

Activation of intestinal Peroxisome Proliferator-Activated Receptor α increases HDL production

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Abbreviations:

ABCA1, ATP-Binding Cassette A1 transporter; ABHD5, AB-Hydrolase Domain containing 5; ACAT-2, Acyl-coenzyme A: Cholesterol Acyltransferase-2; ACOX1, Acyl-Coenzyme A oxidase 1; ACSL5, Acyl-CoA Synthetase Long-chain family member 5; apo, apolipoprotein; CE, Cholesteryl Ester; CPT-1, Carnitine Palmitoyl Transferase I; CREB3L3, cAMP responsive element binding protein 3 like 3; FC, Free Cholesterol; FF, Fenofibrate; HDL, High Density Lipoproteins; LDL, Low Density Lipoproteins; PLIN2, Perilipin 2; PPAR, Peroxisome proliferator-activated receptor;

Disclosures:

No conflicts of interest exist

Abstract

Aims: Peroxisome Proliferator-Activated Receptor (PPAR) α is a transcription factor controlling lipid metabolism in liver, heart, muscle and macrophages. PPAR α -activation increases plasma HDL-cholesterol and exerts hypotriglyceridemic actions via the liver. However, the intestine expresses PPAR α , produces HDL and chylomicrons and is exposed to diet-derived PPAR α ligands. Therefore, we examined the effects of PPAR α -activation on intestinal lipid and lipoprotein metabolism.

Methods and Results: The impact of PPAR α -activation was evaluated in term of HDL-related gene expression in mice, *ex-vivo* in human jejunal biopsies and in Caco-2/TC7 cells. ApoAI/HDL secretion, cholesterol esterification and trafficking were also studied *in-vitro*.

In parallel to improving plasma lipid profiles and increasing liver and intestinal expression of fatty-acid-oxidation genes, treatment with the dual PPAR α/δ -ligand GFT505 resulted in a more pronounced increase of plasma HDL compared to fenofibrate in mice. GFT505, but not fenofibrate, increased the expression of HDL-production genes such as apolipoprotein-AI and ATP-Binding-Cassette-A1 transporter in murine intestines. A similar increase was observed upon PPAR α -activation of human biopsies and Caco-2/TC7 cells. Additionally, HDL secretion by Caco-2/TC7 cells increased. Moreover, PPAR α -activation decreased the cholesterol-esterification capacity of Caco-2/TC7 cells, modified cholesterol trafficking and reduced apolipoprotein-B secretion.

Conclusions: PPAR α -activation reduces cholesterol esterification, suppresses chylomicron- and increases HDL-secretion by enterocytes. These results identify the intestine as a target organ of PPAR α -ligands with entero-hepatic tropism to reduce atherogenic dyslipidemia.

Key words:

PPAR α ; intestine; HDL; dyslipidemia

Introduction

High levels of low-density-lipoprotein (LDL)-cholesterol and low levels of high-density-lipoprotein (HDL)-cholesterol are risk factors for cardiovascular disease (1). In association with lifestyle intervention, LDL-cholesterol lowering therapy with statins is highly efficient to reduce cardiovascular risk, but has only modest effects on HDL-cholesterol (2). Furthermore, low HDL-cholesterol is an independent risk factor which contributes to the persistent residual cardiovascular risk in statin-treated patients (3; 4). Therefore, raising HDL may be a promising strategy to attenuate residual cardiovascular risk in statin-treated patients.

In enterocytes, dietary lipids are packaged with apolipoprotein-B48 (apoB48) into chylomicrons secreted into the lymph (5). Several studies demonstrated that post-prandial chylomicrons contribute to lipid accumulation in the arterial wall and to atherogenesis (6). Animal studies have shown that the intestine produces HDL (7). Indeed, intestinal-specific deletion of ATP-Binding-Cassette-A1 transporter (ABCA1) in mice results in a 30% decrease of HDL-cholesterol levels (8). Furthermore, exercise-training enhances HDL-cholesterol in rats in correlation with increased intestinal ABCA1 mRNA expression (9). Interestingly, human intestinal mucosa was shown to produce apolipoprotein-AI (apoAI) (10) and HDL (11).

