DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Oleuropein-induced acceleration of CYP-catalyzed drug metabolism: central role for nuclear receptor PPARα

Foteini Malliou[1#], Christina E. Andriopoulou[1#], Frank J. Gonzalez[2], Aristeidis Kofinas[1], Alexios-Leandros Skaltsounis[3], Maria Konstandi[1]*

[1]University of Ioannina, School of Health Sciences, Faculty of Medicine, Department of Pharmacology, Ioannina GR-45110, Greece;; [2]Laboratory of Metabolism, National Cancer Institute, National Institutes of Health, Bethesda 20892, Maryland, USA; [3]Faculty of Pharmacy, School of Health Sciences, University of Athens, Greece

Corresponding author: Maria Konstandi, E-mail: mkonstan@uoi.gr; Tel: +3026510 07554, Fax: +3026510 07859.

#These authors have equally contributed

Keywords: CYP induction; CYP1A; CYP2C; CYP2D; CYP3A; peroxisome proliferator-activated receptors; drug metabolism

Running title: Oleuropein induces major CYPs via PPARα activation

Number of text pages: 22

Number of tables: 1

Number of figures: 8

Number of references: 105

Number of words in abstract: 218

Number of words in introduction: 925

Number of words on discussion:1824

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Abbreviations: ACN, acetonitrile; CYP, cytochrome P450; C, control; CAR, constitutive androstane receptor; CREB, cAMP responsive element-binding protein; FOXO1, Forkhead box protein O1; HNF4α, hepatocyte nuclear factor 4α; JNK, c-Jun N-terminal kinase; PRL, prolactin; PXR, pregnane X receptor; RXR, retinoic X receptor; rCYP, recombinant CYP; WORT, wortmannin. EIA, Elisa; OLE, oleuropein; FEN, fenofibrate; PKA, protein kinase A; PPARα, peroxisome proliferator-activated receptor α; PCR, polymerase chain reaction; RIA, radioimmunoassay.

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Oleuropein (OLE), the main constituent of *olea oleuropea*, displays pleiotropic beneficial effects in health and disease, which are mainly attributed to its antiinflammatory and cardioprotective properties. Several food supplements and herbal medicines contain OLE and are available without a prescription. This study investigated the effects of OLE on the main CYPs catalyzing the metabolism of many prescribed drugs. Emphasis was given on the role of peroxisome proliferator-activated receptor alpha (PPARα), a nuclear transcription factor regulating numerous genes including *CYPs*. 129/Sv wild-type and *Ppara*-null mice were treated with OLE for six weeks. OLE induced *Cyp1a1*, *Cyp1a2*, *Cyp1b1*, *Cyp3a14*, *Cyp3a25*, *Cyp2c29*, *Cyp2c44*, *Cyp2d22* and *Cyp2e1* mRNAs in liver of wild-type mice, whereas no similar effects were observed in *Ppara*-null mice, indicating that the OLE-induced effect on these *CYPs* is mediated by PPARα. Activation of the PI3k/AKT/FOX01, JNK, AKT/p70 and ERK related pathways participates in CYP induction by OLE. These data indicate that consumption of herbal medicines and food supplements containing OLE could accelerate the metabolism of drug stubstrates of the above mentioned CYPs, thus reducing their efficacy and the outcome of pharmacotherapy. Therefore, OLE-induced activation of PPARα could modify the effects of drugs due to their increased metabolism and clearance, which should be taken into account when consuming OLE-containing products with certain drugs, in particular those of narrow therapeutic window.

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Significance statement

This study indicated that oleuropein, which belongs to the main constituents of the leaves and olive drupes of *Olea europaea*, induces the synthesis of the major CYPs metabolizing the majority of prescribed drugs via activation of PPARα. This effect could modify the pharmacokinetic profile of co-administered drugs-substrates of the CYPs, thus altering their therapeutic efficacy and toxicity.

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Globally, there is an upsurge in the use of medicines coming from herbs as many people claim that they are non-toxic based on their natural origin and their use in popular medicine for centuries. Nonetheless, herbs contain many active substances that could induce adverse effects, toxicity or even cancer (Bensoussan et al., 2002; De Smet, 1995; Deng, 2002; Eisenberg et al., 1993; Ernst and Pittler, 2002a; Ernst and Pittler, 2002b; Greensfelder, 2000; Haller and Benowitz, 2000; Kennedy and Seely, 2010; Klepser and Klepser, 1999; Koh and Woo, 2000; Malliou et al., 2018; Mckenna et al., 2012; McRae et al., 2002; Stedman, 2002). When all foreign substances (xenobiotics) enter the body, they undergo biotransformation primarily in the liver, which accelerates their elimination. In particular, the metabolism of xenobiotics, such as drugs, pre-carcinogens and toxic agents during Phase I is mainly catalyzed by CYPs and can result either in activation of pro-drugs or in inactivation of pharmacologically active drugs, or in activation of pre-carcinogens to carcinogens (Gonzalez and Gelboin, 1994b; Ingelman-Sundberg, 2004a). It is estimated that CYPs belonging to the CYP3A, CYP2C, CYP2D and CYP1A subfamilies catalyze hepatic metabolism of over than 95% of the most widely prescribed drugs (Daskalopoulos et al., 2012a; Guengerich, 2003; Ingelman-Sundberg, 2004a; Konstandi, 2013). From a toxicological point of view, it is worth noting that CYP1A1/2 and CYP1B1 catalyze the bioactivation of the major groups of pre-carcinogens, the polycyclic aromatic hydrocarbons (PAHs), polycyclic arylamines and aflatoxin B1 to electrophilic DNA-binding derivatives (Cheng and Morgan, 2001; Flint et al., 2010; Harkitis et al., 2015; Kawajiri, 1999; Konstandi et al., 2006; Konstandi et al., 2005; Pasanen and Pelkonen, 1994). Interestingly, the

biotransformation of steroids, fatty acids and several other endogenous compounds is also catalyzed by CYPs (Guengerich, 2003; Spatzenegger and Jaeger, 1995).

It is well-documented that the traditional Mediterranean diet has various beneficial effects in health and longevity, and olive oil and olives are substantial ingredients of this diet (Impellizzeri et al., 2012). The main compounds found in the leaves and olive drupes of *Olea europaea* are oleuropein (OLE) and its hydrolysis product, hydroxytyrosol. There is accumulating evidence based on preclinical studies that OLE displays significant cardioprotective properties (Andreadou et al., 2006; Briante et al., 2001; Malliou et al., 2018; Oi-Kano et al., 2008; Tuck and Hayball, 2002; Visioli and Galli, 1994; Visioli et al., 2002a; Visioli et al., 2002b), which could be attributed to activation of peroxisome proliferator-activated receptor α (PPARα) (Harkitis et al., 2015; Malliou et al., 2018), a ligand-activated nuclear receptor that controls lipid homeostasis (Fruchart and Duriez, 2006; Hansen and Connolly, 2008; Kuusisto et al., 2007; Robillard et al., 2005). The anti-inflammatory properties of OLE may also contribute to the drug's cardioprotective effects (Shimada et al., 1996; Spatzenegger and Jaeger, 1995). Preliminary data also indicated a pleotropic effect of OLE on several vital functions of the body including stimulation of neural plasticity and protection against neurodegenerating disorders, among others, effects potentially mediated by activation of PPARα (data not shown).

It is well defined that OLE activates PPARα (Malliou et al., 2018; Spatzenegger and Jaeger, 1995), which controls several genes participating in the regulation of inflammatory responses, the metabolism of lipids and glucose, as well as the adipose differentiation and cancer, among others (Malliou et al., 2018; Yang et al., 2008; YUMUK, 2006). It is of interest that PPARα apparently holds a substantial regulatory role for CYPs (Choi and Waxman, 1999; Tauber et al., 2020). In

particular, the hepatic sexual dimorphism of the CYP expression pattern is largely regulated by PPARs (Leuenberger et al., 2009).

Drug-drug interactions are of critical clinical significance, because they markedly determine the outcome of pharmacotherapy, the side effects of the drugs and pharmacotoxicity (Konstandi, 2013). They are usually dependent on the drugs' effect on CYPs acting either as inducers, inhibitors or substrates. On one hand, induction of the most important CYPs that catalyze the metabolism of the majority of prescribed drugs, may accelerate the biotransformation of their drug-substrates and in most cases, result in their reduced pharmacological efficacy (Daskalopoulos et al., 2012a; Daskalopoulos et al., 2012b; Ingelman-Sundberg et al., 2007; Konstandi et al., 2020; Konstandi et al., 2005; Zhou et al., 2004), whereas in some cases, in perturbation of several endogenous regulatory circuits, often associated with pathophysiological states (Choi and Waxman, 1999). On the other hand, inhibition of CYPs may lead to accumulation of their drug-substrates in the blood, followed by adverse side effects of varying severity that may reach the level of toxicity and this is of particular clinical interest when drugs of low therapeutic index are administered (Daskalopoulos et al., 2012b; Konstandi et al., 2020; Spatzenegger and Jaeger, 1995). Therefore, it should be noted that food supplements or herbal medicines containing pharmacologically active compounds, such as OLE, which act either as substrates of CYPs, or even as their inducers or inhibitors (Zhou et al., 2004), may modify the efficacy of co-administered drugs in multi-drug therapeutic schemes and potentially, induce pharmacotoxicity (Daskalopoulos et al., 2012a; Daskalopoulos et al., 2012b; Gonzalez and Gelboin, 1994a; Guengerich, 2003; Harkitis et al., 2015; Konstandi et al., 2014; Pelkonen et al., 2008).

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

In the light of the above considerations, current study investigated the potential regulatory role of OLE for the main CYPs that are involved in drug metabolism, emphasizing the role of PPARα in this regulation. To approach this issue, wild-type (WT) and *Ppara*-null mice were treated with OLE and *Cyp* mRNA and protein expressions were analyzed. OLE markedly upregulated several genes encoding the most significant drug metabolizing CYP isozymes in the liver, a process profoundly mediated by PPARα activation.

Materials and Methods

Animals and treatment: Adult male 129/Sv wild-type (WT) and *Ppara*-null mice that were used in this study were bred in the Animal House of the University of Ioannina (Ioannina, Greece) and were housed up to five mice per cage. All animals (5-6 per group of treatment) had access to a standard chow diet for rodents (1324 TPF, Altromin Spezial futter GmbH & Co. KG) and water *ad libitum*. Throughout the experiments, all mice were housed in their cages under a standard 12-h light/12-h dark cycle (lights on at 6:00 AM). The experimental protocols employing procedures with animals received the approval of the Ethics Committee of the University of Ioannina- Faculty of Medicine. The procedure followed was in compliance with the European Commission ethical standards for the care and use of experimental animals (Directive 86/609-EEC). Both, WT and *Ppara*-null mice were treated with food pellets containing OLE (100 mg/kg) daily for 6 consecutive weeks (Andreadou et al., 2006; Andreadou et al., 2014; Impellizzeri et al., 2012). For the isolation of OLE the leaves of Olea europaea were used following a previously described method (Andreadou et al., 2014). The choice of the dose of OLE that was used in this study was based on information from the literature and our previous findings (Andreadou et

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

al., 2006; Andreadou et al., 2014; Impellizzeri et al., 2012). It corresponds approximately to the average consumption of olive oil and olive drups in the Mediterranean countries during a day (Abdel-aleem et al., 1997), and represents total polyphenol consumption from these olive products and is estimated to be approximately 100mg/day (Abdel-aleem et al., 1997; Del Boccio et al., 2003). Mice were treated with OLE in food pellets, because it is known that the drug is slightly absorbed in intestinal lumen even under normal iso-osmotic conditions. The absorption of OLE can be markedly improved by solvent flux through paracellular junctions, an effect that is facilitated by hypotonic luminal conditions (Edgecombe et al., 2000). A significant factor stimulating the water flux through the opening of paracellular junctions is the postprandial presence of glucose or amino acids in the intestinal lumen. This mechanism regulating the absorption of OLE in intestinal lumen appears to have similar effects with those of hypotonic solutions (Pappenheimer and Reiss, 1987). The pharmacokinetic profile of OLE in mice has not been determined, but only that in rats, when one dose of OLE (100 mg/kg per os) reached 200 ng/ml in tmax of 2 h (Boccio et al., 2003). Controls received regular rodent food for 6 weeks. Wild type and *Pparα*-null mice also recieved intraperitoneally either fenofibrate (FEN, 100mg/kg), a selective PPARα agonist (Ghonem et al., 2015; Hu et al., 2019) or normal saline for 7 consecutive days. Upon completion of the experiments, all mice were euthanized using CO_2 asphyxiation, and parts from the liver were dissected for the extraction of total RNA, nuclear/cytosolic and microsomal proteins. All liver tissue samples were preserved at -80°C until assayed.

