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## Molecular Actions of PPAR $\alpha$ in Lipid Metabolism and Inflammation

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29 **Abstract**

30 Peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ) is a nuclear receptor of  
31 clinical interest as a drug target in various metabolic disorders. PPAR $\alpha$  also exhibits  
32 marked anti-inflammatory capacities. The first generation PPAR $\alpha$  agonists, the  
33 fibrates, have however been hampered by drug-drug interaction issues, statin drop-in  
34 and ill-designed cardiovascular intervention trials. Notwithstanding, understanding the  
35 molecular mechanisms by which PPAR $\alpha$  works will enable control of its activities as  
36 a drug target for metabolic diseases with an underlying inflammatory component.  
37 Given its role in reshaping the immune system, the full potential of this nuclear  
38 receptor subtype as a versatile drug target with high plasticity becomes increasingly  
39 clear, and a novel generation of agonists may pave the way for novel fields of  
40 applications.

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50 **Outline**

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## 94 **I. Introduction to PPAR family members**

95 The peroxisome proliferator-activated receptors (PPARs) are ligand-inducible  
96 transcription factors that belong to the nuclear receptor (NR) superfamily. Three  
97 PPAR subtypes have been identified: PPAR $\alpha$  (NR1C1), PPAR $\beta/\delta$  (NR1C2) and  
98 PPAR $\gamma$  (NR1C3) (1-5).

99 The first member of the PPAR family, PPAR $\alpha$ , was identified in 1990 and named by  
100 its ability to become activated by chemicals known to induce peroxisome proliferation  
101 in rodents (6). Peroxisomes are subcellular organelles found in most plant and animal  
102 cells and harbor a complex set of enzymes acting on various metabolic functions  
103 including  $\beta$ -oxidation of fatty acids (FA), bile acid and cholesterol metabolism (7).

104 Peroxisome proliferators cover a various set of synthetic and structurally diverse  
105 industrial and pharmaceutical chemicals, including herbicides, industrial solvents and  
106 hypolipidemic drugs, that cause significant increases in the size and number of  
107 peroxisomes in liver, liver hypertrophy, liver hyperplasia and hepatocarcinogenesis in  
108 rodents (8). Following the initial discovery of mouse PPAR $\alpha$ , the two other PPAR  
109 subtypes, PPAR $\beta/\delta$  and PPAR $\gamma$  were identified by homology screens (2,9).  
110 Contradicting their name, the latter two subtypes do not cause peroxisome  
111 proliferation in mice (8). The PPAR subtypes are encoded by distinct genes located on  
112 different chromosomes (reviewed in (10)) and share a high degree of structural and  
113 sequence homology, but each subtype displays a divergent pattern of tissue-specific  
114 expression and ligand-binding specificity (9,11).

115 PPARs were originally described as orphan nuclear receptors, but shortly thereafter a  
116 plethora of potential endogenous ligands were described (12), acknowledging PPARs  
117 to be activated by a wide range of endogenous ligands including fatty acids and fatty  
118 acid metabolites, such as hydroxylated eicosanoids, prostaglandins and leukotrienes,

119 and by many synthetic compounds including fibrates and antidiabetic  
120 thiazolidinedione drugs (10,13).

121 Most of the physiological functions of PPARs can be explained by their activity as  
122 transcription factors that modulate the expression of specific target genes (14-17).

123 PPARs can bind to sequence-specific target elements in the promoter region of target  
124 genes following heterodimerization with the retinoid receptor to drive several aspects  
125 of normal cellular physiology as well as pathology (15,18). This includes lipid  
126 metabolism, glucose homeostasis, cell differentiation, proliferation, obesity, cancer  
127 and inflammation (19,20). In addition to positively regulating gene expression,  
128 activated PPARs can inhibit gene expression by negatively interfering with the  
129 activity of pro-inflammatory transcription factors including signal transducer and  
130 activator of transcription (STATs), activator protein-1 (AP-1), and nuclear factor- $\kappa$ B  
131 (NF- $\kappa$ B) (21-23). Such transrepression mechanisms are likely to participate in the  
132 anti-inflammatory actions of PPARs (24).

133 In this review, we will focus mainly on the molecular biology of the PPAR $\alpha$  subtype  
134 and its role in inflammation control and inflammation-related disorders. Hereby, the  
135 focus is on liver, adipose, cardiovascular and immune system as prime tissues/organ  
136 systems of interest.

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## 144 **II. PPAR $\alpha$ expression profile, gene and protein structure**

145 PPAR $\alpha$  controls the expression of numerous genes involved in a plethora of lipid  
146 metabolic pathways, including microsomal, peroxisomal and mitochondrial fatty acid  
147 oxidation (FAO), fatty acid binding and activation, fatty acid elongation and  
148 desaturation, synthesis and breakdown of triglycerides (TGs) and lipid droplets,  
149 lipoprotein metabolism, gluconeogenesis, bile acid metabolism, and various other  
150 metabolic pathways and genes (25). Consistent herewith, PPAR $\alpha$  is widely expressed  
151 in tissues with high fatty acid oxidation rates, such as heart, liver and skeletal muscle,  
152 and functions as a major regulator of fatty acid homeostasis (26-28). PPAR $\alpha$   
153 expression is also significant in brown adipose, adrenal and kidney tissue, as well as  
154 in several inflammatory/immune cell types such as monocytes/macrophages  
155 (26,27,29).

156 It is generally agreed that active PPAR $\alpha$  is predominantly localized in the nucleus  
157 (30). However, PPAR $\alpha$  was reported to be localized in the cytoplasm of rat  
158 chondrocyte primary cultures (31). Similarly, in differentiated human macrophages,  
159 PPAR $\alpha$  was also much more localized in the cytoplasm (32). Hence, as with other  
160 nuclear receptors, PPAR $\alpha$  displays a dynamic shuttling between the cytosol and the  
161 nucleus. This shuttling is influenced by Ca<sup>2+</sup> signaling and ligand binding, the latter  
162 enhancing nuclear import of PPAR $\alpha$  (33). In this way, internal and/or external cell  
163 signals can be translated into changes in subcellular localization and turnover/stability  
164 of PPAR $\alpha$  and, as a consequence, affect its regulation of cellular functions (33). The  
165 observation of a nucleo-cytoplasmic shuttling is not only restricted to PPAR $\alpha$ , but  
166 also applies to PPAR $\gamma$ . MEK-dependent export of PPAR $\gamma$  from the nucleus reduces its  
167 transcriptional activity and therefore represents a mechanism by which nuclear  
168 activities of PPAR $\gamma$  and possibly also other NR are downregulated (34). Further



169 investigations are required to fully elucidate the underlying molecular mechanism of  
170 nucleo-cytoplasmic shuttling as well as the subcellular functions of this receptor.  
171 Moreover, a better understanding of where and how PPARs are activated, turned over  
172 and regulated is of the utmost importance.

173 The human and mouse PPAR $\alpha$  genes, located on chromosome 22 and chromosome 15  
174 respectively, encode 468 amino acid (AA) polypeptides with 91% homology (35). As  
175 in the mouse, the human PPAR $\alpha$  gene encoded mRNA is derived from 8 exons with a  
176 5' untranslated region encoded by exons 1, 2, and part of exon 3. The remainder of  
177 exon 3 and exons 4-8 contribute to the coding region of PPAR $\alpha$  (35,36) (**Fig. 1A**).  
178 The last 232 bp of exon 8 contribute to the 3'-untranslated region (37). In  
179 correspondence with other nuclear receptors and PPARs, the encoded PPAR $\alpha$  protein  
180 displays four distinguishable functional domains whose cooperation modulate  
181 receptor activity (38). These domains are: an N-terminal region (A/B domain), a  
182 central DNA-binding domain (DBD, C domain), a flexible hinge region (D domain)  
183 and a C-terminal ligand-binding domain (LBD, E domain) (39,40) (**Fig. 1B**) (**Table**  
184 **1**).

185 The N-terminal A/B domain, which is the least conserved domain among PPARs,  
186 harbors a ligand-independent activation function-1 (AF-1) region. This region,  
187 enriched with acidic amino acids, has a low level of basal transcriptional activity and  
188 can function independently of ligand-binding (tested via GAL4-fusion proteins in  
189 one-hybrid assays) (35,40). Although the A/B domain suffers from a poor structural  
190 organization, secondary structure formation in this domain is suggested as an  
191 important step towards AF-1-mediated transactivation (38,40). The importance of the  
192 A/B domain is emphasized by the fact that its deletion results in a gene-dependent  
193 alteration in PPAR $\alpha$  transcriptional activity. For example, A/B domain deletion

194 interrupts PPAR $\alpha$ -mediated transactivation of the acyl-CoA oxidase (ACOX)  
195 promoter, however, transactivation of the cytochrome P450 4A6 (CYP4A6) promoter  
196 is unaffected (40,45). In addition, AF-1 has also been proposed to be a key  
197 determinant of subtype-selective target gene expression amongst the PPAR subtypes.  
198 For example, addition of the PPAR $\alpha$  A/B domain to PPAR $\gamma$  $\Delta$ AB (A/B domain  
199 truncated) enhances its ability to activate PPAR $\alpha$ -specific target genes (38). Vice  
200 versa, addition of the PPAR $\gamma$  A/B domain to the non-adipogenic PPAR $\beta/\delta$  $\Delta$ AB was  
201 sufficient to provide adipogenic potential to the resulting PPAR $\beta/\delta$  chimeric protein  
202 (44). Post-translational modifications (PTMs) further fine-tune PPAR $\alpha$  functionality.  
203 PPAR $\alpha$  AF-1 domain phosphorylation leads to changes in transcriptional activity and  
204 even ligand binding of the receptor (43). For example, MAPK-mediated  
205 phosphorylation of serine 12 and 21 in the human PPAR $\alpha$  A/B domain increases the  
206 transcriptional activity in response to mitogenic stimuli, such as high concentration of  
207 insulin (58). On the other hand, glycogen synthase kinase 3 (GSK3)-mediated  
208 phosphorylation of serine 73 increases the ubiquitination and degradation of PPAR $\alpha$   
209 (42). Concerning its relation/association with other domains in the protein, PPAR $\alpha$   
210 AF-1 synergistically activates transcription together with AF-2, as found for other  
211 nuclear receptors (40).

212 The DBD, which is highly conserved and rich in cysteine and basic amino acids,  
213 consists of two zinc-finger binding motifs (45). This domain is responsible for  
214 physical interaction with DNA. Like other PPAR subtypes, PPAR $\alpha$  binds DNA as an  
215 obligate heterodimer with retinoid X receptors (RXR) (NR2B) (49). The receptor can  
216 be activated not only by phosphorylation of the A/B domain or via ligand binding to  
217 PPARs themselves, but also via associating with RXR, which for example binds 9-cis  
218 retinoic acid. The latter activation mechanism is referred to as the permissive effect.

219 Ligand binding of either receptor thus activates the PPAR-RXR complex; however,  
220 simultaneous binding of both ligands can lead to a more potent or even synergistic  
221 activation (59-65). These different activation mechanisms allow fine-tuning of PPAR  
222 activity.