Peroxisome Proliferator-Activated Receptor (PPAR) α is a transcription factor belonging to the nuclear receptor superfamily and activated by fibrates, eicosanoids and fatty-acids (12; 13). PPAR α controls lipid and glucose metabolism in several tissues and cell types including liver, heart, kidney, adipose tissue and macrophages (14-16). PPAR α -activation enhances hepatic fatty-acid-oxidation and peripheral triglyceride clearance, thus reducing hypertriglyceridemia (13; 17). PPAR α increases hepatic apoAI synthesis and ABCA1 expression, resulting in increased plasma HDL-cholesterol (18; 19). In addition, PPAR α -activation controls cholesterol trafficking in human macrophages and raises cholesterol efflux *via* ABCA1 contributing to increased reverse cholesterol transport (15; 20). Since diet-derived compounds (fatty-acids and phospholipids) can activate PPAR α and since the intestine is the site of dietary lipid absorption, it is highly exposed to PPAR α -ligands (21; 22). The role of PPAR α in the intestine has been addressed in only a few studies. Previous studies in rodents did not evidence pronounced effects of the PPAR α -ligand fenofibrate (FF) in the intestine contrary to the liver, possibly

because of its rapid urinary excretion and hence relatively low intestinal exposure (24). However, a genome wide analysis of intestinal RNA of mice treated with Wy14643 revealed that PPAR α controls numerous metabolic pathways and in particular lipid metabolism (25), pointing to a role for PPAR α in this organ.

We thus investigated whether intestinal PPAR α -activation regulates HDL production. Treatment of mice with two different PPAR-agonists, FF, a pure PPAR α -agonist and GFT505 (26), a PPAR α/δ -modulator, which, unlike FF, undergoes extensive enterohepatic cycling, resulted in comparable hypotriglyceridemic effects. However, only GFT505 treatment increased apoAI and ABCA1 mRNA levels in the murine intestine, an effect which was associated with a more pronounced increase of plasma HDL levels. These effects were not observed in PPAR α -deficient mice. Using the human Caco-2/TC7 model cultured on porous filters, we found that PPAR α -activation reduces secretion of chylomicrons while enhancing HDL production probably by increasing ABCA1 expression, apoAI secretion and by reducing cholesterol esterification. The physiological relevance of these regulatory processes was verified in human jejunal biopsies. Collectively, our data suggest that the intestine is a target organ for entero-hepato-tropic PPAR α -ligands to increase HDL, an effect which may result in a reduction of residual cardiovascular risk.

Materials and methods

For details, see supplementary materials and methods (supp.M&M).

Animal study

Wild-type (+/+) and homozygous (-/-) PPAR α -deficient female mice in the apoE2-KI background (12 week-old) fed a western-diet were treated for 14d with GFT505 (10 or 30mpk) or FF (200mpk) or carboxy-methyl-cellulose (0.5%) (27).

Human intestine culture

Intestinal biopsies were recovered during gastric bypass surgery from obese patients enrolled in the ABOS study (ClinicalTrials.gov; NCT01129297).

Caco-2/TC7 cell culture and PPAR α knock-down

Caco-2/TC7 were grown as described (28). For stable PPAR α invalidation (29) and culture details, see supp.M&M.

HDL preparation

HDL (d=1.12-1.21g/ml) from human plasma and basolateral media of Caco-2/TC7 were prepared by sequential ultracentrifugations. For details in preparation, electron microscopy and apolipoprotein quantification, see supp.M&M.

Post-prandial micelle preparation

Synthetic micelles prepared as described (28).

Confocal microscopy

For details, see supp.M&M and (30).

Cholesterol esterification assay

Caco-2/TC7 were incubated with [³H]-cholesterol-micelles (7.5μCi/well), lipids were extracted and separated by TLC.

Macrophage cell culture and cholesterol efflux

Mononuclear cells were isolated from blood of healthy donors, cholesterol-loaded with [³H]-cholesterol-AcLDL and efflux assays performed as described (15).

Statistics

For details, see supp.M&M.

Results

PPAR α activation in mice and in human jejunal biopsies increases the expression of genes involved in intestinal HDL production.