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Isolation of microsomal proteins: Liver tissue was homogenized in homogenization buffer containing 0.15 M KCl, 10 mM K_2EDTA and 1 mM dithiothreitol (pH 7.4) at +4^{0}C, for the isolation of microsomal fractions. The homogenates were then centrifuged for 20 min at 15,000 rpm (+4^o C). The upper phase was transferred into clean vials followed by centrifugation at 27,500 rpm (+4^{o}C) for 60 min. The formed microsomal pellet was resuspended by homogenization in the specific ice-cold homogenization buffer and centrifuged at 27,500 rpm for 45 min. Temperature was always kept at +4^{o}C. Finally, the formed microsomal pellet was resuspended in the specific ice-cold storage buffer containing K_2HPO_4/KH_2PO_4 (pH 7.4), K_2EDTA (1 mM), dithiothreitol (0.1 mM) and 20% glycerol. Aliquots of microsomal suspensions were stored at -80^o C until assayed (Lang et al., 1981).

CYP2D Activity, 1´-Bufuralol hydroxylation: 1´-Bufuralol hydroxylation is mainly catalysed by CYP2D isozymes (Matsunaga et al., 1990). Liver microsomal proteins (40 μg ~ 20μl of sample) were pre-incubated in a 180 μl reaction mixture containing potassium phosphate (0.1 M, pH 7.4) at 37°C for 5 min, in the presence of 50 μM bufuralol (substrate) and NADPH (0.5 mM, Sigma-Aldrich). The duration of the reaction was 7.5 min and was terminated using 20 μl of perchloric acid (60%). Following a ten min centrifugation at 14,075g , the supernatant containing the main metabolite of bufuralol (1´-hydroxy-bufuralol) was analysed using an HPLC method. The fluorescence detection was set at 252 nm (excitation wavelength) and 302 nm (emission wavelength) and a specific column (reverse-phase Luna C18, 5 μm, 150X 3 mm; Phenomenex, Torrance, CA) was used for this purpose. The mobile phase consisted of 30% acetonitrile/70% perchlorate buffer (20 mM, pH 2.5). The elution of each sample took place at a flow rate of 1 ml/min for 14 min. The recombinant rat

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

CYP2D1 and CYP2D2, enriched with CYP reductase BD Supersomes, was used as a positive control of bufuralol 1′-hydroxylation (BD Gentest, Woburn, MA). Bufuralol (50 μM) was used as substrate, which was pre-incubated at 37°C for 2 min with approximately, 200 μl of 0.1 M potassium phosphate buffer (pH 7.4). Recombinant CYPs (50 μM) along with NADPH (1 mM) were added in the mixture, which was incubated at 37°C for 30 min. The termination of the reaction was achieved with 20μl acetonitrile. Then, all samples were placed on wet ice and left undisturbed for 10 min, before their injection into the HPLC for analysis.

Western Blot Analysis: Alterations in CYP apoprotein levels were assessed with immunoblot analysis using liver microsomes, while nuclear extracts were used for PPARα and HNF4α analysis. pJNK, pP38, pERK, pP70, PXR, CAR and pFOX01 were analyzed using total cellular extracts. For the preparation of nuclear and cytosolic extracts, the corresponding cytosolic and nuclear extraction kit of Thermo Fisher Scientific, Waltham, MA was used. The content of proteins in the sample was determined using the bovine serum albumin (BSA) assay (Thermo Fisher Scientific). All proteins run on SDS-polyacrylamide gel electrophoresis followed by immunoblotting and for this purpose the following antibodies were used: mouse/rat polyclonal CYP3A, CYP2C and CYP2D IgGs, mouse/rat monoclonal HNF4α, CAR, PXR IgGs and mouse polyclonal PPARα IgG (Santa Cruz Biotechnology). The rabbit phosphorylated polyclonal IgGs CREB-1 (Ser133), P38 (Thr180/ Tyr182) (Santa Cruz Biotechnology), MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signaling Technology), p70S6K (Thr389) (Cell Signaling Technology), FOX01 (Ser256) (Santa Cruz Biotechnology), AKT (Ser473) (Santa Cruz Biotechnology) and the mouse phosphorylated monoclonal JNK (Thr183 and Tyr185) IgG (Santa Cruz

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Biotechnology). The secondary antibodies used in this study were conjugated with horseradish peroxidase (Santa Cruz Biotechnology). For the detection of proteins in the blot the enhanced chemiluminescence detection kit (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK) was used. As a loading control, immunoblotting employing the specific antibodies, Histone H3 or actin (Santa Cruz Biotechnology), and the secondary antibody, anti-mouse horseradish peroxidase-conjugated IgG was used.

Quantitative Real-Time Polymerase Chain Reaction Assays: TRIzol reagent (Invitrogen) was used for the isolation of total RNA from liver tissue following the instructions in the manufacturer's protocol. Following a spectrophotometric method the concentration of total RNA in each sample was determined. Total RNA (1 μg) and a SuperScript II reverse transcriptase kit (Invitrogen) were used to generate cDNA, which was used in quantitative and reverse transcription-polymerase chain reaction (PCR) assays. Table 1 shows all sequences of the forward and reverse gene-specific primers that were used in this study. For the real-time reactions, the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) was used, and these reactions were performed employing the C1000 Touch thermal cycler with a real-time detection system (Bio-Rad Laboratories, Hercules, CA). Relative mRNA expression was estimated using β-actin mRNA levels to normalize mRNA expression levels of each gene (QuantiTect primer assay; QIAGEN, Valencia, CA). The comparative threshold cycle method was used to quantify all values.

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Statistical Analysis: For the analysis of data, the one-way analysis of variance followed by multiple comparisons employing Bonferroni's and Tukey's least honest significant difference methods was used. All values are presented as mean ± S.E. and p values of <0.05 were considered significant.

Results

OLE-induced effect on CYP1s

OLE markedly increased hepatic *Cyp1a1* and *Cyp1a2* mRNA expression and CYP1A protein expression in WT mice (Fig. 1A and 1B, respectively); *Cyp1b1* mRNA levels were also increased by OLE (Fig. 1C).

Oleuropein-induced effect on CYP2 and CYP3s

Oleuropein markedly increased hepatic *Cyp2c29* and *Cyp2c44* mRNA and CYP2C protein expression in WT mice (Fig. 2A & 2B, respectively). Similarly, OLE increased the hepatic Cyp2d22 mRNA, CYP2D protein and activity levels (Fig. 2C) and the hepatic *Cyp2e1* (Fig. 3A), *Cyp3a14* and *Cyp3a25* expression (Fig. 3B) at mRNA and protein levels.

Assessment of OLE effect on transcription factors and signal transduction pathways related to CYP regulation

The OLE-induced effect on aryl hydrocarbon receptor (AHR), aryl hydrocarbon receptor repressor (AhrR) and heat shock protein 90 (HSP90) (Fig. 4A, 4B and 4C,

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

respectively), critical transcription factors in *Cyp1a1*, *Cyp1a2* and *Cyp1b1* regulation (Nebert et al., 2013), is apparently followed by upregulation of these CYPs, an effect profoundly associated with the OLE-induced PPARα activation (Malliou et al., 2018), because OLE did not increase Ahr, AhrR and Hsp90 mRNAs in *Ppara*-null mice, but the drug rather slightly repressed the expression of these transcription factors (Fig. 4A-4C).

The OLE-induced effect on *Cyp2c29, Cyp2c44* and *Cyp3a25* mRNA expression in the liver of WT mice is apparently mediated by induction of *Pxr*, *Car*, *Rxra* and *Rxrb*, which encode critical transcription factors regulating the above CYP genes (Daskalopoulos et al., 2012a) (Fig. 5A-5D). The drug's upregulating effect on these transcription factors is probably mediated by PPARα, because, OLE had a repressing effect on *Pxr*, *Car* and *Rxrb* in *Ppara*-null mice (Fig. 5A, 5B and 5D, respectively).

Long term treatment of WT mice with OLE induced hepatic *Ppara* mRNA expression by 4-5 fold, a stimulating effect that was also evident at protein level (Malliou et al., 2018),(Supplemental Fig. 1). Further analysis employing molecular docking experiments suggests that OLE is a PPARα ligand and the luciferase reporter gene assay revealed significant activation of this nuclear receptor and transcription factor by OLE (Malliou et al., 2018), followed by upregulation of various PPARα target genes including *Acox1*, *Acot1*, *Cyp4a10* and *Cyp4a14* (Malliou et al., 2018). *Hnf4a*, encoding a nuclear transcription factor, which holds a determinant role in the regulation of several CYP genes (Daskalopoulos et al., 2012a; Konstandi et al., 2020), was also upregulated by OLE in the liver of mice (Malliou et al., 2018), (Supplemental Fig. 1). It is assumed that the OLE upregulating effect on all Cyps analyzed in this study is profoundly mediated by PPARα, because no similar effects

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

on these *Cyps* were observed in *Ppara*-null mice (Fig. 1A-1C, Fig. 2A-2C, Fig. 3A-3C). In particular, OLE either repressed *Cyp1a1*, *Cyp1a2*, *Cyp1b1* (Fig. 1A-1C) *Cyp2c29*, *Cyp2c44*, *Cyp2d22* (Fig. 2A-2C) and *Cyp3a25* (Fig. 3C) or had no effect on *Cyp2e1* (Fig. 3A) and *Cyp3a14* (Fig. 3B) in the liver of *Ppara* deficient mice. The determinant role of PPARα in CYP induction was also confirmed in mice treated with the selective PPARα agonist, FEN (Ghonem et al., 2015; Hu et al., 2019), which increased CYP1A1, CYP1A2, CYP1B1, CYP2C, CYP2D, CYP2E1 and CYP3A protein expression in the liver of WT mice, whereas FEN had no similar effects in the liver of PPARα-deficient mice (Fig 6).

The role of nitric oxide synthases, endothelial NOS (NOS3) and inducible NOS (NOS2), in the regulation of hepatic *Cyp1a, Cyp1b, Cyp3a, Cyp2c, Cyp2d* and *Cyp2e1* by OLE in WT mice appears to be less significant, because although OLE induced *Nos3* and *Nos2* mRNA expression (Fig. 7A and 7B), this effect was not followed by inhibition of the afore-mentioned CYPs (Hara and Adachi, 2002). Instead, OLE induced the synthesis of these CYPs (Fig 1A-1C, Fig. 2A-2C and Fig. 3A-3C), indicating that the NOS inhibiting effect on CYPs was probably overlapped by the inducing effect of other regulatory factors.

At signal transduction level, OLE increased FOX01 and JNK phosphorylation, along with that of p70 in the livers of WT mice (Fig. 8). These findings indicate that the OLE-induced activation of PI3k/AKT/FOX01, JNK and AKT/p70 pathways (Fig. 8) is profoundly related to the induction of CYPs that belong to the CYP1A, CYP1B, CYP3A, CYP2C, CYP2D and CYP2E subfamilies. Activation of ERK could also participate in the regulation of CYP induction by OLE (Fig. 8).

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Comparatively, OLE and FEN at the doses given, induced hepatic protein expression of CYP1A1, CYP2C and CYP3A to a similar extent, whereas the induction of CYP1A2, CYP1B1, CYP2D and CYP2E1 by FEN was markedly higher than that by OLE (Figs 1-3 and Fig. 6).

Discussion

There is an accumulating amount of evidence that supports the beneficial effects of OLE in preservation of good health and in the outcome of various disease states, including those related to the cardiovascular system (Ahamad et al., 2019; Andreadou et al., 2006; Araki et al., 2019; Lockyer et al., 2017; Vogel et al., 2014), which are mainly attributed to the drug's anti-inlammatory properties (Malliou et al., 2018), to its effects on lipid homeostasis (Andreadou et al., 2014; Araki et al., 2019; Lockyer et al., 2017; Malliou et al., 2018) and the protection of myocardium in conditions related to ischemia (Andreadou et al., 2014). Based on these OLE-related beneficial effects, the pharmaceutical industry produced several food supplements and herbal medicines containing the drug, which are available in the market without a prescription, a potentially high risk practice for health and disease.