223 The PPAR $\alpha$ -RXR heterodimer recognizes and binds to PPAR response elements  
224 (PPREs), which are localized in gene regulatory regions and organized as a direct  
225 repeat type 1 (DR-1). DR-1 consists of two copies of the hexameric nucleotide  
226 recognition motif 5'-AGGTCA-3' separated by a single nucleotide (47,48). An  
227 additional consensus AACT motif is positioned 5' to the DR1 (66,67). Binding to the  
228 PPRE occurs in such a way that PPAR $\alpha$  binds to the 5' extended half-site of the  
229 response element, while RXR occupies the 3' half-site (5,66,68). The PPAR $\alpha$ -RXR  
230 heterodimers can form independently of the PPAR $\alpha$  ligand. These unliganded  
231 heterodimers recruit the corepressor protein complex and inhibit target gene  
232 transcription (69). Upon ligand binding, the corepressor complex is released from the  
233 PPAR $\alpha$ -RXR heterodimer and the coactivator complexes will be recruited to the  
234 promoter region of target genes, thereby initiating transcription (70) (**Fig. 2A**).

235 The first natural PPRE was found in the promoter region of the ACOX gene (36,71).  
236 In addition, recent genome-wide profiling of PPAR $\alpha$  binding sites revealed about  
237 46% of PPAR $\alpha$ -RXR binding sites within intronic regions (72). Using PPAR $\gamma$  as  
238 study case, chromatin accessibility in general was identified as a major determinant  
239 for transcriptional activity, as PPAR-RXR heterodimers were found to primarily bind  
240 in open chromatin regions (73-75).

241 While PPAR $\alpha$  binds DNA only as a heterodimer and not as a monomer, deletion of its  
242 N-terminal A/B domain generates a truncated protein that binds DNA non-specifically  
243 as a monomer in *in vitro* assays (45). Although the physiological significance of such

244 binding is not completely understood, it may serve as evidence of an interdomain  
245 communication. The DBD of PPAR $\gamma$  was shown to form an interface with its own  
246 LBD as well as with the LBD of its heterodimeric partner, hereby influencing ligand  
247 binding (76). More crystallization efforts are needed to challenge or validate this  
248 interface concept in the case of PPAR $\alpha$ . The DBD of PPAR $\alpha$  (and PPAR $\gamma$ ) is also  
249 subject to phosphorylation, which serves to further modulate the transcriptional  
250 activity of the receptor (46).

251 Like PPARs, many other nuclear receptor members also need RXRs as their  
252 obligatory heterodimerization partner, raising therefore the possibility of a  
253 competition between particular NRs for their common binding partner (77). This  
254 competition may have important biological outcomes. For instance, liver X receptor  $\alpha$   
255 (LXR $\alpha$ ) activation by either overexpression of LXR $\alpha$  or ligand activation suppresses  
256 PPAR $\alpha$  signaling through reduction of active PPAR $\alpha$ -RXR heterodimers in the liver  
257 and thus diminished PPRE occupancy. RXR $\alpha$  supplementation restored the LXR-  
258 mediated inhibition of PPAR $\alpha$  activity (78). Conversely, excess PPAR $\alpha$  in the  
259 presence of its ligands suppresses the sterol regulatory element-binding protein-1c  
260 (SREBP-1c) promoter that contains two LXR response elements (79), indicating that  
261 the mechanism of competition for RXR $\alpha$  between PPAR $\alpha$  and LXR may be reciprocal  
262 (78). This relationship between PPAR $\alpha$ , LXR and RXR could be crucial for a mutual  
263 regulation of both LXR and PPAR $\alpha$  activities, and thus for the nutritional regulation  
264 of their downstream target genes. However, the physiological validation of these  
265 mechanisms still remains to be demonstrated. Another example of competition for  
266 RXR $\alpha$  between RXR-partnering NRs was described for the thyroid hormone receptor  
267 (TR) with LXR (80).

268 Of note, Silent information regulator 1 (SIRT1) was also shown to bind PPAR $\alpha$ ,  
269 through a region homologous to the PPAR $\alpha$  binding domain in RXR $\alpha$ . Interestingly,  
270 the balance between SIRT1 and RXR appears to determine whether PPAR $\alpha$  is a  
271 positive or negative regulator of genes involved in fatty acid metabolism (81).  
272 Additionally, the PPAR $\alpha$ -SIRT1 complex would directly bind a single hexameric  
273 motif embedded in estrogen-related receptor (ERR) elements (ERREs), thus  
274 competitively downregulating ERR target genes, at least in the heart. These findings  
275 suggest a PPAR $\alpha$ -dependent transcriptional repression mechanism in the control of  
276 ERR-driven mitochondrial respiration and cardiac contraction (82,83). In addition,  
277 exposure to a high-fat diet (HFD) also induces the formation of a PPAR $\alpha$ -SIRT1  
278 complex, impairing an association of PPAR $\alpha$  with the pro-inflammatory transcription  
279 factor p65. Collectively, this shift is expected to lead to the repression of PPAR $\alpha$ -  
280 target genes involved in fatty acid oxidation and enhancement of pro-inflammatory  
281 pathways in the heart (84). Albeit intriguing, more experimental evidence is necessary  
282 to prove that PPAR $\alpha$  can bind DNA as an RXR-independent monomer and whether  
283 this mechanism is also operative in other tissues such as the liver.

284 Immediately adjacent to the DBD is the highly flexible hinge domain (D), connecting  
285 the DBD and the LBD (35). This region binds corepressor proteins, such as the  
286 nuclear receptor corepressor (NCoR), to the receptor in its inactive, unliganded state  
287 (51) and serves as a target for PTMs including phosphorylation on serines 179 and  
288 230 catalyzed by PKC and the SUMOylation at lysine 185 that promote NCoR  
289 recruitment (52,53). In addition, mutation of a potential phosphorylation site within  
290 the PPAR $\alpha$  hinge was shown to prevent dimerization capacities (46). Another study  
291 on truncated PPAR $\alpha$  lacking part of the hinge region and LBD suggested that the  
292 hinge region might contain a nuclear localization signal (NLS) (50). Consistently, a

293 series of truncation mutants revealed that PPAR nuclear transport is mediated by at  
294 least two nuclear localization signals, i.e. one in the DBD-hinge region and one in the  
295 AF-1 domain (33). It was suggested that the hinge region also interferes with PPAR $\alpha$   
296 binding to DNA and hence takes up an important suppressor role (85). More  
297 specifically, the ribosomal protein L11 (rpL11) was shown to interact with the D-  
298 domain of PPAR $\alpha$  and to inhibit PPRE-driven reporter activity (85). Furthermore,  
299 heat shock protein 90 (Hsp90) was reported to associate with both the hinge and LBD  
300 of PPAR $\alpha$ . Using several approaches to disrupt Hsp90 complexes within the cell,  
301 Hsp90 was confirmed as a repressor of PPAR $\alpha$  (and PPAR $\beta/\delta$ ) activity (54).

302 Next, the PPAR $\alpha$ -LBD harbors a ligand-dependent transactivation function (AF-2)  
303 and has a structure consisting of an  $\alpha$ -helical sandwich and a four-stranded  $\beta$ -sheet,  
304 forming a Y-shaped cavity of 1400 Å called the ligand-binding pocket (LBP) (55).  
305 The volume of the PPAR $\alpha$ -LBP is quite comparable to other PPAR subtypes, but is  
306 substantially larger as compared to most other NRs. Among the PPARs, The PPAR $\alpha$   
307 LBP is more lipophilic which potentially explains the greater affinity of PPAR $\alpha$  to  
308 bind the more saturated fatty acids (55). Following ligand-binding, the AF-2 domain  
309 undergoes conformational changes, which allows for the interaction with various  
310 coactivators carrying LXXLL motifs (L–leucine, X–any amino acid) (55), such as  
311 cAMP-response element-binding protein (CBP)/p300 and steroid receptor  
312 coactivator-1 (SRC-1/NCoA-1) (55). In addition, the LBD is crucial for dimerization  
313 with RXR; this was confirmed in studies involving deletion of PPAR $\alpha$  helix 10-12 as  
314 well as a L433R mutation in PPAR $\alpha$ , which caused impaired heterodimerization with  
315 RXR (51,57). PPAR $\alpha$  furthermore possesses two nuclear export signals (NES) in the  
316 DBD and LBD regions, which are recognized by the export receptors calreticulin and  
317 chromosomal maintenance 1 (CRM1), respectively. A recent study confirmed the

318 presence of a functional NES in the LBD, i.e. at AA 300-308 in human PPAR $\alpha$   
319 (86,87).

320 As mentioned earlier, human and murine PPAR $\alpha$  show 85% identity at the nucleotide  
321 level and 91% identity at the amino acid level, however evidence exists for a genetic  
322 heterogeneity in the functional coding sequence of human PPAR $\alpha$  that translates into  
323 functional differences in receptor activity. For example PPAR $\alpha$  L162V, a variant of  
324 the human PPAR $\alpha$  gene resulting in the introduction of an amino acid substitution  
325 within the PPAR $\alpha$  DBD, is found at an allelic frequency of ~0.025–0.073 in  
326 ethnically diverse populations (88-90), while being more frequent (0.745) in North  
327 Indians (91). Functional studies on the L162V gene variant demonstrate enhanced  
328 ligand-induced activity when compared to the wild-type (88,91). Importantly, in  
329 various studies the L162V polymorphism is correlated with dyslipidaemia, coronary  
330 ischemic events (37,88-90,92-94) and adiposity (95,96). However, other studies have  
331 not replicated these correlations (97,98). These inconsistencies could be due to  
332 differing environments, genetic background and disease status between the studied  
333 populations. In this context, the effect of the L162V polymorphism could be  
334 modulated via gene-drug and gene-nutrient interactions (94,99,100). For example, the  
335 association of the L162V genotype with a particular lipoprotein profile may be  
336 modified by dietary fats (94,101).

337 V227A, a natural variant in which the PPAR $\alpha$  hinge region is affected, was identified  
338 in a Japanese population with a frequency of 5.0% (102-104) and in a Chinese  
339 population with a frequency of approx. 4% (105). This variant attenuates the PPAR $\alpha$ -  
340 mediated transcription of CYP4A6 and the mitochondrial 3-hydroxy-3-  
341 methylglutaryl-CoA (mHMG-CoA) synthase genes in the presence of fibrates. This  
342 transcriptional repression was ascribed, at least partially, to increased corepressor

343 binding to PPAR $\alpha$  and defective release of NCoR from chromatin (106). Interestingly,  
344 the V227A variant was associated with perturbations in plasma lipid levels and  
345 modulated the association between dietary polyunsaturated fatty acid intake and high-  
346 density lipoprotein (HDL) cholesterol concentrations (105). Of note, the PPAR $\alpha$   
347 V227A polymorphism may be involved in the pathogenesis of non-alcoholic fatty  
348 liver disease (NAFLD) and may play a protective role in obesity (107). Although  
349 epidemiological and interventional studies demonstrate a link between PPAR $\alpha$   
350 polymorphisms and metabolic parameters, more studies are required to understand  
351 whether and how the response to fibrate drugs and other PPAR $\alpha$ -regulated pathways  
352 are affected by the polymorphisms.