To determine whether PPAR α -activation regulates HDL production in the small intestine, apoE2-KI mice, which display a similar lipid-response to PPAR α -agonists as humans (36), were fed a western-diet with daily oral administration of FF or the dual PPAR α / δ -agonist GFT505 for 14d. As reported (36), FF decreased plasma triglycerides (TG) and total-cholesterol (tab1). Similarly, GFT505 lowered plasma TG and total-cholesterol. Whereas plasma HDL-cholesterol increased approximately 2.5-fold upon treatment with FF, the increase of HDL-cholesterol was significantly more pronounced upon GFT505 (tab1). FF and GFT505 induced the hepatic expression of PPAR α target genes, such as Acyl-CoenzymeA-oxidase-1 (ACOX1) at a similar extent (supp.fig1) indicating equipotent dosing. The effects of GFT505 on TG and HDL-cholesterol were abolished in PPAR α -deficient apoE2-KI mice, demonstrating that the effects are PPAR α -dependent (supp.tab1). Interestingly, a decrease, albeit less pronounced, of total and nonHDL-cholesterol was maintained in GFT505-treated PPAR α -deficient apoE2-KI mice, suggesting a contribution of its PPAR δ activity on these lipid parameters (supp.tab1). GFT505-treated mice displayed a PPAR α dependent higher ABCA1 and apoAI gene expression in the small intestine compared to untreated mice (fig1A, fig1B), whereas FF did not regulate ABCA1 nor apoAI mRNA expression (fig1C, fig1D). Treatment of mice with GW0742, a pure PPAR δ -agonist, did not modify ABCA1 nor ApoAI gene expression (not shown).

To determine whether PPAR α -activation also regulates these genes in human intestine, human jejunal biopsies were treated with GFT505 and a synthetic PPAR α -ligand, GW7647, for 18h. Both compounds significantly increased ABCA1 and apoAI mRNA expression (fig1E, fig1F).

PPAR α -activation increases the production of HDL in Caco-2/TC7.

Based on observations in mice (8), we hypothesized that the up-regulation of ABCA1 and apoAI gene expression observed upon PPAR α -activation would translate in increased intestinal HDL production. To test this, we used an *in-vitro* model of the human intestinal barrier, polarized Caco-2/TC7 cells grown on porous filters (28; 32). PPAR α expression was knocked-down (ShPPAR α) or

not (ShControl) by lentiviral infection and cells were subsequently incubated with GW7647. HDL secreted by Caco-2/TC7 were isolated by ultracentrifugation and analyzed by electron microscopy. PPAR α -activation increased the number of HDL secreted at the basolateral side of Caco-2/TC7 (fig2A). ApoAI secreted in the HDL fraction increased when non-infected Caco-2/TC7 and ShControl-infected cells were treated with GW7647, whereas apoAI secretion was not modified upon treatment of ShPPAR α -infected cells by GW7647 (fig2B), demonstrating that this effect is PPAR α -dependent. Interestingly, the quantity of apoAI in the chylomicron fraction was not modified (not shown). To evaluate the HDL functionality, we investigated their capacity to promote cholesterol efflux from macrophages. Human monocyte-derived macrophages were cholesterol-loaded with [³H]-cholesterol-AcLDL and subsequently incubated with HDL secreted by Caco-2/TC7 treated with GW7647 for 24h. Equivalent volumes of isolated HDL from non-infected or ShControl-infected Caco-2/TC7 treated with GW7647 enhanced macrophage cholesterol efflux compared to HDL isolated from vehicle-treated cells (fig2C). HDL from ShPPAR α -infected cells treated with GW7647 did not show enhanced efflux capacity compared to HDL from untreated cells. ABCA1 gene expression increased upon GW7647 treatment in a PPAR α -dependent manner (fig2D). A similar up-regulation was observed with GFT505 (supp.fig2). In contrast to the *in-vivo* and *ex-vivo* response, apoAI mRNA was not induced by PPAR α -agonists in Caco-2/TC7 (not shown).

PPAR α -activation modulates cholesterol distribution, increases cytoplasmic lipid droplet formation and reduces apoB secretion in Caco-2/TC7.

