implications for definition, diagnosis, and management. *Lancet Diabetes Endocrinol* **2**, 655-666.

13 Lalloyer, F. & Staels, B. (2010) Fibrates, glitazones, and peroxisome proliferator-activated receptors. *Arterioscler Thromb Vasc Biol* **30**, 894-899.

14 Balanarasimha, M., Davis, A.M., Soman, F.L., Rider, S.D., Jr. & Hostetler, H.A. (2014) Ligand-regulated heterodimerization of peroxisome proliferator-activated receptor alpha with liver X receptor alpha. *Biochemistry* **53**, 2632-2643.

15 Gao, Y., Shen, W., Lu, B., Zhang, Q., Hu, Y. & Chen, Y. (2014) Upregulation of hepatic VLDLR via PPARalpha is required for the triglyceride-lowering effect of fenofibrate. *J Lipid Res* **55**, 1622-1633.

16 Burczynski, M.E. & Dorner, A.J. (2006) Transcriptional profiling of peripheral blood cells in clinical pharmacogenomic studies. *Pharmacogenomics* **7**, 187-202.

17 D'Amore, S., Vacca, M., Graziano, G., D'Orazio, A., Cariello, M., Martelli, N., Di Tullio, G., Salvia, R., Grandaliano, G., Belfiore, A., Pellegrini, F., Palasciano, G. &

Moschetta, A. (2013) Nuclear receptors expression chart in peripheral blood mononuclear cells identifies patients with Metabolic Syndrome. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1832**, 2289-2301.

18 Bouwens, M., Afman, L.A. & Muller, M. (2007) Fasting induces changes in peripheral blood mononuclear cell gene expression profiles related to increases in fatty acid beta-oxidation: functional role of peroxisome proliferator activated receptor alpha in human peripheral blood mononuclear cells. *Am J Clin Nutr* **86**, 1515-1523.

19 Frazier-Wood, A.C., Aslibekyan, S., Borecki, I.B., Hopkins, P.N., Lai, C.Q., Ordovas, J.M., Straka, R.J., Tiwari, H.K. & Arnett, D.K. (2012) Genome-wide association study indicates variants associated with insulin signaling and inflammation mediate lipoprotein responses to fenofibrate. *Pharmacogenet Genomics* **22**, 750-757.

20 Expert Panel on Detection, E., and Treatment of High Blood Cholesterol in Adults (2001) Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *Jama* **285**, 2486-2497.

21 Cardona, F. & Tinahones, F.J. (2011) Composition useful for the detection of postprandial hypertriglyceridemia. *Patent number P201030776*.

22 Reiner, Z., Catapano, A.L., De Backer, G., Graham, I., Taskinen, M.R., Wiklund, O., Agewall, S., Alegria, E., Chapman, M.J., Durrington, P., Erdine, S., Halcox, J., Hobbs, R., Kjekshus, J., Filardi, P.P., Riccardi, G., Storey, R.F. & Wood, D. (2011) ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J* **32**, 1769-1818.

23 Cardona, F., Tunez, I., Tasset, I., Garrido-Sanchez, L., Collantes, E. & Tinahones, F.J. (2008) Circulating antioxidant defences are decreased in healthy people after a high-fat meal. *Br J Nutr* **100**, 312-316.

24 Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. & Turner, R.C. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412-419.

25 Klotz, L., Schmidt, M., Giese, T., Sastre, M., Knolle, P., Klockgether, T. & Heneka, M.T. (2005) Proinflammatory stimulation and pioglitazone treatment regulate peroxisome proliferator-activated receptor gamma levels in peripheral blood mononuclear cells from healthy controls and multiple sclerosis patients. *J Immunol* **175**, 4948-4955.

26 Bouwens, M., Afman, L.A. & Muller, M. (2008) Activation of peroxisome proliferator-activated receptor alpha in human peripheral blood mononuclear cells reveals an individual gene expression profile response. *BMC Genomics* **9**, 262.