353 In addition to polymorphic variants, a truncated splice variant of PPAR $\alpha$ , generated  
354 by alternative splicing resulting in the skipping of exon 6, has been detected in human  
355 PPAR $\alpha$  (**Fig. 1A**) (50,108). This variant lacks the hinge region and the entire LBD  
356 and is unable to bind to PPREs. As a consequence, it exerts a dominant negative  
357 transcriptional repressive activity on full-length PPAR $\alpha$  through titration of the  
358 coactivator CBP by competing for coactivators. It was speculated that differences in  
359 the expression level of truncated hPPAR $\alpha$  might be implicated in the heterogeneity in  
360 response to fibrates among different patients (50). Of interest, genome-wide  
361 correlation analysis with subsequent pathway enrichment analysis indicated a  
362 selective role for truncated PPAR $\alpha$  as an anti-proliferative and anti-inflammatory  
363 factor (109). Further experimental manipulation of human and mouse hepatocytes by  
364 specific knock-down and overexpression analysis substantiated this hypothesis (109).  
365 *In vivo* evidence for a dominant negative transcriptional repressive activity is lacking  
366 so far. Therefore, whether this variant has any specific physiological significance with



367 regard to metabolic processes, inflammation as well as its relevance for  
368 hepatocarcinogenesis still needs to be revealed and requires further investigation.

369

### 370 **III. Modulating factors of PPAR $\alpha$ activity**

#### 371 **PPAR $\alpha$ gene and protein expression**

372 The expression of the PPAR $\alpha$  gene is regulated by various physiological conditions  
373 such as stress, glucocorticoids (GCs), growth hormones, insulin and leptin. As an  
374 important physiological response, PPAR $\alpha$  expression is induced during fasting  
375 resulting in an enhanced expression of PPAR $\alpha$  target genes in liver to operate fatty  
376 acid oxidation and to form substrates that can be metabolized by other tissues. Vice  
377 versa, PPAR $\alpha$  also controls, directly or indirectly, lipogenic pathways (which will be  
378 further discussed in detail in section V). Liver lipogenesis particularly allows FA  
379 synthesis in a fed state when dietary carbohydrates are abundant (110,111).  
380 Adipocyte-derived factors may further influence the expression level and subsequent  
381 PPAR $\alpha$  activity. Consistent with this, administering leptin, considered pro-  
382 inflammatory, correlated with an increased PPAR $\alpha$  hepatic expression in ob/ob mice  
383 (112). The mechanisms underlying the induction of PPAR $\alpha$  transcription by leptin  
384 may be connected to changes in fatty acid flux mediated by leptin signaling (112).  
385 Leptin-stimulated PPAR $\alpha$  gene expression was also observed in muscle cells, through  
386 the activation of and changes in the subcellular localization of AMPK- $\alpha$ 2 (113).  
387 Additionally, leptin was found to enhance PPAR $\alpha$  gene transcription in pancreatic  $\beta$ -  
388 cells (114) but not in adipose tissue (115). The basis for this discrepancy is not  
389 known, but findings generated using isolated model systems will definitely require  
390 further confirmation *in vivo*.

391 On the other hand, the anti-inflammatory adipokine adiponectin also increased  
392 PPAR $\alpha$ -responsive promoter activity in H4IIEC3 cells, an effect that required co-  
393 expression of PPAR $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ), suggesting that adiponectin-induced  
394 PPAR $\alpha$  activity may be mediated through up-regulation of PGC-1 $\alpha$  expression (116).

395 In contrast, growth hormone (GH) has been shown to rather decrease PPAR $\alpha$  mRNA  
396 in cultured rat hepatocytes (117,118). A similar effect was shown in  
397 hypophysectomized rats (119). In addition, GH also decreases PPAR $\alpha$  transcriptional  
398 activity (120) as well as the expression level of PPAR $\alpha$  (121). Similarly, treatment of  
399 primary hepatocytes for 3 days with another anabolic signal, insulin, also led to  
400 reduced PPAR $\alpha$  levels (122), whereas short-term exposure to this hormone increased  
401 PPAR $\alpha$  transactivation via phosphorylation of the ligand-independent AF-1 domain  
402 (123).

403 Next to tissue-specific differences in PPAR $\alpha$  levels, the expression of PPAR $\alpha$  in a  
404 given tissue further oscillates under the control of the circadian rhythm. For example,  
405 PPAR $\alpha$  expression peaks during daytime in mice, which correlates with the timing of  
406 fasting in these nocturnal rodents (124). These diurnal patterns thus correspond well  
407 with known functions of PPAR $\alpha$  in physiology but also pathophysiology (125).  
408 PPAR $\alpha$  is under the transcriptional control of the GC-activated glucocorticoid  
409 receptor (GR) in rat hepatocytes (126). As such, PPAR $\alpha$  expression follows a diurnal  
410 rhythm, which parallels that of circulating corticosterone and the levels of PPAR $\alpha$  are  
411 also up-regulated during times of stress (127). Moreover, the circadian expression of  
412 PPAR $\alpha$  was reported to be directly regulated by the core-clock transcription factors  
413 brain and muscle Arnt-like protein (BMAL) and circadian locomotor output cycles  
414 kaput (CLOCK) both *in vivo* and *in vitro* (128). In another study, the proline- and  
415 acidic amino acid-rich basic leucine zipper (PARbZip) proteins were also shown to  
416 modulate the rhythmic expression and activity of PPAR $\alpha$ . The mechanism was  
417 proposed to be indirect and involved a cyclic generation of PPAR $\alpha$  ligands which  
418 initiate a feed-forward loop, in which PPAR $\alpha$  enhances transcription from its own  
419 gene (129). In addition, enhanced PPAR $\alpha$  transcript levels were observed in both B

420 and T cells upon GCs treatment (130), indicative of a function of PPAR $\alpha$  in the  
421 immune system (see further below under section 6).

422 Additionally, PPAR $\alpha$  expression is also reported to be related to aging. In this line of  
423 reasoning, examination of mRNA expression in mouse spleens revealed an age-  
424 associated decline in the levels of mRNA encoding PPAR $\alpha$  and its concomitant target  
425 genes ACOX and catalase, which was accompanied by a constitutive pro-  
426 inflammatory cytokine production (131). Similarly, a recent study has shown that  
427 aging inhibits PPAR $\alpha$  activation as assessed by diminished mRNA levels of its target  
428 genes ACOX and carnitine palmitoyl transferase 1 (CPT1) in aged rat livers (132).

429 Metabolite levels also affect PPAR $\alpha$  expression; intriguingly, glucose and metabolites  
430 (glucose-1-phosphate and glucose-6-phosphate) were shown to bind PPAR $\alpha$  with high  
431 affinity resulting in a significantly altered PPAR $\alpha$  secondary structure (133). In this  
432 report, in vitro addition of glucose alone reduced the interaction of PPAR $\alpha$  with its  
433 coactivator SRC-1. Conversely, in the presence of the activating ligands arachidonic  
434 acid and clofibric acid, glucose rather increased PPAR $\alpha$  interaction with SRC-1, DNA  
435 binding and activation of the  $\beta$ -oxidation pathway (133). In addition, high glucose  
436 levels regulate PPAR $\alpha$  expression in human macrophages. Incubation of murine J774  
437 macrophages with high glucose concentrations increased the expression of PPAR $\alpha$  at  
438 the mRNA and protein levels and enhanced its binding to the PPRE of the lipoprotein  
439 lipase (LPL) promoter (134). In contrast, glucose decreases PPAR $\alpha$  expression in  
440 pancreatic  $\beta$ -cells leading to a reduced FAO and increased phospholipids and TG  
441 synthesis, a mechanism believed to underlie  $\beta$ -cell glucolipototoxicity (135).

442 NF- $\kappa$ B-driven cytokines such as TNF $\alpha$ , IL-1 $\beta$  and IL-6 also affect the expression of  
443 PPAR $\alpha$ . In TNF $\alpha$ -treated male Sprague Dawley-rats, PPAR $\alpha$  mRNA and  
444 corresponding PPAR $\alpha$  protein levels were significantly reduced, likely contributing to

445 the decrease of peroxisomal  $\beta$ -oxidation (136). IL-1 $\beta$  and IL-6 both repress the  
446 induction of PPAR $\alpha$  expression exerted by the combined action of clofibric acid and  
447 the GC dexamethasone in fetal rat hepatocytes (137).

448 The presence or absence of the activity of other transcription factors also impacts  
449 PPAR $\alpha$  levels and activity. In hepatocytes, Krüppel-like factor 6 (KLF6) deficiency  
450 reduces PPAR $\alpha$  protein expression and downstream targets resulting in attenuation of  
451 lipid and glucose abnormalities associated with a HFD. KLF6 mediated induction of  
452 PPAR $\alpha$  protein expression through miRNA 10b repression (138). PPAR $\alpha$  is also  
453 regulated at the transcriptional level by other nuclear receptors such as hepatocyte  
454 nuclear factor 4 (HNF4), chicken ovalbumin upstream promoter-transcription factor II  
455 (COUP-TFII), and others. While HNF4 positively regulates hPPAR $\alpha$  expression via a  
456 DR1 element present in the hPPAR $\alpha$  promoter, COUP-TFII also bound this site but  
457 antagonized this effect (139). The regulation of PPAR $\alpha$  by HNF4, consistent with the  
458 tissue-expression pattern of PPAR $\alpha$ , was also demonstrated *in vivo* using HNF4-  
459 deficient mice that display significantly lower PPAR $\alpha$  mRNA levels compared with  
460 the wild-type mice (140). Exemplary of another metabolite, bile acids stimulate  
461 hPPAR $\alpha$  expression in a species-specific manner through a mechanism involving the  
462 binding of Farnesoid X receptor (FXR) on its promoter (141). Moreover, PPAR $\alpha$   
463 expression in the mouse small intestine was shown to be under the control of LXR  
464 and the pregnane X receptor (PXR) (142).

465 Cross-talk with other nuclear receptor signaling pathways may also modulate PPAR  
466 activity, as illustrated by the direct control of the PPAR $\beta$  promoter by the vitamin D  
467 receptor (143). In this respect, and as mentioned earlier, nuclear receptors that  
468 function as heterodimers with RXR can potentially compete with PPAR $\alpha$  signaling in  
469 tissues in which the amount of RXR is limiting (77).

470 Furthermore, PPAR $\alpha$  turnover is controlled by ligand specificity and availability (**Fig.**  
471 **3**). In this context, Blanquart *et al.* demonstrated that synthetic PPAR $\alpha$  ligands such as  
472 Wy14,643, GW7647 or fibrates prolonged half-life and increased stability of the  
473 receptor, preventing thereby its ubiquitination and its subsequent degradation via the  
474 proteasome (41). Besides, PPAR $\alpha$  agonists, such as fenofibrate, markedly up-  
475 regulated hPPAR $\alpha$  mRNA levels in primary cultures of human hepatocytes as well as  
476 hPPAR $\alpha$  promoter activity suggesting that PPAR $\alpha$  positively autoregulates its own  
477 expression (139).