It is well documented that cytochromes belonging to CYP families 1-3 are involved in the metabolism of a plethora of diverse endogenous and exogenous compounds, such as drugs, pre-carcinogens, carcinogens, environmental pollutants, food additives, prostaglandins, fatty acids, lipid hydroperoxides, steroid hormones, biogenic amines and numerous other xenobiotics (Cribb et al., 2005; Gonzalez, 2005; Gonzalez and Gelboin, 1994b; Gonzalez and Yu, 2006; Guengerich, 2003; Ingelman-Sundberg, 2004b; Xu et al., 2005), in order to increase their water solubility and

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

prepare them for the subsequent conjugation and elimination (Gonzalez, 2005). Their biotransformation takes place primarily in the liver and usually, results in inactive metabolites, or in some cases, in active molecules, a process that usually depends on the structure of the parent compound. These active metabolic products may induce several serious toxic manifestations, such as teratogenesis, carcinogenicity, cell death, and oxidative stress, among others (Cribb et al., 2005; Gonzalez, 2005; Gonzalez and Gelboin, 1994b; Gonzalez and Yu, 2006; Guengerich, 2003; Ingelman-Sundberg, 2004a; Konstandi, 2013; Xu et al., 2005). The findings of the current study clearly showed that OLE induced the expression of several CYPs in the liver of WT mice, including CYP1A1, CYP1A2, CYP1B1, CYP3A14, CYP3A25, CYP2C29, CYP2C44, CYP2D22 and CYP2E1 via activation of major nuclear transcription and other cellular factors, such as AHR, CAR, RXR and PXR (Dalton et al., 2000; Daskalopoulos et al., 2012a; Harkitis et al., 2015). Induction of CYPs is a part of the regulatory mechanisms aimed at maintaining homeostasis. It is important for the adaptation of the organism to a modified biological and chemical internal milieu and environment. Nonetheless, it is not always feasible to predict the extent of the effect of CYP induction on drug efficacy and toxicological risk assessment, determinant parameters in clinical drug therapy (Pelkonen et al., 2008). According to the regulatory stadards of the Food and Drug Administration (FDA), a drug is considered as CYP inducer with clinical significance if the fold change of *CYP* mRNA expression relative to the vehicle control is ≥ 2-fold and then, is efficient to accelarate the metabolism of CYP-substrates (Fahmi and Ripp, 2010); *https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers*). It is considered that induction of CYP1A2, CYP3A14, CYP3A25, CYP2C29, CYP2C44, CYP2D22 and CYP2E1

may result in reduced concentrations of their drugs-substrates in blood, potentially below their therapeutic levels and therefore, in failure of pharmacotherapy (Dalton et al., 2000; Kawajiri and Fujii-Kuriyama, 2007; Konstandi, 2013; Okey, 1990; Zhang et al., 1999), whereas induction of CYP1A1, CYP1A2 and CYP1B1 is followed by bioactivation of the major groups of pre-carcinogens, the polycyclic aromatic hydrocarbons, polycyclic arylamines and aflatoxin B1, to DNA-binding metabolites (Cheng and Morgan, 2001; Kawajiri, 1999; Mulder et al., 2001; Pasanen and Pelkonen, 1994). In contrast to the present findings related to the induction of CYP3A14/25 by OLE, previous *in vitro* studies indicated that OLE and its metabolite hydroxytyrosol, inactivated androstenedione 6beta-hydroxylase (CYP3A4-dependent) activity in microsomal preparations of human liver (Stupans et al., 2001; Stupans et al., 2000; Zhou et al., 2007). This discrepancy could be explained primarily, on the basis of a species-specific regulation of the main CYPs (Konstandi et al., 2020; Visioli et al., 2002a) and the different experimental approaches followed in these studies. Current *in vivo* study evaluated the OLE effect on CYP3A14/25 in mouse liver microsomes, whereas the *in vitro* studies mentioned above used human liver microsomes (Stupans et al., 2001; Stupans et al., 2000).

In order to investigate the mechanisms underlying the OLE-induced upregulating effect on CYPs, the role of the nuclear transcription factor, PPARα that regulates a variety of genes enconding CYPs was assessed (Tauber et al., 2020). For this purpose, WT and *Pparα*-null mice were employed, which received OLE in their diet, for six weeks. The findings indicated that, in contrast to what happened in WT mice, OLE did not induce the expression of CYP1A1, CYP1A2, CYP1B1, CYP3A14, CYP3A25, CYP2C29, CYP2C44, CYP2D22 and CYP2E1 in the liver of *Pparα*-null mice. It is appearent that the OLE-mediated induction of these CYPs in

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

the liver of WT mice is mediated by the drug's stimulating effect on PPARα activation. In order to further evaluate the involvment of PPARα in the regulation of CYPs and in particular, of CYP induction, WT mice were treated with the selective PPARα agonist, fenofibrate (Ghonem et al., 2015; Hu et al., 2019), which induced CYP1A1, CYP1A2, CYP1B1, CYP2C, CYP2D, CYP2E1 and CYP3A protein expression in their livers. No similal effects were observed in the liver of *Pparα*-null mice following treatment with fenofibrate, and these findings confirm the role of PPARα in CYP induction. This hypothesis is also supported by other studies reporting the up-regulating effects of PPARs on several CYPs, and in particular of PPARα on CYPs displaying epoxygenase activities on polyunsaturated fatty acids, including isozymes that belong to CYP1A, CYP3A, CYP2C and CYP2E subfamilies (Tauber et al., 2020; Thomas et al., 2013). Interestigly though, findings from the current and previous studies (Shi et al., 2017) showed that basal CYP expresion levels in the liver of *Pparα*-null mice are markedly higher than those in WT mice. This observation indicates a distinct role of PPARα in the regulation of constitutive and inducible CYP expression. It is well established that CYP induction and constitutive expression are regulated by distinct mechanisms (Hahn et al., 2009; Nebert, 2000; Zanger and Schwab, 2013), including various transcription factors, co-activators, and co-repressors, with both positive and negative regulatory roles that cross-talk between several regulatory pathways (Pelkonen et al., 2008). In this regard, the diverse role of PPARα in CYP regulation at basal and induced states may be attributed in the cross-talk between this trancription factor and AhR, CAR, PXR and/or other transcription factors with significant regulatory roles in CYP induction by xenobiotics (Honkakoski and Negishi, 2000; Pelkonen et al., 2008; Zanger and Schwab, 2013). This hypothesis is supported by the finding that several functional

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

PPARα-binding regions within the CYP promoters were detected (Makia and Goldstein, 2016; Oshida et al., 2016a; Thomas et al., 2013; Yao et al., 2007) (Oshida et al., 2016b). It should be noted that the mechanism underlying the PPARα-mediated CYP induction by OLE could also include modifications at PPARα phosphorylation status. It is well established that ligand-induced PPARα activation is mediated by increased expression of this nuclear receptor at transcriptional and protein level along with alterations at its phosphorylation state (Ning et al., 2016; Tamasi et al., 2008)((Barger et al., 2001; Passilly et al., 1999).

The nuclear transcription factor, HNF4α, profoudly participates in the OLE-induced upregulation of the above mentioned CYPs, because OLE induced hepatic *Hnf4a* expression in WT mice. It is known that the transcription factors, HNF4α, RXR and HNF1α (Weltman et al., 1998; Wiwi and Waxman, 2004), along with FOX01 and the nuclear receptors, CAR and PXR (Kodama et al., 2004), belong to a complex cross-talk mechanism displaying central regulatory roles in the expression of various CYPs.

In CYP regulation there is also an interplay between PPARα and inducible nitric oxide synthase (iNOS or NOS2), efficient enough to modify *CYP* expression. In particular, it has been reported that PPARα ligads reduced the LPS-induced iNOS expression and subsequent nitric oxide (NO) synthesis in macrophages by increasing the proteasome pathway mediated iNOS protein degradation (Paukkeri et al., 2007). Notably, NO displays down-regulating effects on several CYP genes, including CYP1A1/2, CYP2B1/2, CYP2D6, CYP2E1 and CYP3A4, but the underlying mechanism of this regulation remains blurred (Gergel et al., 1997; Hara and Adachi, 2002; Wink et al., 1993). Current findings indicated that the OLE-induced effect on NOS has a weak impact on CYPs compared to other regulatory factors. It appears

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

that the up-regulating effect of OLE on NOS, which should be followed by CYP downregulation (Gergel et al., 1997; Hara and Adachi, 2002; Wink et al., 1993), was overlapped by the upregulating effects of other transcription factors. Further analysis indicated that at signal transduction level, activation of the PI3k/AKT/FOX01, JNK, AKT/p70S6K and ERK related signaling pathways profoudly participate in CYP induction by OLE (Kim and Novak, 2007). It is well documented that upon activation, AKT stimulates the phosporylation of the nuclear transcription factor, forkhead box 01 (FOX01), which in turn translocates into the cytoplasm. This process is usually followed by termination of FOX01 transcriptional activity in CYP genes. But then, the OLE-mediated activation of c-Jun N-terminal kinases (JNK) promotes the nuclear localization of FOX01, an effect that restores its transcriptional activity in CYP genes and counteracts the downregulating effect of AKT (Daskalopoulos et al., 2012a; Hay, 2011). Current findings indicated that OLE decreased JNK phosphorylation in the liver of *Pparα*-null mice, an effect that likely participates in the OLE-mediated downregulation of CYPs in these mice.

Taken together the above data clearly show the upregulating effect of OLE on various CYPs encoding the main drug-metabolizing enzymes of Phase I, an effect that is apparently mediated by PPARα activation. Current and previous findings indicate that diverse and distinct mechanisms participate in the regulation of constitutive and inducible CYP expression by PPARα agonists. Apparently, PPARα displays a negative regulatory role in constitutive *CYP* expression and a positive role in CYP induction by OLE. Although, it is not always feasible to accurately predict the clinical impact of the OLE-induced effect on CYP3A14, CYP3A25, CYP2C29, CYP2C44, CYP2D22, CYP2E1, CYP1A1, CYP1A2 and CYP1B1, under certain conditions it could modify the pharmacokinetic profile of drug-substrates of these

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

CYPs and subsequently, affect the outcome of pharmacotherapy and the severity of drug-related adverse reactions. Although these data come from a preclinical study, and cannot be extrapolated directly to humans, they underscore the necessity of taking into consideration the consumption of herbal medicines and food supplements containing drugs, such as OLE that may affect the pharmacokinetic profiles of co-administered drugs. This parameter should be considered predominately, in multiple drug therapeutic schemes, in particular those of vital significance for the patient, or in drugs with narrow therapeutic windows or with severe side effects. This concern is of particular importance, because herbal medicines and food supplements are widely used and freely available in the market. All of these concerns indicate that further investigation, pharmacovigilance, better regulatory control and seek of advice from health professionals is essential to ensure the safety when using herbal medicines and food supplements.

Acknowledgments

This study has been funded by the European Union (European Regional Development Fund- ERDF) and Greek national funds coming from the Operational Program "THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013, Grant 346985/80753).

Author contributions

Participated in research design: Konstandi, Gonzalez

Conducted experiments: Malliou, Andriopoulou, Konstandi, Skaltsounis

Performed data analysis: Konstandi, Andriopoulou, Malliou

Wrote or contributed to the writing of the manuscript: Konstandi, Gonzalez

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

References

Abdel-aleem S, el-Merzabani MM, Sayed-Ahmed M, Taylor DA and Lowe JE (1997) Acute and chronic effects of adriamycin on fatty acid oxidation in isolated cardiac myocytes. *J Mol Cell Cardiol* **29**(2): 789-797.

Ahamad J, Toufeeq I, Khan MA, Ameen MSM, Anwer ET, Uthirapathy S, Mir SR and Ahmad J (2019) Oleuropein: A natural antioxidant molecule in the treatment of metabolic syndrome. *Phytotherapy research : PTR* **33**(12): 3112-3128.

Andreadou I, Iliodromitis EK, Mikros E, Constantinou M, Agalias A, Magiatis P, Skaltsounis AL, Kamber E, Tsantili-Kakoulidou A and Kremastinos DT (2006) The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. *The Journal of nutrition* **136**(8): 2213-2219.

Andreadou I, Mikros E, Ioannidis K, Sigala F, Naka K, Kostidis S, Farmakis D, Tenta R, Kavantzas N, Bibli S-I, Gikas E, Skaltsounis L, Kremastinos DT and Iliodromitis EK (2014) Oleuropein prevents doxorubicin-induced cardiomyopathy interfering with signaling molecules and cardiomyocyte metabolism. *Journal of Molecular and Cellular Cardiology* **69**: 4-16.

Araki R, Fujie K, Yuine N, Watabe Y, Nakata Y, Suzuki H, Isoda H and Hashimoto K (2019) Olive leaf tea is beneficial for lipid metabolism in adults with prediabetes: an exploratory randomized controlled trial. *Nutr Res* **67**: 60-66.