To test the effect of PPAR α -activation on intestinal cholesterol absorption known to occur in sequential steps, from uptake at the brush border membrane and transport into the cell to packaging and secretion of lipoproteins at the basolateral side of enterocytes (33), Caco-2/TC7 were incubated for 24h with [³H]-cholesterol-micelles and activated with GW7647 24h before and 24h after cholesterol-loading. Cholesterol content in the apical medium, the cellular compartment and the basolateral medium of Caco-2/TC7 was not modulated by GW7647 (not shown), suggesting that PPAR α regulates neither total cholesterol uptake nor efflux by enterocytes. Next, GW7647-treated Caco-2/TC7 were incubated with fluorescent-NBD-cholesterol-containing micelles for 4h, followed

by non-fluorescent micelles for an additional 4h period. Quantitative analysis of fluorescence intensity at each optical slice showed that, without affecting the total amount of absorbed cholesterol (assessed by radioactive assay), GW7647 treatment modifies cholesterol distribution inducing retention of cholesterol in the subapical compartments (fig3A). Interestingly, as shown by confocal microscopy (fig3B), GW7647 also increased the size of cholesterol-containing lipid droplets in the apical and basolateral compartments.

Since PPAR α -activation in Caco-2/TC7 does not affect the total amount of cholesterol uptake and efflux, we hypothesized that PPAR α -activation may decrease apoB-containing lipoprotein secretion to counterbalance the increase of secreted HDL. Indeed, GW7647 decreased apoB secretion in the basolateral compartment of Caco-2/TC7 (fig3C) as previously shown (34).

PPAR α -activation decreases cholesterol esterification capacity of Caco-2/TC7.

Cholesterol trafficking from the plasma membrane to the endoplasmic reticulum is a limiting step for its esterification and subsequent secretion in chylomicrons (35). To address whether, by interfering with cholesterol trafficking, PPAR α reduces cholesterol esterification, Caco-2/TC7 were incubated for 24h with [³H]-cholesterol-micelles and activated with GW7647 24h before and 24h after cholesterol loading. PPAR α -activation decreased the quantity of [³H]-cholesteryl esters (CE) assayed by TLC both in the cells (fig4A) and secreted in the basolateral compartment (fig4B). The decreased CE production does not result only from a perturbation of cholesterol trafficking since ACAT-2 (acyl-coenzymeA:cholesterol-acyltransferase-2) gene expression was also decreased (fig4C).

PPAR α -activation increases the expression of lipid droplet formation-genes in Caco-2/TC7 cells.

To investigate the mechanism behind the increase of cytoplasmic lipid droplet size upon PPAR α -activation (fig3B), we analyzed the expression of lipid droplet-associated proteins. PPAR α -activation increased mRNA levels of perilipin-2 (PLIN2), which coats lipid droplets with its partner ABHD5 (AB-hydrolase-domain-containing-5) (fig5A) (36). In line, ABHD5 gene expression was also increased (fig5B). Furthermore, the increase of PLIN2 was strongly reduced and the increase of

ABHD5 was totally abolished in ShPPAR α -infected cells treated with GW7647 (supp.fig3A, supp.fig3B). In addition, PLIN2 and ABHD5 mRNA levels increased only in intestines of GFT505-treated PPAR α ^{+/+}, but not PPAR α ^{-/-} apoE2-KI mice (supp.fig3C, suppl.fig3D). The increased PLIN2 and ABHD5 gene expression was also observed, albeit less pronounced, in human jejunal biopsies treated with GW7647 or GFT505 (fig5C, fig5D).

PPAR α -activation increases fatty-acid-oxidation genes in human enterocytes and murine intestines.

In addition to the decreased apoB secretion (fig3C) and in accordance with previous data showing that intestinal PPAR α -activation suppresses postprandial lipidemia by increasing fatty-acid-oxidation (25; 34), PPAR α ligands increased mRNA levels of fatty-acid-oxidation genes including Carnitine-Palmitoyl-Transferase-1 (CPT-1A), ACOX1 and long-chain Acyl-CoA synthetase family member 5 (ACSL5) (fig6A, fig6B, fig6C) in Caco-2/TC7. CPT-1A and ACOX1 mRNA levels were increased in human biopsies treated with PPAR α -ligands (fig6D, fig6E). The effect of GW7647 on CPT-1A and ACSL5 was strongly reduced in treated ShPPAR α -infected cells and totally abolished for ACOX1 (supp.fig4A, supp.fig4B, supp.fig4C). GFT505 treatment increased the intestinal expression of fatty-acid-oxidation genes in a PPAR α -dependent manner in apoE2-KI mice (supp.fig4D, supp.fig4E). The expression of fatty-acid-oxidation genes was induced in intestines of FF-treated mice, in contrast to the lack of regulation of HDL-related genes, suggesting that FF acts as a selective PPAR modulator in the intestine (supp.fig4F, supp.fig4G, supp.fig4H).