27 Moreno-Santos, I., Pavon, F.J., Romero-Cuevas, M., Serrano, A., Cano, C., Suardiaz, M., Decara, J., Suarez, J., de Fonseca, F.R. & Macias-Gonzalez, M. (2014) Computational and biological evaluation of N-octadecyl-N'-propylsulfamide, a selective PPARalpha agonist structurally related to N-acylethanolamines. *PLoS One* **9**, e92195.

28 Moreno-Santos, I., Pavón, F.J., Romero-Cuevas, M., Serrano, A., Cano, C., Suardíaz, M., Decara, J., Suarez, J., de Fonseca, F.R. & Macías-González, M. (2014) Computational and Biological Evaluation of N-octadecyl-N′-propylsulfamide, a Selective PPARα Agonist Structurally Related to N-acylethanolamines. *PLoS One* **9**, e92195.

29 Kimura, R., Takahashi, N., Murota, K., Yamada, Y., Niiya, S., Kanzaki, N., Murakami, Y., Moriyama, T., Goto, T. & Kawada, T. (2011) Activation of peroxisome proliferator-activated receptor-alpha (PPARalpha) suppresses postprandial lipidemia through fatty acid oxidation in enterocytes. *Biochem Biophys Res Commun* **410**, 1-6.

30 Staels, B., Dallongeville, J., Auwerx, J., Schoonjans, K., Leitersdorf, E. & Fruchart, J.C. (1998) Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* **98**, 2088-2093.

31 Prieur, X., Coste, H. & Rodriguez, J.C. (2003) The human apolipoprotein AV gene is regulated by peroxisome proliferator-activated receptor-alpha and contains a novel farnesoid X-activated receptor response element. *J Biol Chem* **278**, 25468-25480.

32 Nakamura, M.T., Yudell, B.E. & Loor, J.J. (2014) Regulation of energy metabolism by long-chain fatty acids. *Prog Lipid Res* **53**, 124-144.

33 Hussain, M.M., Nijstad, N. & Franceschini, L. (2011) Regulation of microsomal triglyceride transfer protein. *Clinical lipidology* **6**, 293-303.

34 Huang, Z., Zhou, X., Nicholson, A.C., Gotto, A.M., Hajjar, D.P. & Han, J. (2008) Activation of peroxisome proliferator-activated receptor-α in mice induces expression of the hepatic low-density lipoprotein receptor. *British Journal of Pharmacology* **155**, 596-605.

35 Rakhshandehroo, M., Knoch, B., Müller, M. & Kersten, S. (2010) Peroxisome Proliferator-Activated Receptor Alpha Target Genes. *PPAR Res* **2010**, 20.

36 Frazier-Wood, A.C., Ordovas, J.M., Straka, R.J., Hixson, J.E., Borecki, I.B., Tiwari, H.K. & Arnett, D.K. (2013) The PPAR alpha gene is associated with triglyceride, low-density cholesterol and inflammation marker response to fenofibrate intervention: the GOLDN study. *Pharmacogenomics J* **13**, 312-317.

37 Price, E.T., Welder, G.J. & Zineh, I. (2012) Modulatory effect of fenofibrate on endothelial production of neutrophil chemokines IL-8 and ENA-78. *Cardiovasc Drugs Ther* **26**, 95-99.

Table 1. Anthropometric and biochemical study variables

	Before fenofibrate treatment		After fenofibrate treatment	
	basal	**postprandial**	**basal**	**postprandial**
BMI (kg/m^2)	29.15±3.36		28.95±3.53	
Uric acid (mg/dL)	6.53±1.38*		4.92±1.17*	
Glucose (mg/dL)	93.07±7.61		94.76±8.86	
Cholesterol (mg/dL)	243.50±45.93*		221.65±44.51*	
Triglycerides (mg/dL)	376.70±215.67*	503.61±248.27Ψ	238.79±144.99*	339.67±162.92Ψ
HDL cholesterol (mg/dL)	39.67±8.21*	38.45±8.0 Ψ	44.06±10.39*	41.79±10.20 Ψ
Apolipoprotein A1(mg/dL)	155.91±19.06*	153.59±18.80 Ψ	162.06±28.82*	157.06±29.45 Ψ
Apolipoprotein B (mg/dL)	150.44±23.07		143.13±27.41	
GGT (mg/dL)	117.42±95.83		75.50±110.21	
Insulin (µUI/mL)	14.52±9.73		12.89±5.39	
HOMA-IR	3.33±2.17		3.04±1.40	

Values are presented as means ± SD. N= 50 subjects. *p<0.05 significant differences between basal levels before and after fenofibrate treatment; Ψ p<0.05 significant differences between postprandial levels before and after fenofibrate treatment.