Barger PM, Browning AC, Garner AN and Kelly DP (2001) p38 Mitogen-activated Protein Kinase Activates Peroxisome Proliferator-activated Receptor α: A POTENTIAL ROLE IN THE CARDIAC METABOLIC STRESS RESPONSE*. *Journal of Biological Chemistry* **276**(48): 44495-44501.

Bensoussan A, Myers S, Drew A, Whyte I and Dawson A (2002) Development of a Chinese Herbal Medicine Toxicology Database * 1. *Journal of toxicology Clinical toxicology* **40**: 159-167.

Boccio P, Deo A, Curtis A, Celli N, Iacoviello L and Rotilio D (2003) Liquid chromatography-tandem mass spectrometry analysis of oleuropein and its metabolite hydroxytyrosol in rat plasma and urine after oral administration. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences* **785**: 47-56.

Briante R, La Cara F, Tonziello MP, Febbraio F and Nucci R (2001) Antioxidant activity of the main bioactive derivatives from oleuropein hydrolysis by hyperthermophilic beta-glycosidase. *J Agric Food Chem* **49**(7): 3198-3203.

Cheng PY and Morgan ET (2001) Hepatic cytochrome P450 regulation in disease states. *Curr Drug Metab* **2**(11469724): 165-183.

Choi HK and Waxman DJ (1999) Growth hormone, but not prolactin, maintains, low-level activation of STAT5a and STAT5b in female rat liver. *Endocrinology* **140**(11): 5126-5135.

Cribb AE, Peyrou M, Muruganandan S and Schneider L (2005) The endoplasmic reticulum in xenobiotic toxicity. *Drug metabolism reviews* **37**(3): 405-442.

Dalton TP, Dieter MZ, Matlib RS, Childs NL, Shertzer HG, Genter MB and Nebert DW (2000) Targeted knockout of Cyp1a1 gene does not alter hepatic constitutive expression of other genes in the mouse [Ah] battery. *Biochem Biophys Res Commun* **267**(1): 184-189.

Daskalopoulos EP, Lang MA, Marselos M, Malliou F and Konstandi M (2012a) D(2)-dopaminergic receptor-linked pathways: critical regulators of CYP3A, CYP2C, and CYP2D. *Molecular pharmacology* **82**(4): 668-678.

Daskalopoulos EP, Malliou F, Rentesi G, Marselos M, Lang MA and Konstandi M (2012b) Stress is a critical player in CYP3A, CYP2C, and CYP2D regulation: role of adrenergic

receptor signaling pathways. *American journal of physiology Endocrinology and metabolism* **303**(1): E40-54.

De Smet PA (1995) Health risks of herbal remedies. *Drug safety* **13**(2): 81-93.

Del Boccio P, Di Deo A, De Curtis A, Celli N, Iacoviello L and Rotilio D (2003) Liquid chromatography-tandem mass spectrometry analysis of oleuropein and its metabolite hydroxytyrosol in rat plasma and urine after oral administration. *J Chromatogr B Analyt Technol Biomed Life Sci* **785**(1): 47-56.

Deng J-F (2002) Clinical and laboratory investigations in herbal poisonings. *Toxicology* **181-182**: 571-576.

Edgecombe SC, Stretch GL and Hayball PJ (2000) Oleuropein, an Antioxidant Polyphenol from Olive Oil, Is Poorly Absorbed from Isolated Perfused Rat Intestine. *The Journal of Nutrition* **130**(12): 2996-3002.

Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR and Delbanco TL (1993) Unconventional medicine in the United States. Prevalence, costs, and patterns of use. *The New England journal of medicine* **328**(4): 246-252.

Ernst E and Pittler MH (2002a) Herbal medicine. *Medical Clinics of North America* **86**(1): 149-161.

Ernst E and Pittler MH (2002b) Risks Associated with Herbal Medicinal Products. *Wiener Medizinische Wochenschrift* **152**(7-8): 183-189.

Fahmi OA and Ripp SL (2010) Evaluation of models for predicting drug-drug interactions due to induction. *Expert Opin Drug Metab Toxicol* **6**(11): 1399-1416.

Flint MS, Hood BL, Sun M, Stewart NA, Jones-Laughner J and Conrads TP (2010) Proteomic analysis of the murine liver in response to a combined exposure to psychological stress and 7,12-dimethylbenz(a)anthracene. *Journal of proteome research* **9**(1): 509-520.

Fruchart JC and Duriez P (2006) Mode of action of fibrates in the regulation of triglyceride and HDL-cholesterol metabolism. *Drugs Today (Barc)* **42**(1): 39-64.

Gergel D, Misik V, Riesz P and Cederbaum AI (1997) Inhibition of rat and human cytochrome P4502E1 catalytic activity and reactive oxygen radical formation by nitric oxide. *Arch Biochem Biophys* **337**(2): 239-250.

Ghonem NS, Assis DN and Boyer JL (2015) Fibrates and cholestasis. *Hepatology* **62**(2): 635-643.

Gonzalez FJ (2005) Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. *Mutat Res* **569**(15603755): 101-110.

Gonzalez FJ and Gelboin HV (1994a) Role of Human Cytochromes P450 in the Metabolic Activation of Chemical Carcinogens and Toxins. *Drug metabolism reviews* **26**(1-2): 165-183.

Gonzalez FJ and Gelboin HV (1994b) Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. *Drug metabolism reviews* **26**(1-2): 165-183.

Gonzalez FJ and Yu A-M (2006) Cytochrome P450 and xenobiotic receptor humanized mice. *Annu Rev Pharmacol Toxicol* **46**(16402898): 41-64.

Greensfelder L (2000) Alternative medicine. Herbal product linked to cancer. *Science (New York, NY)* **288**: 1946.

Guengerich FP (2003) Cytochromes P450, drugs, and diseases. *Molecular interventions* **3**(4): 194-204.

Hahn ME, Allan LL and Sherr DH (2009) Regulation of constitutive and inducible AHR signaling: complex interactions involving the AHR repressor. *Biochem Pharmacol* **77**(4): 485-497.

Haller CA and Benowitz NL (2000) Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. *The New England journal of medicine* **343**(25): 1833-1838.

Hansen M and Connolly T (2008) Nuclear receptors as drug targets in obesity, dyslipidemia and atherosclerosis. *Current opinion in investigational drugs (London, England : 2000)* **9**: 247-255.

Hara H and Adachi T (2002) Contribution of hepatocyte nuclear factor-4 to down-regulation of CYP2D6 gene expression by nitric oxide. *Molecular pharmacology* **61**(1): 194-200.

Harkitis P, Daskalopoulos EP, Malliou F, Lang MA, Marselos M, Fotopoulos A, Albucharali G and Konstandi M (2015) Dopamine D2-Receptor Antagonists Down-Regulate CYP1A1/2 and CYP1B1 in the Rat Liver. *PloS one* **10**(10): e0128708.

Hay N (2011) Interplay between FOXO, TOR, and Akt. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1813**(11): 1965-1970.

Honkakoski P and Negishi M (2000) Regulation of cytochrome P450 (CYP) genes by nuclear receptors. *Biochem J* **347**(Pt 2): 321-337.

Hu DD, Zhao Q, Cheng Y, Xiao XR, Huang JF, Qu Y, Li X, Tang YM, Bao WM, Yang JH, Jiang T, Hu JP, Gonzalez FJ and Li F (2019) The Protective Roles of PPARalpha Activation in Triptolide-induced Liver Injury. *Toxicological sciences : an official journal of the Society of Toxicology*.

Impellizzeri D, Esposito E, Mazzon E, Paterniti I, Di Paola R, Bramanti P, Morittu VM, Procopio A, Perri E, Britti D and Cuzzocrea S (2012) The effects of a polyphenol present in olive oil, oleuropein aglycone, in an experimental model of spinal cord injury in mice. *Biochem Pharmacol* **83**(10): 1413-1426.

Ingelman-Sundberg M (2004a) Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn-Schmiedeberg's archives of pharmacology* **369**(1): 89-104.

Ingelman-Sundberg M (2004b) Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. *Trends Pharmacol Sci* **25**(4): 193-200.

Ingelman-Sundberg M, Sim SC, Gomez A and Rodriguez-Antona C (2007) Influence of cytochrome P450 polymorphisms on drug therapies: Pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacology & Therapeutics* **116**(3): 496-526.

Kawajiri K (1999) Cyp1a1. *IARC Sci Publ*(148): 159-172.

Kawajiri K and Fujii-Kuriyama Y (2007) Cytochrome P450 gene regulation and physiological functions mediated by the aryl hydrocarbon receptor. *Archives of biochemistry and biophysics* **464**: 207-212.

Kennedy DA and Seely D (2010) Clinically based evidence of drug–herb interactions: a systematic review. *Expert Opinion on Drug Safety* **9**(1): 79-124.

Kim SK and Novak RF (2007) The role of intracellular signaling in insulin-mediated regulation of drug metabolizing enzyme gene and protein expression. *Pharmacol Ther* **113**(1): 88-120.

Klepser TB and Klepser ME (1999) Unsafe and potentially safe herbal therapies. *American Journal of Health-System Pharmacy* **56**(2): 125-138.

Kodama S, Koike C, Negishi M and Yamamoto Y (2004) Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. *Mol Cell Biol* **24**(18): 7931-7940.

Koh H-L and Woo S (2000) Chinese proprietary medicine in Singapore: Regulatory control of toxic heavy metals and undeclared drugs. *Drug safety : an international journal of medical toxicology and drug experience* **23**: 351-362.

Konstandi M (2013) Psychophysiological stress: a significant parameter in drug pharmacokinetics. *Expert Opinion on Drug Metabolism & Toxicology* **9**(10): 1317-1334.

Konstandi M, Andriopoulou CE, Cheng J and Gonzalez FJ (2020) Sex steroid hormones differentially regulate CYP2D in female wild-type and CYP2D6-humanized mice. **245**(2): 301.

Konstandi M, Johnson EO and Lang MA (2014) Consequences of psychophysiological stress on cytochrome P450-catalyzed drug metabolism. *Neuroscience & Biobehavioral Reviews* **45**: 149-167.

Konstandi M, Kostakis D, Harkitis P, Johnson EO, Marselos M, Adamidis K and Lang MA (2006) Benzo(α)pyrene-induced up-regulation of CYP1A2 gene expression: Role of adrenoceptor-linked signaling pathways. *Life Sciences* **79**(4): 331-341.

Konstandi M, Kostakis D, Harkitis P, Marselos M, Johnson EO, Adamidis K and Lang MA (2005) Role of adrenoceptor-linked signaling pathways in the regulation of CYP1A1 gene expression. *Biochemical Pharmacology* **69**(2): 277-287.

Kuusisto J, Andrulionyte L and Laakso M (2007) Atherosclerosis and cardiovascular risk reduction with PPAR agonists. *Current Atherosclerosis Reports* **9**(4): 274-280.

Lang MA, Gielen JE and Nebert DW (1981) Genetic evidence for many unique liver microsomal P-450-mediated monooxygenase activities in heterogeneic stock mice. *The Journal of biological chemistry* **256**(23): 12068-12075.

Leuenberger N, Pradervand S and Wahli W (2009) Sumoylated PPARα mediates sex-specific gene repression and protects the liver from estrogen-induced toxicity in mice. *The Journal of Clinical Investigation* **119**(10): 3138-3148.

Lockyer S, Rowland I, Spencer JPE, Yaqoob P and Stonehouse W (2017) Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: a randomised controlled trial. *European journal of nutrition* **56**(4): 1421-1432.

Makia NL and Goldstein JA (2016) CYP2C8 Is a Novel Target of Peroxisome Proliferator-Activated Receptor alpha in Human Liver. *Molecular pharmacology* **89**(1): 154-164.

Malliou F, Andreadou I, Gonzalez FJ, Lazou A, Xepapadaki E, Vallianou I, Lambrinidis G, Mikros E, Marselos M, Skaltsounis AL and Konstandi M (2018) The olive constituent oleuropein, as a PPARalpha agonist, markedly reduces serum triglycerides. *The Journal of nutritional biochemistry* **59**: 17-28.

Matsunaga E, Zeugin T, Zanger UM, Aoyama T, Meyer UA and Gonzalez FJ (1990) Sequence requirements for cytochrome P-450IID1 catalytic activity. A single amino acid change (Ile380 Phe) specifically decreases Vmax of the enzyme for bufuralol but not debrisoquine hydroxylation. *The Journal of biological chemistry* **265**(28): 17197-17201.

Mckenna DJ, Jones K, Hughes K and Tyler VM (2012) *Botanical Medicines: The Desk Reference for Major Herbal Supplements, Second Edition*. Taylor & Francis.