Interestingly, PPAR α -ligands increased cAMP-responsive-element-binding protein-3-like-3 (CREB3L3) mRNA levels in Caco-2/TC7 (supp.fig5A), a transcription factor involved in the regulation of TG metabolism for which mutations were recently associated with hypertriglyceridemia in humans (37). The induction of CREB3L3 was observed in human biopsies treated with GW7647 and GFT505 (supp.fig5B). Furthermore, PPAR α -ligands did not regulate this gene in ShPPAR α -infected cells nor in PPAR α -deficient mice (supp.fig5C, supp.fig5D). Collectively, these results indicate that PPAR α -activation increases the fatty-acid-oxidation pathway, the transcription factor CREB3L3 and increases lipid droplet size.

Discussion

In addition to the liver, the intestine has emerged more recently as a central organ participating in lipid metabolism by regulating lipid absorption, trafficking and lipoprotein metabolism through its involvement in the production of chylomicrons (5; 32), HDL (8) and in trans-intestinal cholesterol excretion (38). Thus, both the liver and intestine are involved in the regulation of whole body lipoprotein metabolism and play a role in the pathophysiology of atherogenic dyslipidemia (39; 40). Fibrates, PPAR α -agonists, are often used in the treatment of dyslipidemia characterized by high TG and low HDL-cholesterol levels. Until now, the effects of PPAR α -agonists on the improvement of lipid profiles were essentially attributed to their action on the liver and the arterial wall (41; 42). However, PPAR α is also expressed in the intestine. In a genome wide analysis of intestinal RNA isolated from PPAR α -ligand-treated mice, intestinal PPAR α was found to regulate fatty-acid metabolism (25). Moreover, PPAR α -activation decreased the secretion of apoB by enterocytes (34). Therefore, we studied whether intestinal PPAR α -activation could contribute to the control of HDL metabolism.

Using a humanized mouse model of mixed dyslipidemia, the apoE2-KI mouse, we show in line with previous studies that FF and GFT505-treatments improve the lipid profile by decreasing TG, total cholesterol and by increasing HDL-cholesterol (27; 31). We show that PPAR α mediates the effects of GFT505 on TG and HDL-cholesterol levels. Although all clinical studies illustrate the hypotriglyceridemic efficiency of PPAR α -agonists, the effects on HDL levels are variable. Such discrepancies could be explained by differences in population selection and heterogeneity or in the molecule used (43-45). In our study, the HDL-increasing effect of GFT505 was more pronounced than FF at concentrations (10mpk vs 100mpk) inducing similar expression levels of ACOX1 in the liver. Although FF did induce intestinal fatty-acid-oxidation-related genes, suggesting that FF can activate intestinal PPAR α , only GFT505 induced expression of intestinal HDL production-related genes such as apoAI and ABCA1. Thus, GFT505 and FF differ in the spectrum of regulated target genes, giving the rationale to design new selective PPAR modulators. Furthermore, the different activity of fenofibrate vs GFT505 on intestinal gene expression is due to the extensive enterohepatic recycling

and fecal excretion of GTF505, whereas fenofibrate is excreted in the urine (Rubenstrunk A and al., manuscript in preparation).

Using the Caco-2/TC7 model (28), we confirmed that PPAR α -ligands decrease apoB secretion (34), suggesting a decrease of TG-rich lipoprotein secretion. Moreover, we showed that PPAR α -activation increased ABCA1 gene expression and apoAI secretion. Although the previously reported absence of apoAI mRNA regulation (46) demonstrates the limits of the Caco-2/TC7 model (apoAI mRNA was increased in PPAR α -agonist-treated human jejunal biopsies), PPAR α -activation increased the number of HDL secreted, which in turn enhanced cholesterol efflux from human monocyte-derived macrophages.