478 Of note, hPPAR $\alpha$  mRNA levels in the liver vary among individuals (50,109),  
479 suggesting that PPAR $\alpha$  is strongly regulated at the gene level in human liver by  
480 genetic and/or environmental factors (50).

481 Last but not least, the function of PPAR $\alpha$  as a transcription factor is controlled by its  
482 PMTs and cofactor recruitment (**Fig. 3**), which will be further discussed in detail in  
483 the next sections.

484 Together, regulation of the PPAR $\alpha$  expression levels efficiently modulates PPAR $\alpha$   
485 activator responses and thus may affect downstream pathways involved in metabolism  
486 and inflammatory processes.

487

#### 488 **PPAR $\alpha$ cofactors**

489 As with other nuclear receptors, PPAR $\alpha$  transactivation activity is regulated by the  
490 presence of coregulators, which may function as corepressors or coactivators  
491 depending on the context. In the absence of a specific ligand, PPAR $\alpha$  binds co-  
492 repressors, such as NCoR and silencing mediator for retinoid and thyroid hormone  
493 receptors (SMRT) (26). The receptor interacting protein 140 (RIP140) is a PPAR $\alpha$   
494 corepressor, which is known to repress the activity of NRs by competing with

495 coactivators and by recruiting downstream effectors such as histone deacetylases  
496 (HDACs) (144). Interestingly, mice devoid of the corepressor protein RIP140 show  
497 resistance to HFD-induced obesity and hepatic steatosis in association with  
498 upregulation of genes involved in energy dissipation. Such phenotype may be  
499 suggestive of a role for this corepressor in PPAR $\alpha$  signaling (145). Whether or not  
500 PPAR $\alpha$  is directly involved remains to be shown. On the other hand, known  
501 coactivators that have been found in a large complex with PPAR $\alpha$ /RXR heterodimers  
502 include PGC-1 $\alpha$ , PGC-1 $\beta$ , CBP/p300, SRC-1, PPAR-interacting protein (PRIP),  
503 PPAR-binding protein (PBP/TRAP220/DRIP205/Med1) as well as coactivator-  
504 associated proteins PIMT (NCoA6IP) and coactivator-associated arginine  
505 methyltransferase-1 (CARM-1). Some of the coactivators, including the SRC-1  
506 family and CBP/p300, contain intrinsic histone acetyltransferase (HAT) activities  
507 required to facilitate chromatin remodeling (Reviewed in (146)). The PPAR $\alpha$ -  
508 interacting cofactor (PRIC) complex isolated from rat liver nuclear extracts reveals  
509 the presence of many of these coregulators, likely forming one mega complex (147).  
510 Most cofactors are not receptor-exclusive, yet, emerging gene knockout mouse  
511 models show that some of the coactivators are essential for PPAR $\alpha$  signaling. For  
512 example, conditional deletion of PBP/MED1 gene in liver results in the abolition of  
513 PPAR $\alpha$  ligand-induced pleiotropic effects, indicating that PBP/MED1 is essential for  
514 the function of PPAR $\alpha$  (148).

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518 **PPAR $\alpha$  post-translational modifications**

519 As for other nuclear receptors, PPAR $\alpha$  activity is finely tuned by various PTMs  
520 including phosphorylation, ubiquitination and SUMOylation (41,53,149).

521 PPAR $\alpha$  is a phosphoprotein of which the transcriptional activity is affected by cross-  
522 talk with several kinases, including MAPK (ERK1/2), c-Jun N-terminal kinases  
523 (JNK)/p38), AMPK, protein kinase A and C (PKA, PKC) and GSK3 (**Table 2**). The  
524 effects of phosphorylation on PPAR $\alpha$  activity depend on a number of features such as  
525 the nature of the modified residue, as well as the stimulus and the kinase involved.

526 PPAR $\alpha$  phosphorylations may also have different functional outcomes depending on  
527 the context, with effects on receptor stability, susceptibility to proteosomal  
528 degradation, enhanced recruitment of cofactors and transactivation. PPAR $\alpha$  was first  
529 shown to be phosphorylated in primary rat adipocytes in culture in response to insulin,  
530 resulting in the stimulation of basal as well as ligand-dependent transcriptional  
531 activity (149). Along the same line of interest, insulin treatment of human HepG2  
532 hepatoma cells increases PPAR $\alpha$  phosphorylation enhancing thereby its  
533 transcriptional activity. This insulin-mediated phosphorylation of PPAR $\alpha$  via the  
534 ERK1/2 MAPK pathway involves amino acids Ser12 and Ser21 in the AF-1 domain  
535 resulting in transcriptional activation probably by relieving inhibition by corepressors  
536 (42,58). In line with this, in rat neonatal cardiac myocytes, stress-activated p38  
537 MAPK phosphorylates Ser 6, 12 and 21 residues located within the N-terminal A/B  
538 domain of the PPAR $\alpha$  protein leading to an increase in ligand-dependent  
539 transactivation of targets and enhanced cooperativity with the transcriptional  
540 coactivator PGC-1. It was speculated that PPAR $\alpha$  phosphorylation and the subsequent  
541 specific recruitment of PGC-1 favor cardiac mitochondrial fatty acid  $\beta$ -oxidation  
542 during periods of stress (150). In contrast, during cardiac hypertrophic growth, the  
543 ERK-mediated phosphorylation of PPAR $\alpha$  was shown to have an opposite effect to



544 that of p38 kinase resulting in a reduced capacity for myocardial lipid and energy  
545 homeostasis (151) demonstrating therefore the importance of cellular context. In FAO  
546 hepatic-derived cells PPAR $\alpha$  phosphorylation level is increased by ciprofibrate,  
547 subsequently enhancing its transcriptional activity as demonstrated by a rise in ACOX  
548 gene expression (152).

549 Given that substrates for MAPK phosphatase 1 (MKP-1, also known as DUSP1)  
550 include many members of the MAPK family including ERK1/2 and p38 MAPK,  
551 overexpression of MKP-1 in turn affected the constitutive PPAR $\alpha$  activity, thus acting  
552 in a feedback control mechanism (153). Taken together, these studies hint at pivotal  
553 activating roles of MAPK on PPAR $\alpha$  activity, thereby placing this receptor under the  
554 control of growth factors and various cellular stressors, as already mentioned above.

555 *In vivo* phosphorylation studies show that the level of phosphorylated PPAR $\alpha$  is  
556 increased by treatment with its agonist Wy-14,643 as well as the PKC activator  
557 phorbol myristoyl acetate (PMA) (46). Particular modifications can increase a subset  
558 of PPAR-mediated functions, e.g. phosphorylation at serine 179 and 230 in the  
559 PPAR $\alpha$  hinge region by PKC leads to enhanced transactivation potential, but impairs  
560 its transrepression activity (52). Consistently, PKC inhibition impairs ligand-activated  
561 PPAR $\alpha$  transcriptional activity and decreases PPAR $\alpha$  ligand-induction of its target  
562 genes including PPAR $\alpha$  itself and CPT1, but enhances PPAR $\alpha$  transrepression  
563 properties. Thus, the PKC signaling pathway acts as a molecular switch dissociating  
564 the transactivation and transrepression functions of PPAR $\alpha$  (52). In support, it was  
565 proposed that the capacity of simvastatin to mitigate PKC action indirectly stimulates  
566 PPAR $\alpha$  transrepression activities towards NF- $\kappa$ B and as such explains statin-mediated  
567 acute anti-inflammatory effects (154). As indicated earlier, PKC phosphorylation is  
568 believed to be important for PPAR $\alpha$  and RXR interaction as mutation of the PKC

569 phosphorylation sites T129 and S179 in the hinge region of PPAR $\alpha$  reduces  
570 heterodimerization (46).

571 The fasting-responsive kinase PKA enhances PPAR $\alpha$  activity converging in the  
572 stimulation of PPAR $\alpha$  target genes. It was speculated that under stress, fasting or  
573 exercise, PPAR $\alpha$  phosphorylation in response to PKA leads to an enhancement of  $\beta$ -  
574 oxidation, production of glucose and ketone bodies, which serve as fuel for muscles  
575 and brain (155). Thus, physiological cAMP modulators such as fasting, stress or  
576 exercise potentially modulate PPAR $\alpha$  activity. Of note, PKA-mediated PPAR $\alpha$   
577 phosphorylation occurs mainly in the DBD, while the enhancement of activity  
578 generally involves the AF-2 domain (155), suggesting an effect of phosphorylation on  
579 interdomain communication. As another starvation-linked kinase, AMPK activity may  
580 influence the expression of PPAR $\alpha$  and PPAR $\alpha$  target genes in certain tissues. In the  
581 murine muscle cell line C2C12, for example, leptin stimulates PPAR $\alpha$  gene  
582 expression and subsequent FAO through the activation of  $\alpha$ 2-subunit containing  
583 AMPK (113). Moreover, AMPK activation increases mRNA expression of PPAR $\alpha$   
584 and its target genes such as ACOX and CPT1 as well as PGC-1; this effect was  
585 correlated with an increased FAO in skeletal muscle (156). In addition, AMPK and  
586 p38 MAPK were involved in the activation of PPAR $\alpha$  by adiponectin in muscle cells  
587 (157). However, whether and which residues within PPAR $\alpha$  may be phosphorylated,  
588 remains an unresolved issue. Next to adiponectin, AMPK was also described as a  
589 modulator of PPAR $\alpha$  expression in response to glucose levels (158,159) suggesting  
590 that cellular energy levels might affect the PPAR $\alpha$ -regulated signaling network in a  
591 ligand-independent manner. Recently, a novel mechanism was uncovered by which  
592 AMPK controls PPAR $\alpha$ -driven gene expression in murine liver subjected to a  
593 prolonged fasting. Activated AMPK was recruited to promoters of glucose and lipid

594 metabolism genes that are co-controlled by PPAR $\alpha$  and GR $\alpha$  (160). For the latter  
595 example in liver, it also remains to be discovered which residue(s) of involved  
596 transcription factors, PPAR $\alpha$ , GR or both, may be phosphorylated by AMPK.  
597 PPAR $\alpha$  phosphorylation, besides affecting target gene regulation, also affects the  
598 stability and turnover of PPAR $\alpha$  through ubiquitination (41,161). Ubiquitination  
599 involves the conjugation of a small protein, ubiquitin, to the target protein via a  
600 sequential action of particular enzymes which primarily leads to degradation of the  
601 receptor by the 26S proteasome. PPAR $\alpha$  is targeted by the E3 enzyme murine double  
602 minute 2 (MDM2), which leads, depending on the cellular PPAR $\alpha$  levels, to  
603 stabilization of receptor levels or degradation via the ubiquitin-proteasome pathway  
604 (162). The effect of degradation on the transcriptional activity is still under debate;  
605 Blanquart *et al.* showed an inverse correlation between PPAR $\alpha$  degradation and  
606 activity, which is in contrast to results obtained by the group of Vanden Heuvel  
607 (41,42). Recently, a muscle ring finger-1 (MuRF1)-mediated monoubiquitination of  
608 cardiomyocyte PPAR $\alpha$  was discovered that enhances nuclear export of the receptor  
609 and consequently inhibits PPAR $\alpha$ -induced FAO in cardiac tissue (87).  
610 GSK3 is a key enzyme of glycogen metabolism and able to inactivate glycogen  
611 synthase. Its activation phosphorylates PPAR $\alpha$  predominantly at serine 73 in the A/B  
612 domain. This modification negatively affects PPAR $\alpha$  stability and promotes ubiquitin  
613 proteasome-mediated degradation (42). PPAR $\alpha$  ubiquitination may thus constitute a  
614 regulatory pathway that may contribute to the duration and the magnitude of the  
615 response to PPAR $\alpha$  activators (41,161). Function-wise, when blocking GSK3 by  
616 biliverdin reductase A (BVRA) in the liver, a protective effect against steatosis is  
617 inferred, due to a preservation of hepatic PPAR $\alpha$  activity (163).