McRae CA, Agarwal K, Mutimer D and Bassendine MF (2002) Hepatitis associated with Chinese herbs. *European Journal of Gastroenterology & Hepatology* **14**(5): 559-562.

Mulder H, Breure A and Rulkens W (2001) Prediction of complete bioremediation periods for PAH soil pollutants in different physical states by mechanistic models. *Chemosphere* **43**: 1085-1094.

Nebert DW (2000) Drug-metabolizing enzymes, polymorphisms and interindividual response to environmental toxicants. *Clin Chem Lab Med* **38**(9): 857-861.

Nebert DW, Wikvall K and Miller WL (2013) Human cytochromes P450 in health and disease. *Philos Trans R Soc Lond B Biol Sci* **368**(1612): 20120431-20120431.

Ning LJ, He AY, Li JM, Lu DL, Jiao JG, Li LY, Li DL, Zhang ML, Chen LQ and Du ZY (2016) Mechanisms and metabolic regulation of PPARalpha activation in Nile tilapia (Oreochromis niloticus). *Biochimica et biophysica acta* **1861**(9 Pt A): 1036-1048.

Oi-Kano Y, Kawada T, Watanabe T, Koyama F, Watanabe K, Senbongi R and Iwai K (2008) Oleuropein, a phenolic compound in extra virgin olive oil, increases uncoupling protein 1 content in brown adipose tissue and enhances noradrenaline and adrenaline secretions in rats. *J Nutr Sci Vitaminol (Tokyo)* **54**(5): 363-370.

Okey AB (1990) Enzyme induction in the cytochrome P-450 system. *Pharmacol Ther* **45**(2405437): 241-298.

Oshida K, Waxman DJ and Corton JC (2016a) Chemical and Hormonal Effects on STAT5b-Dependent Sexual Dimorphism of the Liver Transcriptome. *PloS one* **11**(3): e0150284.

Oshida K, Waxman DJ and Corton JC (2016b) Correction: Chemical and Hormonal Effects on STAT5b- Dependent Sexual Dimorphism of the Liver Transcriptome. *PloS one* **11**(8): e0161519.

Pappenheimer JR and Reiss KZ (1987) Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *The Journal of Membrane Biology* **100**(1): 123-136.

Pasanen M and Pelkonen O (1994) The expression and environmental regulation of P450 enzymes in human placenta. *Crit Rev Toxicol* **24**(3): 211-229.

Passilly P, Schohn H, Jannin B, Cherkaoui Malki M, Boscoboinik D, Dauça M and Latruffe N (1999) Phosphorylation of peroxisome proliferator-activated receptor α in rat Fao cells and stimulation by ciprofibrate. *Biochemical Pharmacology* **58**(6): 1001-1008.

Paukkeri EL, Leppanen T, Sareila O, Vuolteenaho K, Kankaanranta H and Moilanen E (2007) PPARalpha agonists inhibit nitric oxide production by enhancing iNOS degradation in LPS-treated macrophages. *Br J Pharmacol* **152**(7): 1081-1091.

Pelkonen O, Turpeinen M, Hakkola J, Honkakoski P, Hukkanen J and Raunio H (2008) Inhibition and induction of human cytochrome P450 enzymes: current status. *Arch Toxicol* **82**(10): 667-715.

Robillard R, Fontaine C, Chinetti G, Fruchart J-C and Staels B (2005) Fibrates, in *Atherosclerosis: Diet and Drugs* (von Eckardstein A ed) pp 389-406, Springer Berlin Heidelberg, Berlin, Heidelberg.

Shi C, Min L, Yang J, Dai M, Song D, Hua H, Xu G, Gonzalez FJ and Liu A (2017) Peroxisome Proliferator-Activated Receptor alpha Activation Suppresses Cytochrome P450 Induction Potential in Mice Treated with Gemfibrozil. *Basic Clin Pharmacol Toxicol* **121**(3): 169-174.

Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP and Sutter TR (1996) Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer research* **56**(13): 2979-2984.

Spatzenegger M and Jaeger W (1995) Clinical importance of hepatic cytochrome P450 in drug metabolism. *Drug metabolism reviews* **27**(3): 397-417.

Stedman C (2002) Herbal hepatotoxicity. *Seminars in liver disease* **22**(2): 195-206.

Stupans I, Murray M, Kirlich A, Tuck K and Hayball PJ (2001) Inactivation of cytochrome P450 by the food-derived complex phenol oleuropein. *Food and Chemical Toxicology* **39**: 1119-1124.

Stupans I, Stretch G and Hayball P (2000) Olive Oil Phenols Inhibit Human Hepatic Microsomal Activity. *The Journal of Nutrition* **130**(9): 2367-2370.

Tamasi V, Miller KK, Ripp SL, Vila E, Geoghagen TE and Prough RA (2008) Modulation of receptor phosphorylation contributes to activation of peroxisome proliferator activated receptor alpha by dehydroepiandrosterone and other peroxisome proliferators. *Molecular pharmacology* **73**(3): 968-976.

Tauber Z, Koleckova M and Cizkova K (2020) Peroxisome proliferator-activated receptor a (PPARa)-cytochrome P450 epoxygenases-soluble epoxide hydrolase axis in ER + PR + HER2- breast cancer. *Med Mol Morphol* **53**(3): 141-148.

Thomas M, Burk O, Klumpp B, Kandel BA, Damm G, Weiss TS, Klein K, Schwab M and Zanger UM (2013) Direct transcriptional regulation of human hepatic cytochrome P450 3A4 (CYP3A4) by peroxisome proliferator-activated receptor alpha (PPARalpha). *Molecular pharmacology* **83**(3): 709-718.

Tuck KL and Hayball PJ (2002) Major phenolic compounds in olive oil: metabolism and health effects. *The Journal of Nutritional Biochemistry* **13**(11): 636-644.

Visioli F and Galli C (1994) Oleuropein protects low density lipoprotein from oxidation. *Life Sciences* **55**(24): 1965-1971.

Visioli F, Galli C, Galli G and Caruso D (2002a) Biological activities and metabolic fate of olive oil phenols. *European Journal of Lipid Science and Technology* **104**(9-10): 677-684.

Visioli F, Poli A and Gall C (2002b) Antioxidant and other biological activities of phenols from olives and olive oil. *Medicinal Research Reviews* **22**(1): 65-75.

Vogel P, Kasper Machado I, Garavaglia J, Zani VT, de Souza D and Morelo Dal Bosco S (2014) Polyphenols benefits of olive leaf (Olea europaea L) to human health. *Nutricion hospitalaria* **31**(3): 1427-1433.

Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M and Liddle C (1998) Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology* **27**(1): 128-133.

Wink DA, Osawa Y, Darbyshire JF, Jones CR, Eshenaur SC and Nims RW (1993) Inhibition of cytochromes P450 by nitric oxide and a nitric oxide-releasing agent. *Arch Biochem Biophys* **300**(1): 115-123.

Wiwi C and Waxman D (2004) Mini Review Role of Hepatocyte Nuclear Factors in Growth Hormone-regulated, Sexually Dimorphic Expression of Liver Cytochromes P450*. *Growth factors (Chur, Switzerland)* **22**: 79-88.

Xu M, Nelson GB, Moore JE, McCoy TP, Dai J, Manderville RA, Ross JA and Miller MS (2005) Induction of Cyp1a1 and Cyp1b1 and formation of DNA adducts in C57BL/6, Balb/c, and F1 mice following in utero exposure to 3-methylcholanthrene. *Toxicol Appl Pharmacol* **209**(1): 28-38.

Yang Q, Nagano T, Shah Y, Cheung C, Ito S and Gonzalez FJ (2008) The PPAR alpha-humanized mouse: a model to investigate species differences in liver toxicity mediated by PPAR alpha. *Toxicol Sci* **101**(1): 132-139.

Yao M, Zhu M, Sinz MW, Zhang H, Humphreys WG, Rodrigues AD and Dai R (2007) Development and full validation of six inhibition assays for five major cytochrome P450 enzymes in human liver microsomes using an automated 96-well microplate incubation format and LC-MS/MS analysis. *J Pharm Biomed Anal* **44**(1): 211-223.

YUMUK VD (2006) Targeting Components of the Stress System as Potential Therapies for the Metabolic Syndrome. *Annals of the New York Academy of Sciences* **1083**(1): 306-318.

Zanger UM and Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* **138**(1): 103-141.

Zhang Q-Y, Dunbar D, Ostrowska A, Zeisloft S, Yang J and Kaminsky LS (1999) Characterization of Human Small Intestinal Cytochromes P-450. *Drug Metabolism and Disposition* **27**(7): 804-809.

Zhou S-F, Xue CC, Yu X-Q, Li C and Wang G (2007) Clinically Important Drug Interactions Potentially Involving Mechanism-based Inhibition of Cytochrome P450 3A4 and the Role of Therapeutic Drug Monitoring. *Therapeutic Drug Monitoring* **29**(6): 687-710.

Zhou S, Chan E, Pan S-Q, Huang M and Lee EJD (2004) Pharmacokinetic Interactions of Drugs with St John's Wort. *Journal of Psychopharmacology* **18**(2): 262-276.

Footnotes

Funding: This study was funded by the European Union (European Regional Development Fund- ERDF) and Greek national funds coming from the Operational Program "THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013, Grant 346985/80753). We certify that there is no involvement of the funding sources in the research conduct and/or preparation of this article, in study design, in the collection, analysis and interpretation of data, in the writing of the article, and the decision to submit the article for publication in DMD.

The authors also state that there is no conflict of interest.

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Figure legends

Figure 1. PPARα-mediated regulation of hepatic CYP1A1, CYP1A2 and CYP1B1. Assessment of the effect of OLE, a PPARα agonist, on (A) CYP1A1, (B) CYP1A2 and (C) CYP1B1 protein levels employing western blotting, and relative gene expression with quantitative PCR analysis. In wild type and *Ppara*-null mice, comparisons were made between Controls and OLE-treated mice. C: Control, OLE: oleuropein; Values are expressed as mean ± SE, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. wild-type mice (n=11), *Ppara* null mice (n=10). Numbers below bands in western blot captures show the ratio of the density of the sample band to that of a-tubulin.

Figure 2. PPARα-mediated regulation of hepatic CYP2C29/2C44 and CYP2D22. Assessment of the effect of OLE, a PPARα agonist, on (A) CYP2C29, (B) CYP2C44 and (C) CYP2D22 protein levels employing western blotting, and on relative gene expression with quantitative PCR analysis. The OLE-mediated effect on 1′-bufuralol hydroxylation was performed using HPLC. In wild-type and *Ppara*-null mice, comparisons were made between Controls and OLE-treated mice. C: Control, OLE: oleuropein; Values are expressed as mean ± SE, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. wild-type mice (n=11), *Pparα* null mice (n=10). Numbers below bands in western blot captures show the ratio of the density of the sample band to that of a-tubulin.

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 3. PPARα-mediated regulation of hepatic CYP2E1 and CYP3A14/3A25. Assessment of the effect of OLE, a PPARα agonist, on (A) CYP2E1, (B) CYP3A14 and (C) CYP3A25 protein level employing western blotting, and on relative gene expression with quantitative PCR analysis. In wild-type and *Ppara*-null mice, comparisons were made between Controls and OLE-treated mice. C: Control, OLE: oleuropein; Values are expressed as mean ± SE, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. wild-type mice (n=11), *Ppara*-null mice (n=10). Numbers below bands in western blot captures show the ratio of the density of the sample band to that of a-tubulin.

Figure 4. Assessment of the effect of OLE, a PPARα agonist, on various factors involved in CYP1A and CYP1B regulation (Matsunaga et al., 1990). (A) aryl hydrocarbon receptor (Ahr), (B) aryl hydrocarbon receptor repressor (AhRR), and (C) heat shock protein 90 (HSP90) relative mRNA expression was estimated with quantitative PCR analysis. In wild-type and *Ppara*-null mice, comparisons were made between Controls and OLE-treated mice. C: Control, OLE: oleuropein; Values are expressed as mean ± SE, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. wild-type mice (n=11), *Ppara*-null mice (n=10).

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 5. Assessment of the effect of OLE, a PPARα agonist, on various factors involved in CYP3A and CYP2C regulation (Konstandi et al., 2006). (A) Pregnane X receptor (*Pxr*), (B) Constitutive androstane receptor (*Car*), (C) Retinoid X receptor a (*Rxra*) and (D) Retinoid X receptor β (*Rxrb*) relative mRNA expression with quantitative PCR analysis. In wild type and *Ppara*-null mice, comparisons were made between Controls and OLE-treated mice. C: Control, OLE: oleuropein; Values are expressed as mean ± SE, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. wild-type mice (n=11), *Pparα* null mice (n=10). Numbers below bands in western blot captures show the ratio of the density of the sample band to that of Histone H3.