Table 2. Gene expression in PBMCs before and after fenofibrate treatment and baseline and posptrandial. Data are expressed as mean ± SD.

	Before fenofibrate		**After fenofibrate**	
	Basal	**Postprandial**	**Basal**	**Postprandial**
CPT1a	0.023±0.008	0.021±0.007	0.021±0.010*	0.028±0.009 *
ACOX1	0.020±0.016	0.020±0.029	0.020±0.009*	0.036±0.026 *
PPARα	0.039±0.056*¥	0.049±0.021*	0.048±0.023¥	0.056±0.029
RXRα	0.106±0.032¥	0.103±0.025	0.149±0.037¥	0.149±0.034
SREBP1c	0.031±0.016	0.040±0.014	0.056±0.030	0.051±0.019
IL8	0.018±0.021¥	0.049 ±0.067	0.046±0.040¥	0.120±0.272
TNFα	0.039±0.021	0.044±0.019	0.040±0.013	0.058±0.029

Abbreviations: carnitine palmitoyltransferase-1A (CPT-1A), acyl-CoA oxidase (ACOX), peroxisome proliferator-activated receptor alpha (PPARα), retinoid X receptor alpha (RXRα), liver X receptor (LXR), sterol regulatory element-binding protein (SREBP1c), Interleukin 8 (IL8) and tumor necrosis factor alpha (TNFα)

*P<0.05 comparing basal and postprandial states and ¥ before and after fenofibrate treatment

Figure legends.

Figure 1. Basal and ligand-induced activity of PPARα and RXRa . Columns represent the mean of at least three experiments and bars indicate standard deviations. Two-tailed, paired Student test was performed, and P values were calculated in reference to the respective solvent control (*P<0.01, **P<0.001) to compare each ligand after 16 h of incubation.

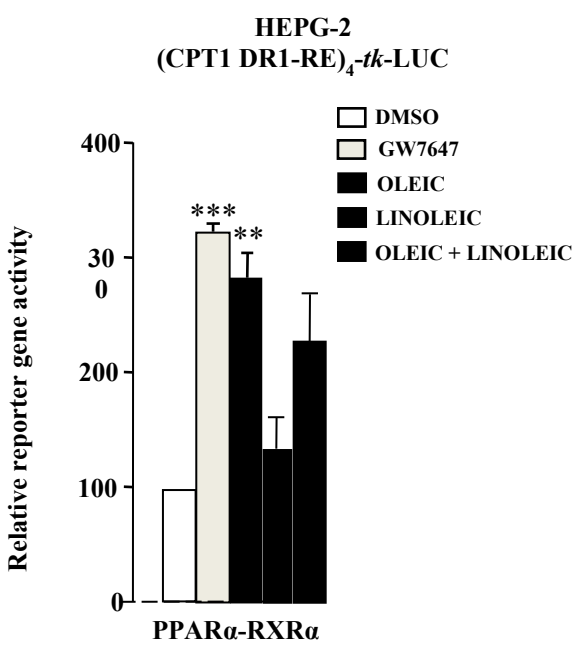

Figure 2. Gene expression fold change in HEPG2 culture submitted to a PPARα agonist (GW7647), because it is a potent and highly selective agonist. Peroxisome proliferator-activated receptor alpha (PPARalpha), carnitine palmitoyltransferase-1 (CPT1), acyl-CoA oxidase (ACOX), apolipoprotein AV (ApoA5), microsomal triglyceride transfer protein (MTP). The data are normalized to vehicle (DMSO) in each experiment. * P<0.05 statistically significant.