618 Next to phosphorylation and ubiquitination, PPARs can be modified by  
619 SUMOylation. SUMOylation is a reversible modification that entails the covalent  
620 attachment of small ubiquitin-related modifier (SUMO) proteins via specific enzymes  
621 (164). Murine PPAR $\alpha$  was shown to be SUMOylated on lysine 358 (56). This  
622 particular SUMOylation triggers the interaction with GA-binding protein  $\alpha$  bound to  
623 the 7 $\alpha$ -hydroxylase cytochrome P450 7b1(*cyp7b1*) promoter resulting in specific  
624 down-regulation of this gene. Hence, SUMO modification can be regarded as a  
625 positive regulator of mPPAR $\alpha$  transrepressive activity (56). Likewise, human PPAR $\alpha$   
626 undergoes SUMOylation by SUMO-1 on lysine 185 in the hinge region, leading to  
627 down-regulation of its transactivation capacity through the selective recruitment of  
628 NCoR (53) (**Table 3**). Both SUMOylation sites are targeted by the E3 enzyme protein  
629 inhibitor of activated STAT (PIAS) $\gamma$  (78,164).

630 Finally, another means of regulating PPAR $\alpha$  activity indirectly includes the  
631 phosphorylation of RXR and of various associated cofactors such as PGC-1 $\alpha$ , SMRT  
632 and NCoR (reviewed in (26)).

633 PTMs play a key role in modulating PPAR $\alpha$  activity in (patho)physiological contexts.  
634 The modifying enzymes themselves are modulated by the cellular metabolic status  
635 and environment, hereby positioning PPAR $\alpha$  into a very complex regulatory network.  
636 The level of complexity is even intensified knowing that PPAR $\alpha$  forms part of a  
637 physical assembly for variable corepressor or coactivator complexes, which are also  
638 on their turn subjected to PTMs (26). Full understanding of these complex patterns of  
639 regulation requires more high-throughput technologies to characterize the PPAR $\alpha$   
640 interactome, its dynamic interactions with modifying enzymes as well as the  
641 mechanisms by which these interactions modulate PPAR $\alpha$  activity. These might point

642 to drugs that can modulate the PPAR $\alpha$  PTMs status with regard to metabolic disorders  
643 and inflammation.

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## 666 **IV. PPAR $\alpha$ natural and synthetic ligands**

### 667 **PPAR $\alpha$ natural ligands**

668 Adoption of orphan NRs through the identification of their endogenous ligands is key  
669 towards understanding their biology (13). The promiscuity of PPAR $\alpha$  for its ligands  
670 can be attributed to the large size of the LBP. PPAR $\alpha$  is activated by a wide range of  
671 endogenous or naturally occurring biological molecules encompassing a variety of  
672 FAs and FA derivatives, including acyl-CoAs, oxidized FAs, eicosanoids,  
673 endocannabinoids, phytanic acids (165-167) and long chain dietary FAs (166,168)  
674 (listed in Table 4). As such, FAs and their metabolites that interact with PPAR $\alpha$  can  
675 be derived from the diet or obtained via *de novo* synthesis. Many of those FAs exhibit  
676 an affinity for PPAR $\alpha$  within a nanomolar range (166). This supports the fact that  
677 PPAR $\alpha$  serves as an intracellular lipid sensor. It was proposed that PPAR $\alpha$  senses  
678 certain endogenous lipid metabolism intermediates as ligands to regulate the  
679 expression of target genes involved in their metabolism (169). *In vivo* evidence for  
680 this came from studies using ACOX1 knockout mice. The disruption of this first and  
681 rate-limiting enzyme of the peroxisomal FAO pathway caused accumulation of long  
682 chain fatty acyl-CoA coinciding with an increased peroxisome proliferation,  
683 hepatocarcinoma and profound activation of PPAR $\alpha$  in rodents (169,170). These  
684 findings suggested that substrates of the ACOX1 enzyme may possibly be PPAR $\alpha$   
685 agonists. Numerous arachidonic acid metabolites serve as ligands for PPAR $\alpha$ ; in this  
686 context, 8(*S*)-HETE, an intracellular hydroxy FA derived from the oxygenation of  
687 arachidonic acid, mainly by lipoxygenases and monooxygenases, was shown to be a  
688 strong activator of PPAR $\alpha$  (171). In addition, 20-hydroxyeicosatetraenoic acid (20-  
689 HETE), a main product of cytochrome P450 (CYP4A and CYP4F) activity that  
690 catalyzes the conversion of arachidonic acid, can bind to and activate PPAR $\alpha$  in

691 HepG2 cells leading to enhanced expression of the PPAR $\alpha$  target gene, apolipoprotein  
692 A-I (APOA1) (172). Leukotriene B4 (LTB<sub>4</sub>) is another putative PPAR $\alpha$  ligand, which  
693 connects the receptor to inflammation and immune responses. Its ability to act as a  
694 PPAR $\alpha$  agonist has been reported in several studies (165,173,174), and the binding  
695 affinity is found to be within the nanomolar range (174). Stimulation of 5-  
696 lipoyxygenase activity leads to activation of PPAR $\alpha$  via production of intracellular  
697 LTB<sub>4</sub> (165,175). In endothelial cells, oxidized phospholipids were found to activate  
698 PPAR $\alpha$  in a phospholipase A<sub>2</sub>-dependent manner suggesting that phospholipids may  
699 be precursor of endogenously generated PPAR $\alpha$  ligands (176).

700 Mice with liver-specific inactivation of fatty acid synthase (FAS), a key lipogenic  
701 enzyme, developed hypoglycemia, fatty liver and defects in expression of PPAR $\alpha$   
702 target genes when subject to a fat-depleted diet. These effects were reversed by a  
703 synthetic PPAR $\alpha$  agonist, identifying thereby the *de novo* synthesized end products of  
704 FAS as endogenous activators of PPAR $\alpha$  in adult liver (177). This phenomenon is not  
705 limited to the liver. Indeed, selective inactivation of FAS in the hypothalamus is  
706 found to affect PPAR $\alpha$ -dependent gene expression and feeding behavior, which are  
707 corrected after hypothalamic infusion of a PPAR $\alpha$  activator (178). These observations  
708 suggest that FAS, which is known to be regulated by nutrition, is involved in the  
709 generation of endogenous PPAR $\alpha$  ligands. The same research group demonstrated  
710 that FAS generates 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (16:0/18:1-  
711 GPC), which seems to be a putative PPAR $\alpha$  ligand (179).

712 Other natural compounds such as polyphenols have been identified as ligands of  
713 PPAR $\alpha$  (180,181). PPAR $\alpha$  activation with resveratrol, a natural polyphenol, and some  
714 of its derivatives and analogs, results in a brain protection against stroke (182,183).  
715 Resveratrol was identified as an AMPK activator, linking this sensor of the nutritional

716 status again to PPAR $\alpha$  functionality (184) (see chapter 3). Since PPAR $\alpha$  acts as an  
717 intracellular sensor of FAs levels, of which an alteration is associated with the  
718 development of several diseases such as diabetes, obesity, atherosclerosis and  
719 inflammation, finding a way of regulating the receptor activity through modulating  
720 (levels of) endogenous ligands may have the potential to generate new therapies.

721

### 722 **PPAR $\alpha$ synthetic ligands**

723 Synthetic PPAR $\alpha$  ligands include fibrates such as clofibrate, fenofibrate, and  
724 bezafibrate (listed in **Table 4**). Fibrates are used in the pharmacological treatment of  
725 hypertriglyceridaemia (48,185) despite the low potencies of these first generation  
726 agonists on PPAR $\alpha$ . Fibrates act by decreasing TG-rich lipoproteins in serum through  
727 an increase in genes involved in FAO and a decrease in apolipoprotein C-III (APOC3)  
728 gene expression (186,187). See further chapter 6 for a current status of PPAR $\alpha$ -  
729 targeted therapies in a clinical setting.

730 Other synthetic compounds that are not approved for clinical use, but are often used  
731 for research purposes to activate PPAR $\alpha$  include GW7647, GW9578 and Wy-14,643  
732 (also known as pirinixic acid) (29). In addition, other chemicals with environmental  
733 impact, particularly plasticizers (example DEHP-used in the manufacturing of  
734 plastics) and herbicides have been shown to function as PPAR $\alpha$  ligands (28). As their  
735 potential impact in human health is unclear, further research is necessary to address  
736 this with respect to lifetime health.

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### 738 **Selective PPAR $\alpha$ modulators (SPPARMs)**

739 The development of a selective PPAR $\alpha$  agonist may provide a good approach to  
740 maximize the therapeutic potential of these drug targets. Accordingly, a new



741 generation of highly potent and selective PPAR $\alpha$ -modulators (SPPARM $\alpha$ ) is being  
742 developed to enable greater pharmacological effects and to dissociate the benefits of  
743 the PPAR $\alpha$  agonists from their side effects (188). A number of SPPARM $\alpha$  have to  
744 date been identified by *in vitro* and preclinical studies and some have entered early  
745 clinical trial studies with promising results (see further **section 6**). Among these  
746 modulators, elafibranor (GFT505), a dual PPAR $\alpha/\delta$  agonist, shows protective effects  
747 on liver steatosis, inflammation and fibrosis (189). Pemafibrate (K-877), a recently  
748 developed highly specific PPAR $\alpha$  agonist with SPPARM properties (190) shows  
749 more potent PPAR $\alpha$ -activating efficacy *in vitro*, better effects on TGs and high-  
750 density lipoprotein-cholesterol (HDL-C) levels in patients with atherogenic  
751 dyslipidaemia and a reduced risk of side effect as compared to fenofibrate. Recently,  
752 pemafibrate was shown to exert beneficial effects on lipid metabolism, reverse  
753 cholesterol transport (RCT) and inflammation, resulting in anti-atherogenic  
754 properties. Therefore, pemafibrate thus seems an ideal next-generation PPAR $\alpha$ -  
755 triggering candidate to target atherogenic dyslipidaemia and cardiovascular disease  
756 risk (190).