Figure 6. *In vivo* assessment of the effects of FEN, a selective PPARα agonist, on CYP protein expression by Western blotting using microsomal proteins extracted from liver samples of wild type and *PPARα*-null mice. FEN-induced hepatic CYP1A1, CYP1A2, CYP1B1, CYP2C, CYP2D, CYP2E1 and CYP3A protein expression. In wild type and *Ppara*-null mice, comparisons were made between Controls and FEN-treated mice. C: Control, FEN: fenofibrate. The samples in the western blot captures are representative of other three that were analyzed in separate blots. Numbers below bands in western blot captures show the ratio of the density of the sample band to that of GAPDH.

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 7. Assessment of the effect of OLE, a PPARα agonist, on (A) endothelial NOS (*Nos3*) and (B) inducible nitric oxide synthase (*Nos2*) relative mRNA expression with quantitative PCR analysis. In wild type and *Ppara*-null mice, comparisons were made between Controls and OLE-treated mice. C: Control, OLE: oleuropein; Values are expressed as mean ± SE, ** $p<0.01$, *** $p<0.001$. wild-type mice (n=11), *Pparα* null mice (n=10).

Figure 8. *In vivo* assessment of the effects of OLE, a PPARα agonist, on several signal transduction pathways involved in CYP regulation by Western blotting using total cellular proteins extracted from liver samples of mice. OLE-induced activation of FOX01, JNK, p70 and ERK but not to p38-MAPK. In wild type and *Ppara*-null mice, comparisons were made between Controls and OLE-treated mice. C: Control, OLE: oleuropein. The samples in the western blot captures are representative of other three that were analyzed in separate blots. Numbers below bands in western blot captures show the ratio of the density of the sample band to that of a-tubulin.

Graphic abstract. Oleuropein (OLE) induced the expression of various important drug metabolizing CYP genes in the liver of mice, including *Cyp1a1*, *Cyp1a2*, *Cyp1b1*, *Cyp2c29*, *Cyp2c44*, *Cyp2d22*, *Cyp2e1*, *Cyp3a14* and *Cyp3a25*. OLE acts as a ligand of the nuclear transcription factor, PPARα, and the complex binds to peroxisome proliferator activated receptor responsive element (PPRE) in the promoter of the CYP gene, thus stimulating its transcription. PXR: pregnane X receptor is a drug activated transcription factor, involved both in constitutive and/or inducible expression of CYP3A CYP2C and CYP2A cytochromes; RXR: retinoid X

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

receptor and CAR: constitutive androstane receptor, are two drug activated transcription factors involved in both constitutive and/or inducible *CYP3A* and *CYP2C* expression Ahr: Aryl Hydrocarbon Receptor and ARNT: Aryl Hydrocarbon Receptor Nuclear Translocator are two transcription factors regulating both, constitutive and inducible *CYP1A* and *CYP1B* expression (Daskalopoulos et al., 2012a; Daskalopoulos et al., 2012b; Harkitis et al., 2015; Nebert et al., 2013).

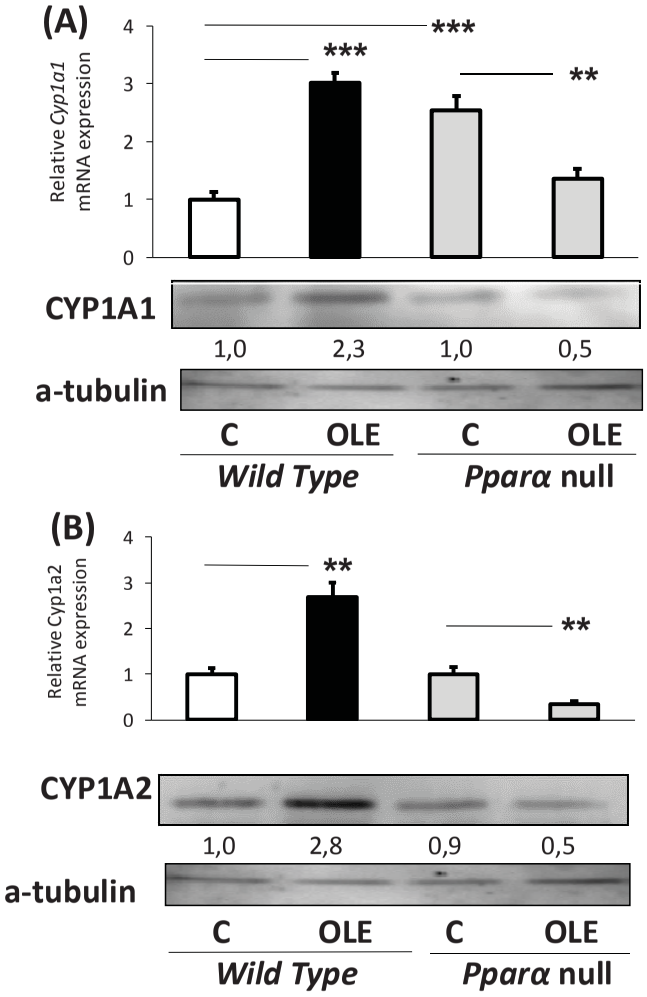
DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1: Oligonucleotide sequences used as primers for quantitation of gene mRNA levels through quantitative PCR assays

Gene	Primer sequence
Ppara	**F:**CAGTGGGGAGAGAGGACAGA **R:**AGTTCGGGAACAAGACGTTG
Cyp2d22	**F:**ACCGGTAAAGGTAGCTGGAGT **R:** CATAGGGCCTGGAGGGTAGT
Hnf4a	**F:**CGGAGCCCCTGCAAAGT **R:**ACTATCCAGTCTCACAGCCCATTC
Cyp2e1	**F:** TGGTCCTGCATGGCTACAAG **R:** CGGGCCTCATTACCCTGTTT
Cyp2c29	**F:** TGTTACAAACCCCCGTGACT **R:** GGATGTGGATAAAGACCTGAGAC
Cyp2c44	**F:** CCTAAAGGCTCTGGTGGAGC **R:** GAAACAAATGCCCACGTGCT
Cyp3a14	**F:**GGCCCAGTGGGGATAATGAG **R:**GGTGCCTTATTGGGCAGAGT
Cyp3a25	**F:**TAGAAACCTGGGTGCTGCTG **R:**GGATGTGGATAAAGACCTGAGAC
Pxr	**F:**AAGAAGCAGACTCTGCCTTGGA **R:**GTGGTAGCCATTGGCCTTGT
Car	**F:**CCTCTTCTCCCCTGGTTTCTG **R:**TCATTGCCACTCCCAAGCTC
Rxra	**F:**CAGTACGCAAAGACCTGACCTACA **R:**GTTCCGCTGTCTCTTGTCGAT
Rxrb	**F:** AAGTGTCTGGAGCACCTGTTCTT **R:**CTCCATGAGGAAGGTGTCAATG
Cyp1a1	**F:**GAAGTGGAAGGGCATAGGCAG **R:**GGCCAAAGCATATGGCACAG
Cyp1a2	**F:**ACTTCGAACCAGTCAGCCAG **R:**GTGCTTGAACAGGGCACTTG
Cyp1b1	**F:**CCAGCTTTTTGCCTGTCACC **R:**TGCACTGATGAGCGAGGATG
Ahr	**F:**TTCAGAACTGACTCCACCGC **R:**CCGGGTGTGATATCGGGAAG
Ahrr	**F:**AGTGTACATACGCCGGTAGG **R:**CAAGACTGGTGCCACAATGC
Hsp90	**F:**CAGACCATGGTGAGCCCATT **R:**TCAACCACACCGCGGATAAA

DMD Fast Forward. Published on June 23, 2021 as DOI: 10.1124/dmd.120.000302
This article has not been copyedited and formatted. The final version may differ from this version.