As we did not observe any difference in the levels of apical cholesterol uptake nor in the levels of cholesterol secreted at the basal side of treated Caco-2/TC7, we hypothesized that PPAR α -activation induces a shift of the absorbed cholesterol from the chylomicron to the HDL production pathway. This hypothesis is strengthened by the fact that less cholesterol was esterified in treated cells. The decrease of esterification may be explained by a decrease of ACAT-2 gene expression which results, as shown in human hepatic cells and macrophages (47; 48), in a decrease of ACAT-2 activity which is also under the control of substrate availability at its site of action, the endoplasmic reticulum (35). By investigating cholesterol trafficking using a fluorescent cholesterol derivative, we observed cholesterol retention in the sub-apical compartment of GW7647-treated Caco-2/TC7 and an increase of lipid droplet size concomitantly with an increase of PLIN2 expression, which is necessary for lipid droplet stabilization, and ABHD5, sequestered by PLIN2 at the lipid surface (49). We did not observe any regulation of Rab proteins which are required for vesicular trafficking, nor NPC1 and NPC2 (not shown) which contrasts to macrophages (47). Thus, the mechanism responsible for the effects of PPAR α -activation on intracellular cholesterol trafficking remains to be clarified. However, the decrease of ACAT-2 gene expression was concomitant to an increase of CPT-1A expression, which could decrease the availability of fatty-acid for cholesterol esterification, as shown in macrophages (47). In line, PPAR α -activation increased the esterase-arylacetamide-deacetylase (AADAC) (not shown) and ACSL5 gene expression, both associated with a reduction of TG accumulation and an

increase of fatty-acid-oxidation in rodent hepatocytes (50). Different mechanisms could thus act in concert to divert cholesterol from esterification: decrease of ACAT-2 gene expression, reduction of fatty-acid availability through an increase of CPT-1A expression and fatty-acid-oxidation enzymes and/or retention of cholesterol in lipid droplets.

It has been recently shown that a mutation of CREB3L3 in humans is associated with hypertriglyceridemia. CREB3L3-deficient mice are hypertriglyceridemic and display defective TG clearance (37). Interestingly, we observed a PPAR α -dependent up-regulation of CREB3L3 expression in GW7647-treated Caco-2/TC7 and in GFT505-treated mice. The importance of the regulation of this gene expression and its implication in the modulation of lipid homeostasis upon PPAR α -activation still remain to be established.

The results of our study collectively show that the increase of ABCA1 expression, the modification in cholesterol trafficking and the decreased cholesterol esterification capacity induced by PPAR α -activation potentially contribute to the overall effects on intestinal secretion of apoAI-containing HDL by Caco-2/TC7. The transcriptional regulation of HDL-related genes was observed in mice and in human jejunal biopsies. Therefore, the decrease of TG and the increase of HDL levels observed in PPAR α -agonist-treated patients could be due, in addition to hepatic actions, to effects in the intestine. Our work strengthens the idea that the intestine is an important target organ for strategies to increase HDL production via activation of intestinal PPAR α . Moreover, our data demonstrate the relevance of designing new specific PPAR α modulators with appropriate tissue and gene selective profiles.

Funding:

This work was supported by grants from Université Lille 2, Région Nord/Pas-de-Calais, the FEDER and the “Fondation Leducq”.

Acknowledgements:

We thank M. Tardivel, C. Allet and A. Loyens of the BICeL (IMPRT- IFR114) for access to confocal microscopy and electron microscopy platforms and technical advices.

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Figure and Table Legends

Table 1: Lipid profiles of apoE2-KI mice treated with FF or GFT505

ApoE2-KI mice on a western-diet were treated by oral gavage with FF (200mpk) or GFT505 (10mpk) or vehicle for 14d (n=6 mice/group). Values are expressed as means \pm SEM. ANOVA; *P<.05; **P<.01; ***P<.001 vs untreated mice; #P<.05 vs FF-treated mice.