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## 764 **V. PPAR $\alpha$ target genes and their function**

765 Numerous studies with PPAR $\alpha$ -deficient mice during the past years have  
766 demonstrated the critical roles played by this receptor in energy metabolism, hepatic  
767 functions, inflammation, cardiac pathophysiology, cell cycle alterations and rodent  
768 hepatocarcinogenesis (27). We will next discuss functionalities of PPAR $\alpha$  target  
769 genes and proteins per tissue type. **Table 5** summarizes the many genes that have  
770 been identified as PPAR $\alpha$  target genes in different organs since the end of the 1990s.  
771 We have indicated whether the regulation was found to be direct or indirect.

772

### 773 **PPAR $\alpha$ target genes in liver**

774 The PPAR $\alpha$  subtype exerts its main functions in the liver, where the receptor is  
775 involved in various aspects of lipid metabolism. These functions range from FA  
776 transport, binding, uptake, synthesis, degradation through mitochondrial or  
777 peroxisomal oxidation, storage, lipoprotein metabolism and ketogenesis under fasting  
778 conditions (111). Hence, although the phenotype of PPAR $\alpha$ -deficient mice is normal,  
779 fasting results in increased plasma free FA (207) levels, accumulation of lipids in the  
780 liver, hypoketonaemia, hypoglycaemia and hypothermia (110,208-210). The wide  
781 range of genes that are expressed after PPAR $\alpha$  activation indicates that this receptor  
782 has a central regulatory role in lipid metabolism (211). Recently, in a hepatocyte-  
783 specific PPAR $\alpha$  knockout mouse model impairment in liver and whole-body FA  
784 homeostasis was observed, resulting in hepatic lipid accumulation and  
785 hypercholesterolemia during aging (212). Still today, novel mechanisms are  
786 discovered. The molecular pathway that leads to the upregulation of PPAR $\alpha$  during  
787 fasting was recently investigated by Maus and coworkers. They found that FFAs bind  
788 to PLC $\gamma$ -coupled cell surface receptors, such as the fatty acid transporter CD36, in the

789 liver and heart. This binding activates a specific pathway that leads to  $\text{Ca}^{2+}$  influx  
790 across the plasma membrane, called store-operated  $\text{Ca}^{2+}$  entry (SOCE). Subsequently,  
791 increased intracellular  $\text{Ca}^{2+}$  levels induce in a cAMP-dependent manner PGC-1 $\alpha$  and  
792 PPAR $\alpha$  expression, ultimately leading to a transcriptional reprogramming of the cell  
793 to FA utilization (213).

794 Altogether these data demonstrate that PPAR $\alpha$  is a master regulator of FA catabolism  
795 by governing the regulation of key enzymes involved in peroxisomal, mitochondrial  
796 and microsomal FAO.

797 Additionally, PPAR $\alpha$  also plays an important role in ketogenesis by regulating  
798 expression of HMG-CoA synthase 2 (Hmgcs2), a rate-limiting enzyme of  
799 ketogenesis, which catalyzes condensation of acetyl-CoA and acetoacetyl-CoA to  
800 generate HMG-CoA (214). Accordingly, the expression of mHMG-CoA synthase  
801 gene, harboring a functional PPRE (214) in its promoter, is impaired in PPAR $\alpha$  null  
802 mouse liver (215). Of note, HMG-CoA synthase specifically stimulates its own  
803 transcription rate by acting as a coactivator of PPAR $\alpha$ . A recent report identified  
804 liver-specific PPAR $\alpha$  as a critical factor for ketogenesis. It was shown that PPAR $\alpha$   
805 protects from fish oil-induced oxidative stress leading to acute liver failure through its  
806 regulatory actions on ketone body levels, hence suggesting a potential therapeutic  
807 advantage of ketone bodies such as  $\beta$ -hydroxybutyrate in preventing lipid  
808 peroxidation-induced liver damage (216). Of particular importance in regulating  
809 ketogenesis, in addition to FAO, is the metabolic fibroblast growth factor 21 (FGF21).  
810 Its hepatic expression is PPAR $\alpha$ -dependent and is induced by fasting, a ketogenic diet  
811 and fenofibrate (217). Moreover, upon fasting, FGF21 production required c-AMP-  
812 responsive element-binding protein H (CREBH), the liver-enriched transcription  
813 factor. Interestingly, it was reported that CREBH and PPAR $\alpha$  act as interactive trans-

814 activators that regulate each other's expression and act in synergy to activate the  
815 expression of their target gene FGF21 to regulate lipolysis, FAO and ketogenesis  
816 upon fasting or under an atherogenic HFD (218). Of note, CREBH acetylation is  
817 required for the fasting-induced CREBH-PPAR $\alpha$  interaction *in vivo* (219). These  
818 findings on synergistic cross-talk between PPAR $\alpha$  and CREBH might provide a  
819 regulatory mechanism for nuclear receptors or transcription factors to extend their  
820 biological actions and thereby coordinate adaptive physiological responses.

821 As far as lipogenesis is concerned, besides examples listed in **Table 5**, Oosterveer *et*  
822 *al.* showed that induction of some lipogenic genes upon fibrate treatment is regulated  
823 transcriptionally by SREBP-1c. Consistently, in SREBP-1<sup>-/-</sup> mice, chronic PPAR $\alpha$   
824 activation with fenofibrate failed to induce lipogenic genes such as stearoyl-CoA  
825 desaturase (Scd1) and FA desaturase 2 (Fads2) suggesting an indirect regulatory  
826 mechanism (220). In line, in primary hepatocytes, the PPAR $\alpha$  agonists Wy-14,643  
827 and GW7647 increased SREBP-1c activity by triggering PPAR $\alpha$  activity on a DR-1  
828 element in the human SREBP-1c promoter (221). Moreover, in PPAR $\alpha$ -deficient  
829 mice, a disturbance in the normal regulation of SREBP-1-sensitive lipogenic genes  
830 such as acetyl-CoA carboxylase (ACC) and FAS was observed (222). The effect of  
831 PPAR $\alpha$  agonists on SREBP targets has been ascribed to increased activation of  
832 SREBP-1c via enhanced proteolytic cleavage (223). PPAR $\alpha$  agonists increase the  
833 protein levels of the mature form of SREBP-1c, by increasing the rate of proteolytic  
834 cleavage of its membrane-bound precursor form, without changing its mRNA levels.  
835 Of note, PPAR $\alpha$  can also indirectly modulate SREBP-1c transcription via cross-  
836 regulation with LXR $\alpha$  signaling pathway. In this line, Hebbachi *et al.* reported that the  
837 intact PPAR $\alpha$  is required to mediate the LXR $\alpha$ -dependent response of Scd-1 and FAS  
838 gene expression to insulin and that an exogenous non-steroidal synthetic ligand of

839 LXR $\alpha$ , TO-901317, was effective in rescuing PPAR $\alpha$  deficiency, suggesting  
840 participation of PPAR $\alpha$  in generating endogenous LXR $\alpha$  ligands (224).

841 In addition, malonyl-CoA decarboxylase (MCD) is transcriptionally upregulated  
842 following PPAR $\alpha$  activation. MCD degrades the lipogenic intermediate malonyl-  
843 CoA, which is both a precursor of FA biosynthesis and an inhibitor of the  
844 mitochondrial fatty acid transporter CPT-1 (225). PPAR $\alpha$  hence might inhibit the  
845 lipogenic pathway indirectly by inducing MCD, in line with its role in promoting  
846 energy expenditure (226,227).

847 Evidence exists for a functional role of PPAR $\alpha$  in bile acid homeostasis; however the  
848 mechanism is not understood. While various studies have reported on the expression  
849 modulation of Cyp7A1, a microsomal cytochrome P450 isoenzyme that regulates *de*  
850 *novo* hepatic biosynthesis of bile acids by PPAR $\alpha$ , contradictory observations were  
851 made (listed in **Table 5**). In transfected cells, both murine and human cholesterol 7 $\alpha$ -  
852 hydroxylase (Cyp7a1) gene promoters were stimulated by FA or Wy-14,643  
853 treatments, however, the murine promoter harboring an additional PPRE displayed a  
854 greater activity (228). In addition, the expression level of Cyp7a1 diminished in  
855 PPAR $\alpha$ -deficient mice, thus revealing the involvement of PPAR $\alpha$  (229). In contrast,  
856 bezafibrate treatment decreases liver bile acids and Cyp7a1 expression in wild-type  
857 mice and PPAR $\alpha$ -deficient mice (230). Moreover, a Cyp7a1 decrease was observed in  
858 wild-type mice upon ciprofibrate treatment but not in PPAR $\alpha$ -deficient mice (231).

859 Besides, other genes involved in bile acid synthesis are regulated by PPAR $\alpha$  including  
860 cholesterol 27 $\alpha$ -hydroxylase (Cyp27a1) whose activity was downregulated by  
861 ciprofibrate treatment in a PPAR $\alpha$ -dependent manner (231) and sterol-12 $\alpha$ -  
862 hydroxylase (rat Cyp8B1), which was reported to be directly upregulated by PPAR $\alpha$   
863 through a functional PPRE (229).

864 PPAR $\alpha$ -deficient mice display severe hypoglycemia after fasting (110), illuminating  
865 the central role of PPAR $\alpha$  in glucose metabolism. Plasma glucose is maintained  
866 during fasting by combination processes including *de novo* glucose synthesis  
867 (gluconeogenesis), glycogen breakdown (glycogenolysis), and control of peripheral  
868 glucose utilization. Fasting-induced hypoglycemia could be caused by inhibition of  
869 either of these pathways. Interestingly, genes involved in gluconeogenesis have been  
870 identified as PPAR $\alpha$  targets including phosphoenolpyruvate carboxykinase (Pck1),  
871 pyruvate carboxylase (Pcx), and lactate dehydrogenase A (Ldha) (232). Although it  
872 was originally proposed that Pck1 may be specifically regulated by PPAR $\alpha$  in human  
873 but not mouse hepatocytes (233), PPAR $\alpha$  was shown to activate Pck1 promoter  
874 activity in murine hepatoma Hepalclc7 cells (234), whereas in the livers of rats  
875 treated with clofibrate a reduced expression level of Pck1 was observed (235). In  
876 addition, PPAR $\alpha$  promotes the metabolic conversion of glycerol into glucose through  
877 the induction of several hepatic enzymes such as glycerol-3-phosphate dehydrogenase  
878 (GPDH) and glycerol kinase, and of glycerol transporters aquaporins 3 and 9; all of  
879 these genes are identified as targets of PPAR $\alpha$  (233). Moreover, PPAR $\alpha$  contributes to  
880 gluconeogenesis by inducing the expression of pyruvate dehydrogenase kinase  
881 isoform 4 (Pdk4) (236) Pdk4 phosphorylates and thereby inactivates the pyruvate  
882 dehydrogenase complex, resulting in a conservation of pyruvate as gluconeogenic  
883 substrate (237). Besides, PPAR $\alpha$  targets glycogen synthesis in liver by regulating its  
884 direct target, glycogen synthase 2 (Gys-2), which is a rate-limiting enzyme in the  
885 storage of glycogen in liver and adipose tissue. In consistence, Wy-14,643 was shown  
886 to stimulate Gys-2 expression in wild-type but not in PPAR $\alpha$ -/- hepatocytes (238).  
887 TRB3, another PPAR $\alpha$ -target gene, is induced in liver during fasting and in diabetic  
888 mice in a PGC-1-dependent manner. Upregulation of TRB3 expression through direct