Nos3	**F:**GCAGAAGAGTCCAGCGAACA **R:**GGCAGCCAAACACCAAAGTC
Nos2	**F:**GTGTTCCACCAGGAGATGTTG **R:**CTCCTGCCCACTGAGTTCGTC



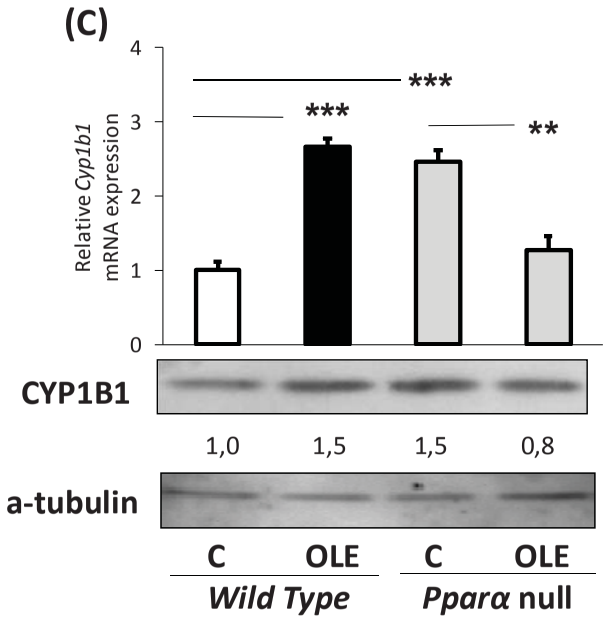


Figure 1

Figure 2

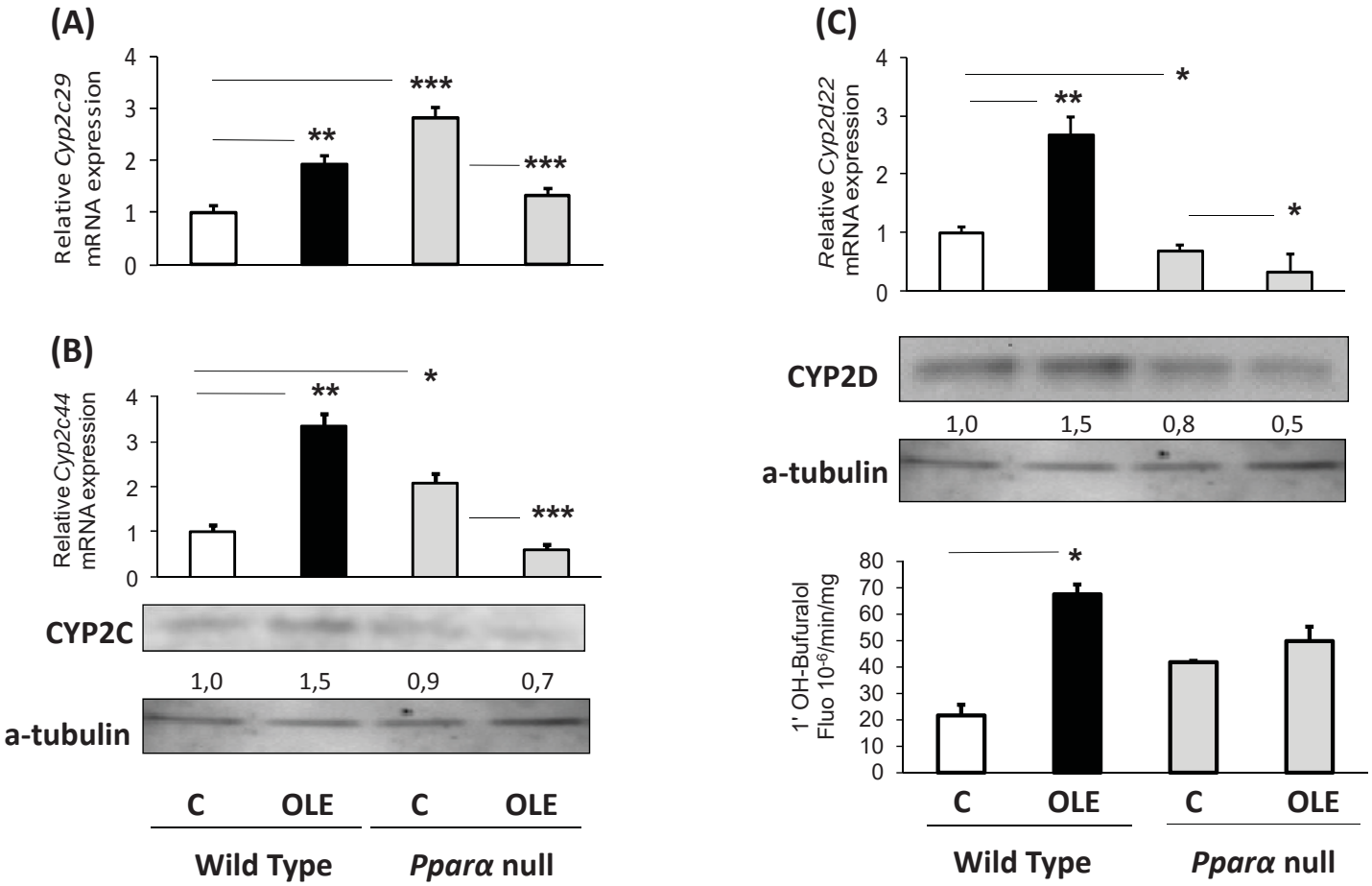


Figure 3

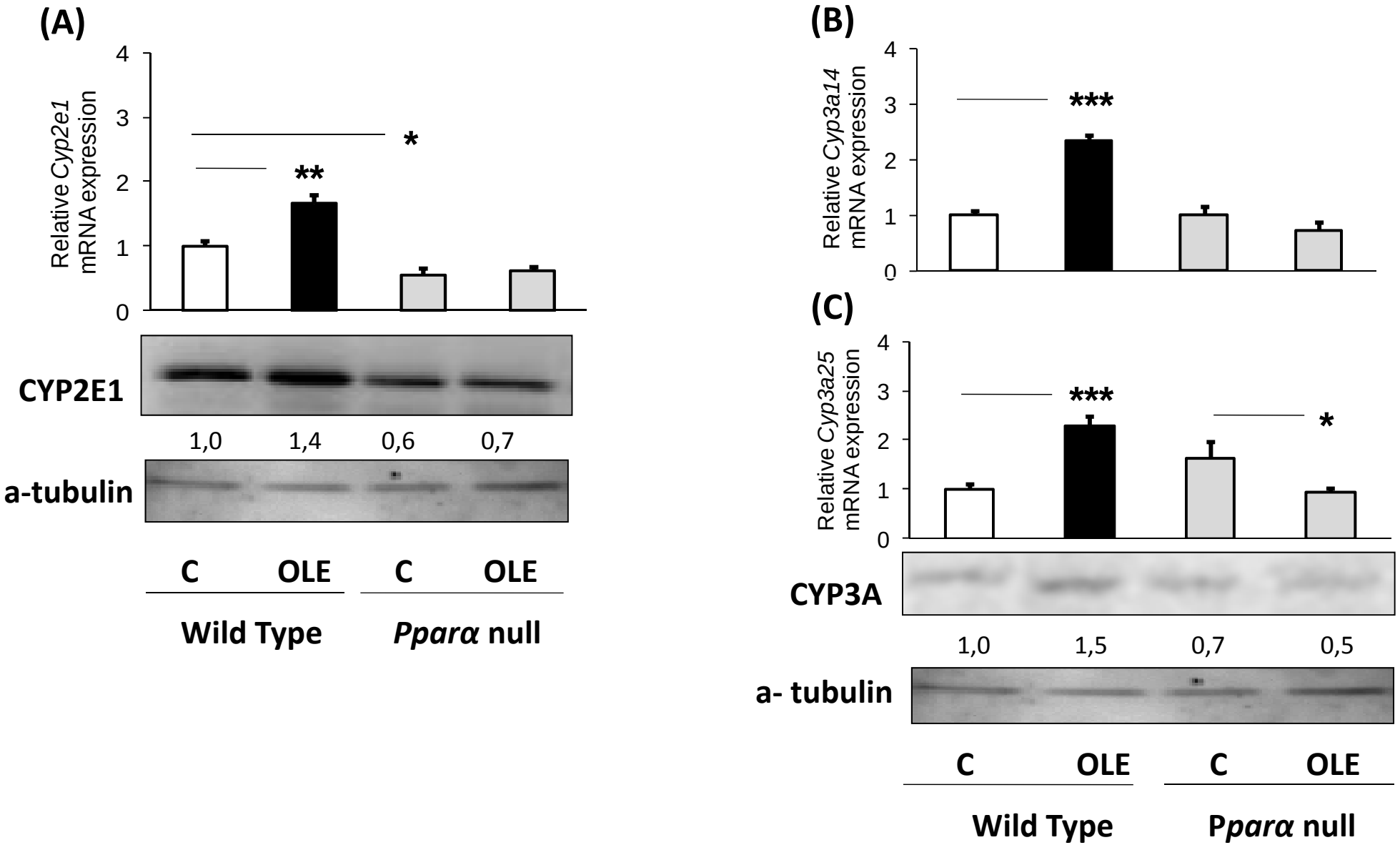
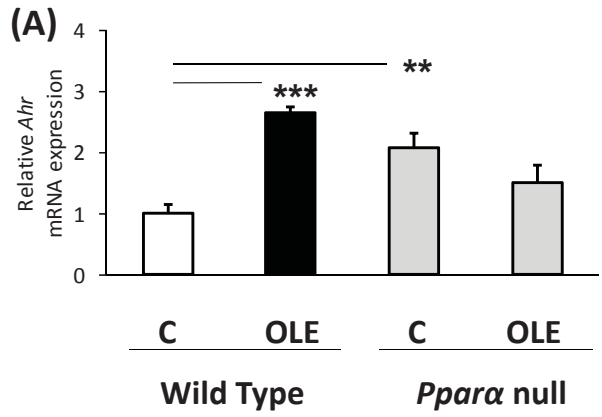
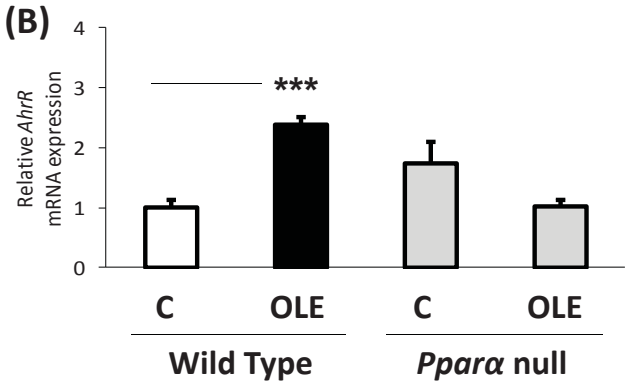
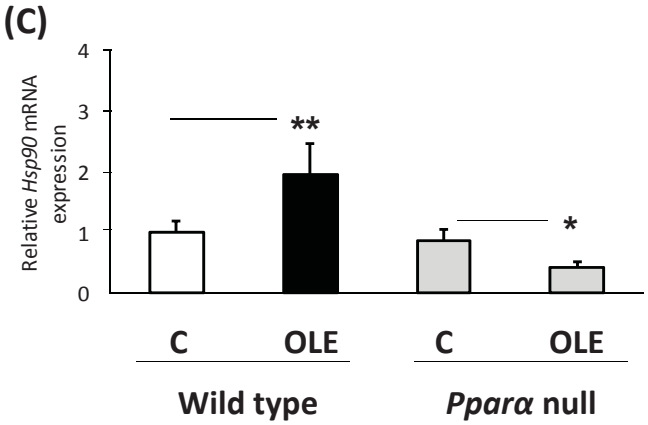
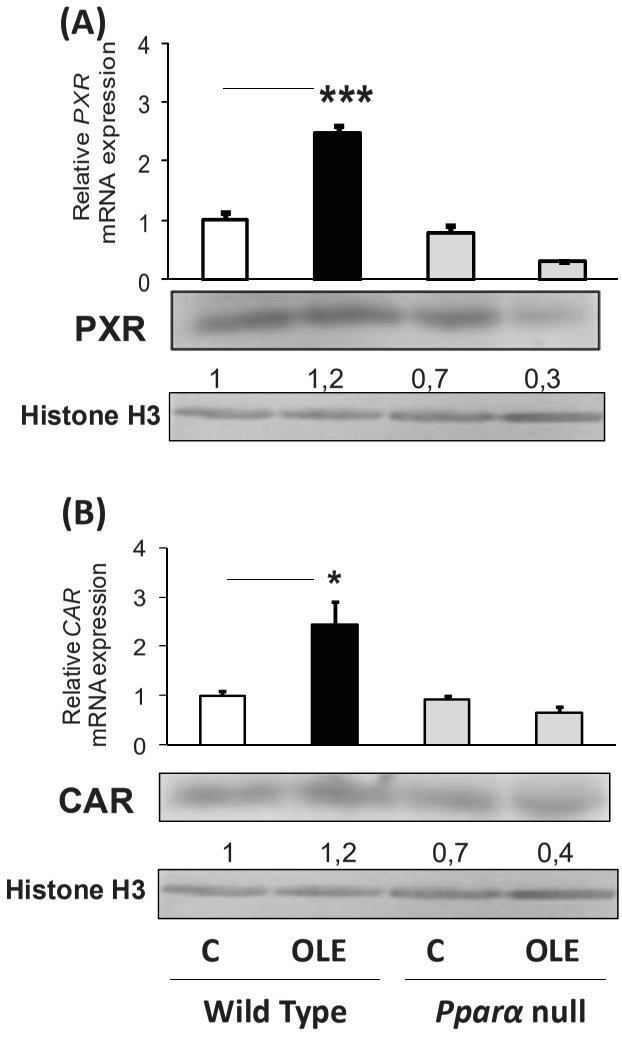