Figure 1: PPAR α -activation with GFT505 *in-vivo* in mice and GFT505 and GW7647 *ex-vivo* in human jejunal biopsies increases the expression of genes involved in HDL production

Q-PCR analysis of ABCA1 (A, C, E) and apoAI (B, D, F) was performed on RNA isolated from scrapped intestinal mucosa (A, B, C, D) of GFT505 (30mpk) or FF (200mpk)-treated apoE2-KI mice for 14d and from human jejunal biopsies (E, F) treated with GW7647 (600nM) and GFT505 (1 μ M) for 18h. *In vivo* values are expressed as means \pm SEM and *ex vivo* values as means \pm SD. t test; *, P<0.05; ***,P<0.001 (Vehicle vs treatment).

Figure 2: GW7647 increases the production of HDL by Caco-2/TC7

Caco-2/TC7 (non-infected, ShControl- or ShPPAR α -infected) were treated with GW7647 (600nM) for 48h (Q-PCR and ELISA analysis) or 72h (cholesterol efflux assay).

(A) Number of HDL secreted by non-infected Caco-2/TC7 per equivalent volume. (B) ELISA analysis of apoAI in the HDL fraction (representative of 3 independent experiments, values are expressed as means \pm SEM). (C) Cholesterol-loaded primary human macrophages were incubated with HDL secreted by Caco-2/TC7 (representative of 2 independent experiments, values are expressed as means \pm SEM). (D) Q-PCR analysis of ABCA1. Values are expressed as means \pm SD. t-test; *P<.05; **P<.01; ***P<.001 (Vehicle vs treatment; #P<.05 treated ShControl- vs treated-ShPPAR α -infected cells).

Figure 3: PPAR α -activation modulates intracellular cholesterol trafficking, increases cytoplasmic lipid droplet formation and reduces apoB secretion by Caco-2/TC7

Caco-2/TC7 were incubated with NBD-cholesterol-micelles (50 μ g/well) for 4h and then for 4h with non-fluorescent micelles. GW7647 (600nM) was added 24h before and during the NBD-cholesterol-loading period (representative of 3 independent experiments). (A) NBD-fluorescence was quantified using Image J at each optical slice. (B) NBD-cholesterol (green), ZO-1 (red) and Hoechst (blue) were visualized by confocal microscopy. (C) ApoB in the basolateral medium was quantified by ELISA. Values are expressed as means \pm SEM. t-test; *P<.05 Vehicle vs treatment.

Figure 4: PPAR α -activation decreases cholesterol esterification in Caco-2/TC7

Caco-2/TC7 were incubated with [³H]-cholesterol-micelles (7.5 μ Ci/well) for 48h. GW7647 (600nM) was added 24h before and during the [³H]-cholesterol-loading. Intracellular lipids (A) and lipids secreted in the basolateral compartment (B) were extracted and separated by TLC. Results are expressed as means of CE percent of total cholesterol (\pm SEM). Q-PCR analysis of ACAT-2 (C) was performed on GW7647 (600nM)-treated Caco-2/TC7 for 24h. Values are expressed as means \pm SD. t-test; *P<.05; **P<.01 Vehicle vs treatment.

Figure 5: PPAR α -activation increases cytoplasmic lipid droplet formation-associated genes in Caco-2/TC7 and in human jejunal biopsies

Q-PCR analysis of PLIN2 (A, C) and ABHD5 (B, D) was performed on RNA isolated from Caco-2/TC7 (A, B) and human jejunal biopsies (C, D) treated with GW7647 (600nM) or GFT505 (1 μ M) for 24h or 18h respectively. Values are expressed as means \pm SD. t test; *P<.05; **P<.01; ***P<.001 Vehicle vs treatment.

Figure 6: PPAR α -activation increases fatty-acid oxidation genes in Caco-2/TC7 and in human jejunal biopsies

Q-PCR analysis of CPT-1 (A, D), ACOX1 (B, E) and ACSL5 (C) was performed on RNA isolated from Caco-2/TC7 (A, B, C) or human jejunal biopsies (D, E) treated with GW7647 (600nM) or GFT505 (1 μ M) for 24h or 18h respectively. Values are expressed as means \pm SD. t-test; *P<.05; **P<.01; ***P<.001 Vehicle vs treatment.