889 transcriptional control by PPAR $\alpha$  inhibits insulin signaling by binding to and  
890 preventing activation of Akt/PKB. The latter kinase is a positive regulator of the  
891 cellular response to insulin (239,240). How this can be reconciled with the profile of  
892 PPAR $\alpha$  agonists in other models or clinical, in man, is as yet unclear.  
893 PPAR $\alpha$  has also a profound impact on amino acid metabolism; specifically, it  
894 downregulates genes involved in major pathways of amino acid metabolism including  
895 transamination, deamination, urea cycle, oxidation of  $\alpha$  keto acids and synthesis of  
896 amino acid-derived-products. In support of a role of PPAR $\alpha$  in amino acids gene  
897 expression, plasma urea levels are elevated in PPAR $\alpha$ -deficient mice. The molecular  
898 mechanisms underlying downregulation of amino acid metabolism by PPAR $\alpha$  remain  
899 elusive; however, some studies proposed that PPAR $\alpha$  may modulate the activity of  
900 other transcription factors that are directly involved in amino acid homeostasis,  
901 including HNF4 $\alpha$  and C/EBP $\alpha$  (241).

902

### 903 **PPAR $\alpha$ target genes in heart**

904 During the transition from fetal to adult cardiomyocytes, both PPAR $\alpha$  and PGC-1 $\alpha$   
905 expression are increased, corresponding with the primary use of FAO as an energy  
906 source (242). In the context of a pathological cardiac hypertrophy, the expression of  
907 PPAR $\alpha$  itself is also downregulated (151,243). Conversely, PPAR $\alpha$  activity is  
908 increased in the diabetic heart, which is characterized by increased lipid oxidation.  
909 Correspondingly, overexpression of PPAR $\alpha$  leads to a cardiomyopathy mimicking a  
910 diabetic heart (244-246). To dissect the *in vivo* role of PPAR $\alpha$  in myocardial  
911 metabolism, transgenic mice with cardiac-specific expression of PPAR $\alpha$  were  
912 generated (245,247). Interestingly, Myosin Heavy Chain (MHC)-PPAR $\alpha$  mice, which  
913 developed hepatic insulin resistance, also displayed enhanced IL-6 levels in the

914 circulation (245). Consistent with the metabolic phenotype of myocardial lipid  
915 accumulation in MHC-PPAR $\alpha$  mice, the expression of genes involved in cellular FA  
916 transport was activated by PPAR $\alpha$ . Conversely, cardiac glucose transport and  
917 glycolytic genes were repressed in MHC-PPAR $\alpha$  mice (247), likely occurring via a  
918 transcriptional mechanism, since fenofibrate also downregulated GLUT4-promoter-  
919 driven luciferase reporter gene expression upon cotransfection with PPAR $\alpha$   
920 expression plasmid into rat neonatal ventricular cardiomyocytes (247). As mentioned  
921 also above, Oka *et al.* have recently demonstrated that SIRT1, which plays an  
922 important role in metabolic adaptation to starvation conditions, suppresses FAO in  
923 failing hearts possibly by inhibiting the heterodimerization of PPAR $\alpha$  and RXR $\alpha$ ,  
924 suggesting thereby that preventing PPAR $\alpha$ -SIRT1 or restoring PPAR $\alpha$ -RXR  
925 heterodimerization may improve the progression of heart failure through modulation  
926 of metabolic remodeling (81). Together, these findings highlight the complexity of a  
927 balanced cardiac energy metabolism and the necessity of distinguishing the etiology  
928 of heart failure to be able to predict the outcome of PPAR $\alpha$  agonists.

929

### 930 **PPAR $\alpha$ target genes in lipid metabolism**

931 PPAR $\alpha$  agonists have a pronounced effect on lipoprotein metabolism, which is  
932 involved in lipid and cholesterol transport. In this regard, PPAR $\alpha$  ligands reduce very  
933 low-density lipoprotein (VLDL) production and enhance the catabolism of TG-rich  
934 particles, which indirectly decreases small dense LDL (sdLDL) particles and enhance  
935 the formation of HDL particles and hepatic elimination of excess cholesterol (27).  
936 More specifically, PPAR $\alpha$  activators decrease plasma TG-rich particles levels by  
937 increasing the activity of LPL (48,248), which is the key enzyme in the hydrolysis of  
938 TGs. PPAR $\alpha$  controls LPL gene expression directly through a functional PPRE



939 located in its promoter region (249), and indirectly by reducing the levels of APOC3,  
940 a natural inhibitor of LPL activity (250), likely via a mechanism involving the  
941 transcription factors Rev-erba, HNF4 $\alpha$  or (Forkhead box protein O1) FOXO1 (251-  
942 253). Rev-erba, itself induced by fibrates via a PPRE in the Rev-erba gene promoter,  
943 negatively interferes with the HNF4-mediated activation of APOC3 gene promoter  
944 (253). FOXO1 stimulates APOC3 expression via specific binding to its target site  
945 (CCAAACA) in the APOC3 promoter. The association of FOXO1 with the APOC3  
946 promoter was significantly reduced in response to PPAR $\alpha$  activation, in line with the  
947 inhibition of APOC3 promoter activity in PPAR $\alpha$ -expressing HepG2 cells (252).  
948 Besides APOC3, LPL activity is tightly modulated by other factors such as  
949 apolipoprotein A-V (APOA5), angiopoietin-like protein 3 (ANGPTL3) and  
950 angiopoietin-like protein 4 (ANGPTL4). In line, PPAR $\alpha$  increases expression levels  
951 of APOA5, which is critical for sustaining normal plasma TG levels, through  
952 functional PPREs located in its promoter (254,255). Moreover, PPAR $\alpha$  upregulates  
953 the expression levels of ANGPTL4 (256), which acts as an inhibitor of LPL activity  
954 by converting active LPL dimers to inactive monomers (257), which may play a role  
955 in tissue-specific directing of FA. PPAR $\alpha$  activators affect also the metabolism of  
956 HDL by regulation the expression of its major apolipoproteins, AI (APOA1) and AII  
957 (APOA2). In humans, it increases the expression of these two genes by binding to  
958 PPREs in their promoters, contributing to increased plasma HDL levels (258,259). In  
959 rodents, however, gene expression of APOA1 is not promoted by PPAR $\alpha$  because of  
960 disabling mutations within the PPRE. In fact, the murine APOA1 gene is even  
961 negatively regulated by PPAR $\alpha$  agonists through an indirect mechanism involving the  
962 PPAR $\alpha$ -dependent induction of the NR Rev-erba, a negative regulator of transcription  
963 (260).

964 **PPAR $\alpha$  target gene expression in immune cells**

965 The functional expression of PPAR $\alpha$  by several immune cell types suggests that this  
966 receptor may play an important role in regulation of immune responses. The exact  
967 role of PPAR $\alpha$  in, for instance, innate host defense mechanisms is however largely  
968 unknown. PPAR $\alpha$  was shown essential for anti-mycobacterial responses in murine  
969 bone marrow-derived macrophage cells, involving gene activation of transcription  
970 factor EB (TFEB) and induction of lipid catabolism (261). PPAR $\alpha$  deficiency resulted  
971 in an increased bacterial load and exaggerated inflammatory responses during  
972 mycobacterial infection (261). In human macrophages, PPAR $\alpha$  activation upregulates  
973 the expression ABCA1, a member of the ATP-binding cassette-transporter family, by  
974 an indirect mechanism involving the induction of LXR $\alpha$  expression. The increase in  
975 ABCA1 expression promotes APOA1-mediated cholesterol efflux (262). Adiponectin  
976 receptor 2 (AdipoR2), an LXR target gene, is positively regulated by PPAR $\alpha$  both in  
977 primary and THP-1 differentiated macrophages (263). Given that AdipoR2 is  
978 expressed in human atherosclerotic lesions and macrophages, modulating the  
979 expression of this gene by PPAR $\alpha$  and LXR $\alpha$  may provide beneficial effects in the  
980 treatment of cardiovascular disease. This finding highlights a mechanism of crosstalk  
981 between adiponectin and PPAR $\alpha$ -LXR $\alpha$  signaling pathways (263). In murine  
982 macrophages, PPAR $\alpha$  activation was also shown to upregulate the expression of the  
983 classic PPAR $\alpha$  target gene CPT1 (154). Further in mice, PPAR $\alpha$  activation in hepatic  
984 K $\ddot{u}$ pfper cells or monocyte-recruited macrophages results in anti-inflammatory effects  
985 via the down-regulation of IL-15 and IL-18 (264).

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989 **PPAR $\alpha$  targets linked to the rodent-only hepatocarcinoma side effect**

990 Sustained induction of the peroxisomal FAO system accounts for the massive  
991 proliferation of peroxisomes, which may contribute to hepatocarcinoma, a property  
992 intrinsic to mouse and rat PPAR $\alpha$  but not observed in humans (25,27). By using  
993 hepatocyte-specific PPAR $\alpha$ -deficient mice, PPAR $\alpha$  agonist treatment was shown to  
994 induce hepatomegaly via hepatocyte PPAR $\alpha$  activation. Interestingly, the proliferative  
995 response was mediated exclusively by PPAR $\alpha$  activation in hepatocytes (264).

996 Several mechanisms have been proposed to elucidate the species difference in  
997 response to peroxisome proliferators. For example, in humans, lower expression  
998 levels of PPAR $\alpha$  or the presence of splice variants was suggested to contribute to the  
999 resistance of humans to peroxisome proliferation upon treatment with fibrate drugs  
1000 (50,108). However, most of the proposed mechanisms have been met with reasonable  
1001 criticism (reviewed in (265)). The work of Cheung *et al.* with “humanized” PPAR $\alpha$   
1002 mice suggests that there are inherent species differences in the receptor that may be  
1003 more critical. In this regard, PPAR $\alpha$ -humanized mice that express human PPAR $\alpha$  in a  
1004 mouse PPAR $\alpha$  null background exhibit, unlike wild-type mice, a diminished  
1005 hepatocellular proliferation while the induction of mitochondrial and peroxisomal  
1006 FAO enzymes was comparable to wild-type (266). Moreover, it was proposed that the  
1007 mechanism by which peroxisome proliferators lead to mitogenic and  
1008 hepatocarcinogenic effects in mouse liver is due to murine PPAR $\alpha$ -dependent  
1009 downregulation of let7c microRNA, which in turn releases the repression of c-myc, an  
1010 important oncogene that is related to liver cancer development. Interestingly, this  
1011 effect does not occur in the humanized PPAR $\alpha$  mice (267), thus the induced  
1012 expression of c-myc protein through species-specific regulation of a microRNA,  
1013 contributes to PPAR $\alpha$  ligand-induced liver hyperplasia in mice (267). Still, some

1014 questions arise regarding the fact that the PPAR $\alpha$ -humanized mouse does not  
1015 precisely model humans because there could be species differences in coactivators,  
1016 corepressor and target gene response elements.