Figure 4









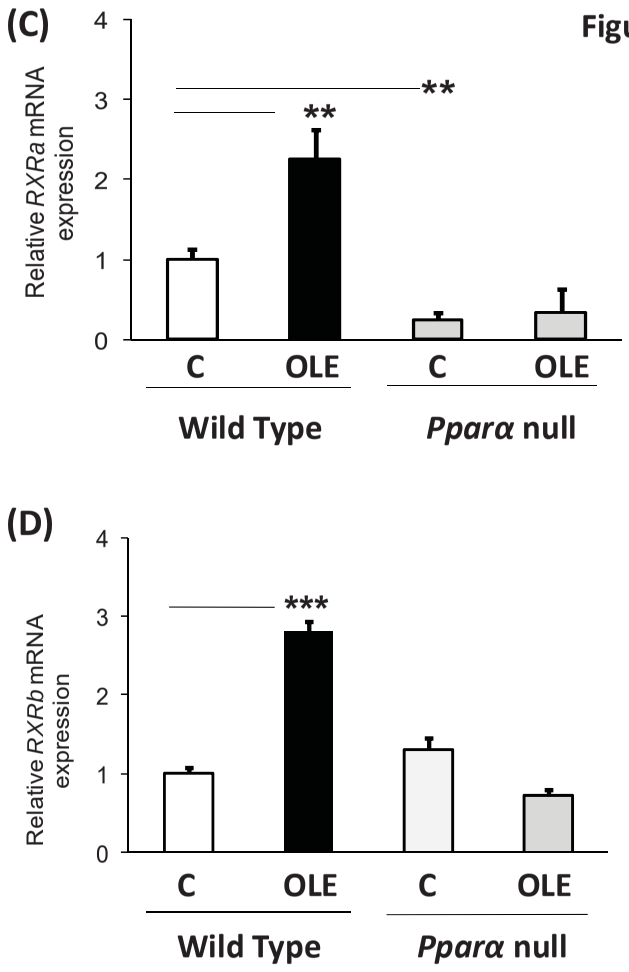


Figure 5

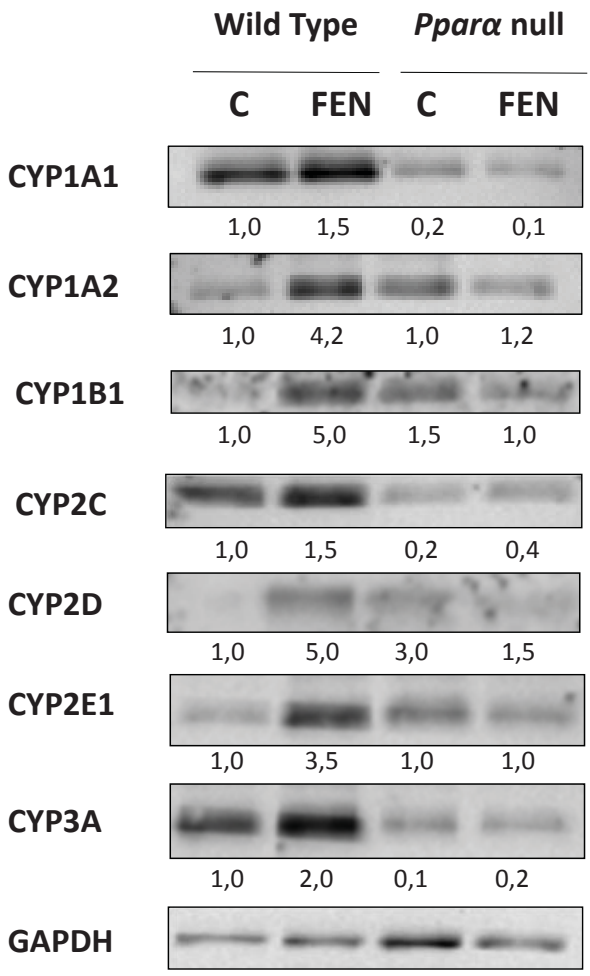


Figure 6

Figure 7

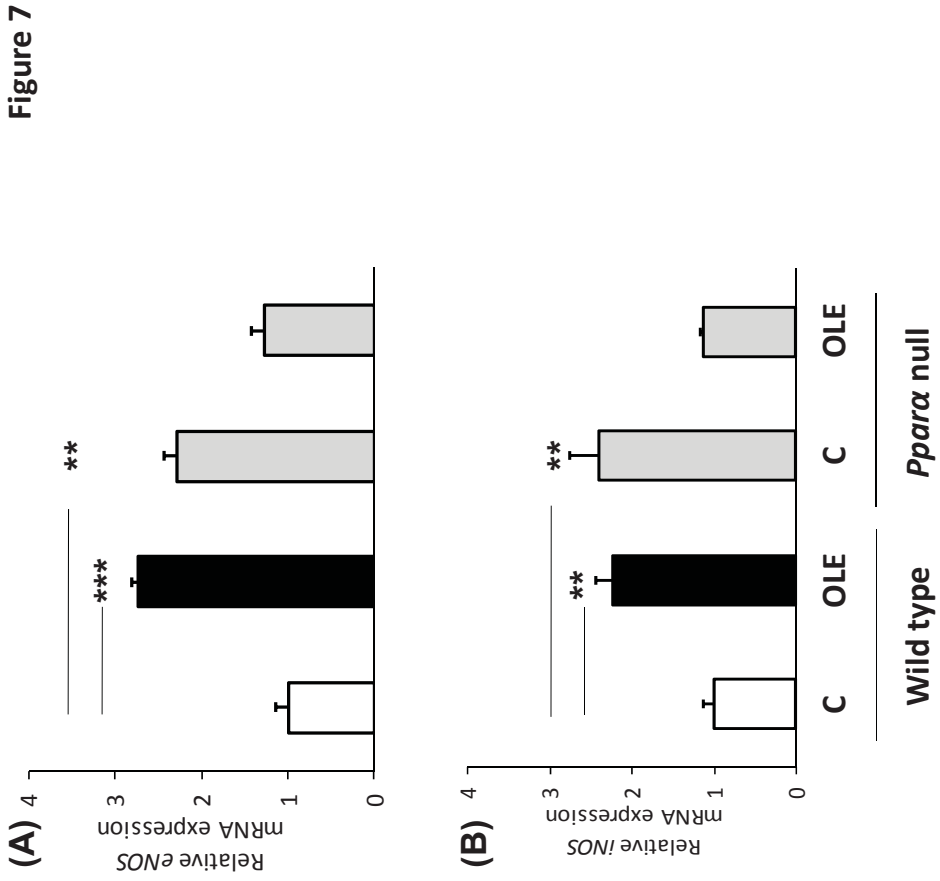
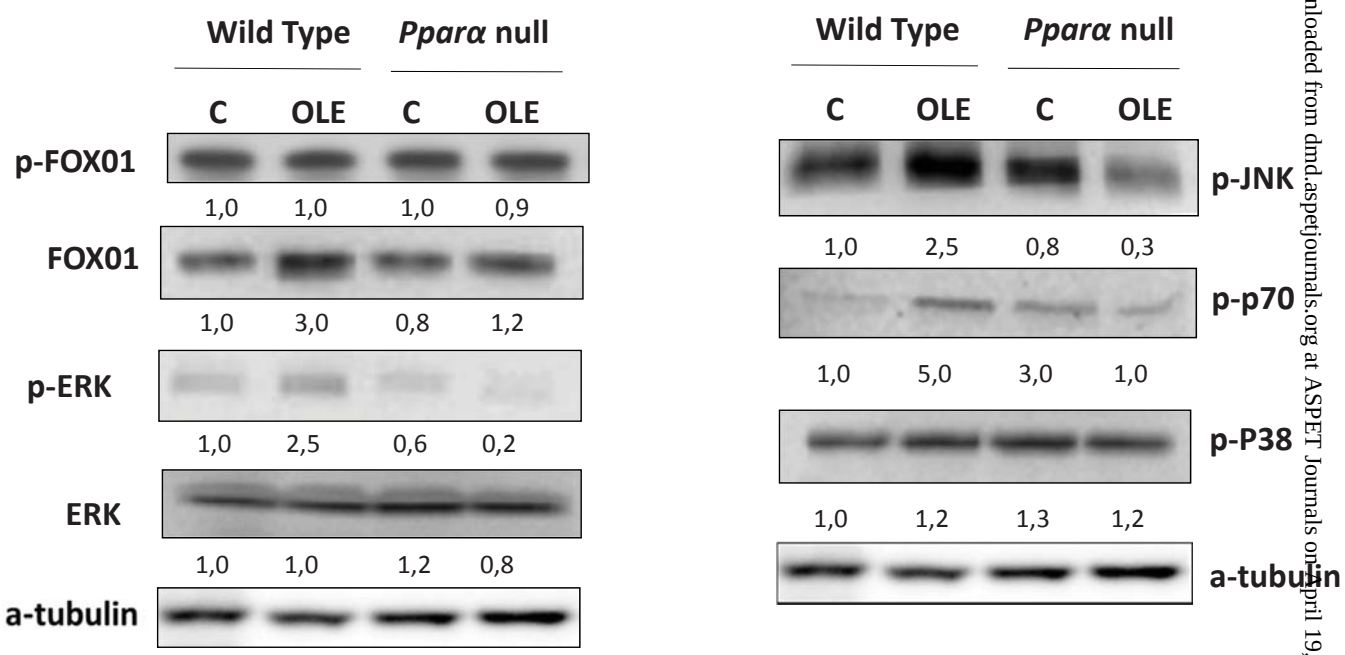
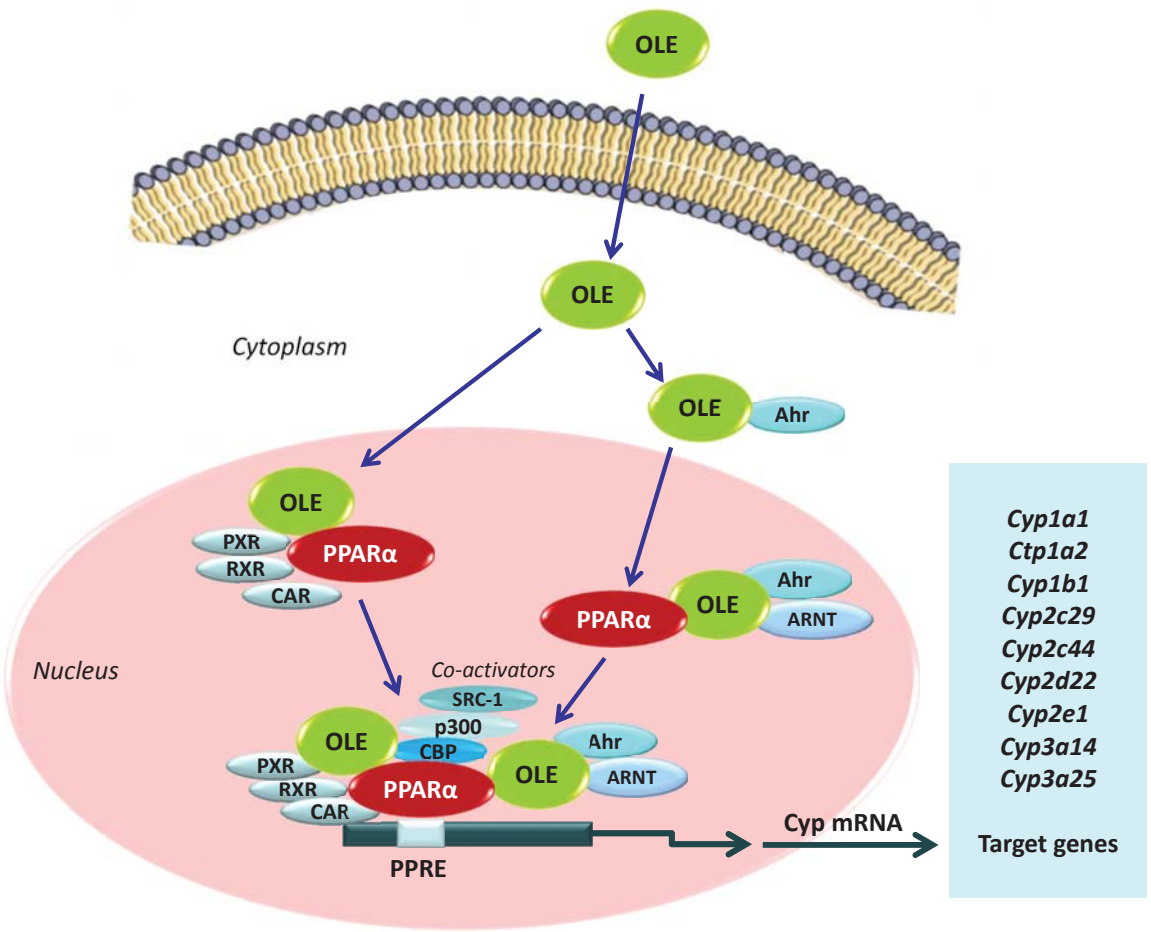


Figure 8



Total cellular proteins



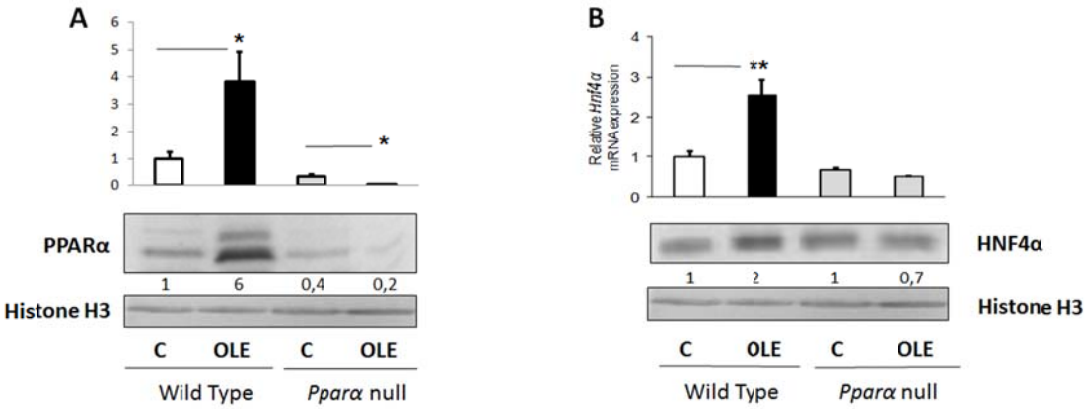
Oleuropein-induced acceleration of CYP-catalyzed drug metabolism: central role for nuclear receptor PPARα

Foteini Malliou[1#], Christina E. Andriopoulou[1#], Frank J. Gonzalez[2], Aristeidis Kofinas[1], Alexios-Leandros Skaltsounis[3], Maria Konstandi[1]*

[1]University of Ioannina, School of Health Sciences, Faculty of Medicine, Department of Pharmacology, Ioannina GR-45110, Greece;; [2]Laboratory of Metabolism, National Cancer Institute, National Institutes of Health, Bethesda 20892, Maryland, USA; [3]Faculty of Pharmacy, School of Health Sciences, University of Athens, Greece

Journal: Drug Metabolism & Disposition

MS #DMD-AR-2020-000302



Supplemental Figure 1. *In vivo* assessment of the effects of OLE, a PPARα agonist, on *Ppara* and *Hnf4a* mRNA expression using quantitative PCR analysis. PPARα and HNF4α protein levels were analyzed by western blot. In wild type and Pparα-null mice, comparisons took place between Controls and OLE-treated mice. C: Control, OLE: oleuropein, WT: wild type; Values are expressed as mean ± SE, ** $p<0.01$, *** $p<0.001$. wild type mice (n=11), *Ppara*-null mice (n=10).