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## 1037 **VI. PPAR $\alpha$ agonists in a clinical setting**

1038 PPAR $\alpha$  is widely expressed and serves to activate FAO pathways in the liver in  
1039 response to fasting. This re-tunes energy metabolism to permit efficient use of  
1040 mobilized lipids in the fasted state. The resulting changes may lower secretion of  
1041 TGs from the liver and promote TG clearance from plasma, and thereby also reduce  
1042 atherogenic lipoprotein particles. It was this activity that prompted therapeutic  
1043 development to reduce cardiovascular risk in patients with raised plasma lipids. In  
1044 clinical trials, a marked reduction in circulating TGs with a rise in HDL-C was  
1045 consistently documented, with reductions in LDL cholesterol (LDL-C) depending on  
1046 the pre-treatment TG levels (reviewed in (27)).

1047 By virtue of their TG-lowering capacity, the official indication for fibrates is  
1048 hypertriglyceridaemia. For hypertriglyceridaemia, fibrates are the first choice when a  
1049 change of diet is not sufficient. For dyslipidaemia and hypercholesterolemia, statins  
1050 remain the first choice because of a higher potency in lowering LDL-C (329).

1051 Fibrates are generally well-tolerated drugs and have a good safety profile irrespective  
1052 of concomitant therapy (330). However, changes in biochemical markers, some of  
1053 which related to pathological states, may occur. Among these are elevations in  
1054 homocysteine, creatinine levels, alanine aminotransferase and aspartate  
1055 aminotransferase. Of note, lowering of homocysteine with folic acid did not affect  
1056 cardiovascular diseases (CVD) (331,332). Along the same line, even after prolonged  
1057 treatment, the increase of creatinine is rapidly reversible upon treatment stop  
1058 indicating absence of irreversible tissue damage (333). Muscle-related adverse effects,  
1059 such as an increase in creatine kinase and myalgia may occur but rhabdomyolysis has  
1060 only been observed upon combining gemfibrozil and cerivastatin, an effect due to

1061 classical P450-mediated drug interaction. Rhabdomyolysis was found to be rare with  
1062 (single) fibrate therapies (334).

1063

#### 1064 **PPAR $\alpha$ agonists and effects on lipid and lipoprotein metabolism in humans**

1065 The prominent role of PPAR $\alpha$  in governing lipoprotein and lipid metabolism in  
1066 humans was demonstrated by the fact that fibrates, widely prescribed drugs to treat  
1067 dyslipidaemia, act by binding to PPAR $\alpha$  and induce PPAR $\alpha$ -dependent gene  
1068 transcription (6,335).

1069 A plethora of clinical studies have shown that fibrate treatment lowers serum TG  
1070 concentration by about 30% (-35% P<0.01 (336); -30% P<0.045 (337); -40-20%  
1071 P<0.001 (338); -37% (339); -38% (329)), reduces VLDL TG by about 40% (-41%  
1072 P<0.01 (336); -44-20% P<0.001 (338)) and reduces (in combination with statins) the  
1073 atherogenic sd-LDL by about 20% (-23% (329)). Additionally, HDL increases  
1074 slightly (+19% P<0.02 (336); +82-106% P<0.005 (338); +14% (329)) and serum  
1075 concentration of APOA1 and APOA2 increase by about 20% (+19% P<0.02  
1076 (258,336)). In obese patients with type 2 diabetes, fenofibrate decreased serum TG  
1077 concentrations, while glycated haemoglobin increased (340). In contrast, the  
1078 Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) and Action to  
1079 Control Cardiovascular Risk in Diabetes (ACCORD) studies show glucose-neutral  
1080 effects of fenofibrate, supporting the notion that the first generations of fibrates are  
1081 not antidiabetic drugs (330,333). In line, insulin sensitivity was not affected by  
1082 fenofibrate administration (337,340). Nevertheless, yet controversial, a more recent  
1083 study reported that fenofibrate may increase insulin sensitivity by inhibiting adipose  
1084 tissue expression of retinol-binding-protein-4 (RBP-4), an adipocytokine (341). For  
1085 LPL, in patients on gemfibrozil (6 weeks), there was no significant change in LPL in

1086 adipose tissue (342), possibly due to pharmacokinetic effects. Nevertheless, plasma  
1087 TG levels decreased by 46% and several patients demonstrated increases in muscle  
1088 LPL activity, though these were inconsistent and not statistically significant (342).  
1089 Previous studies, using other PPAR $\alpha$  agonists did report a significant increase of  
1090 skeletal muscle LPL activity (+39% P<0.05 (336); +50% P<0.005 (339)) and even  
1091 adipose tissue LPL activity (343). It was originally proposed that gemfibrozil  
1092 predominantly decreases TG levels by decreasing hepatic VLDL secretion  
1093 (337,342,344). However increased lipolysis may rather be caused by a primary liver  
1094 effect (e.g. APOC3 decreased and APOA5 increased). Additionally, although  
1095 fenofibrate reduces plasma TG levels, it does not seem to lower concentrations or  
1096 turnover rates of non-esterified FAs in adipose tissue (337), correlating with a lack of  
1097 effect on insulin sensitivity.

1098

#### 1099 **PPAR $\alpha$ agonists and effects on liver in humans**

1100 Dyslipidaemia and insulin resistance are known risk factors for NAFLD. As PPAR $\alpha$   
1101 activation reduced hepatic steatosis in mice, applicability of fibrates was suggested as  
1102 a potential treatment for NAFLD in humans (111). In a relatively small (16 patients)  
1103 and phenotypically heterogeneous cohort study, patients completed 48 weeks of  
1104 therapy with fenofibrate without adverse events (345). Significant decreases in TG,  
1105 glucose, alkaline phosphatase and gamma-glutamyl transpeptidase, and an increase of  
1106 APOA1 levels were found, yet, effects on fibrosis as addressed by liver histology  
1107 were minimal (345). Similar studies with fenofibrate for 3 months confirmed results  
1108 of a lowered TG yet unchanged hepatic fat content (346). Short-term treatment (2-8  
1109 weeks) with bezafibrate combined with diet and exercise of steatosis-suffering donors  
1110 for liver transplantation was found to decrease macrovesicular steatosis (347),

1111 although in this particular study the impact of bezafibrate alone was not addressed.  
1112 Treatment of non-alcoholic steatohepatitis (348) patients with gemfibrozil (for 4  
1113 weeks) lowers ALT, AST and  $\gamma$ GT plasma levels (349), but not in a pilot study with  
1114 clofibrate (for 12 months) (350). The relatively weak potency of these fibrates  
1115 combined with lower expression level of PPAR $\alpha$  in human as compared to mouse  
1116 liver was presented as an explanation (351). However, in all studies, plasma TG levels  
1117 effectively decreased. Novel PPAR $\alpha$  agonists with greater potency and efficacy, such  
1118 as pemafibrate, or with dual (elafibranor) or triple (lanifibranor) agonism, may prove  
1119 to be more useful in the treatment of NASH/NAFLD (352). As mentioned also in  
1120 section 5.5, it is important to emphasize that humans are resistant to the induction of  
1121 neoplasms by fibrate drugs in contrast to rodents (25,353). This implies that following  
1122 ligand activation, the PPAR $\alpha$ -mediated pathways controlling lipid metabolism work  
1123 independent from those controlling cell proliferation pathways (266).

1124

#### 1125 **PPAR $\alpha$ agonists and effects on the cardiovascular system in humans**

1126 As in rodents, PPAR $\alpha$  is downregulated in the failing human heart (354). PPAR $\alpha$   
1127 protein levels were compared between homogenates from left ventricular biopsies  
1128 from five control donor hearts vs biopsies from five patients with compensated end-  
1129 stage heart failure at the time of transplantation (354). Whether augmenting PPAR $\alpha$   
1130 activity would improve the progression of heart failure is an interesting, but to date  
1131 unanswered question. Sub-group analysis of the Helsinki Heart Study (HHS) trial  
1132 suggested potential benefit in preventing heart failure with gemfibrozil compared to  
1133 placebo (13.3% vs. 10.6%, HR 0.76; P=0.04) (355). Potential mechanisms may relate  
1134 to direct cardiac and/or on PPAR $\alpha$ 's lipid lowering effects. PPAR $\alpha$  agonists may also  
1135 improve cardiac performance by decreased transcription of pro-inflammatory



1136 cytokines as shown in vitro (356) and/or as in rodent studies, by reduced activation of  
1137 aldosterone-mediated myocardial apoptosis (357) and/or by decreased endothelin-1-  
1138 mediated cardiac fibrosis (358). Further research is needed to assess whether or not  
1139 fibrates, and especially the novel SPPARMs (see further **section 6.5**) can prevent or  
1140 ameliorate chronic heart failure in man.

1141 Gemfibrozil was shown to decrease the incidence of coronary heart disease in both  
1142 HHS and Veterans Affairs HDL Intervention Trial (VA-HIT). Treatment was  
1143 concomitant with a marked increase in HDL-C and reductions in serum levels of total,  
1144 low-density lipoprotein and non-HDL-C and TGs (199,355), yet there is no definite  
1145 proof that these events are the mode of action leading to a decrease incidence of  
1146 coronary heart disease.

1147 Investigating a role for fibrates in the prevention of cardiovascular disease, a pooled  
1148 meta-analysis of long-term randomized placebo-controlled clinical trials pointed out  
1149 that fibrates significantly reduce the odds of nonfatal myocardial infarction, but not of  
1150 cardiovascular mortality, fatal myocardial infarction or stroke, compared to placebo  
1151 (359). Fibrates can reduce the risk of major cardiovascular events predominantly by  
1152 prevention of coronary events, and might have a role in individuals at high risk of  
1153 cardiovascular events and in those with combined dyslipidaemia (360,361). Further,  
1154 in a small-scale pilot study, bezafibrate was found to induce plaque regression in  
1155 thoracic and abdominal aortas with marked TG reduction and HDL-C increase (362).

1156

### 1157 **Human clinical trials and clinical trial endpoints**

1158 Primary endpoint results from the HHS and VA-HIT studies as well as the post-hoc  
1159 and analysis of the ACCORD and FIELD trials suggest that fibrates may benefit  
1160 patients with high TG ( $\geq 200$ mg/dl) and low HDL-C (330,333). This hypothesis is

1161 now formally being tested in a phase 3 study with pemafibrate, called Pemafibrate to  
1162 Reduce Cardiovascular Outcomes by Reducing Triglycerides IN patients With  
1163 diabetes (PROMINENT).

1164 Clinical trials have found that fibrate treatment of atherogenic dyslipidaemia reduces  
1165 vascular atherosclerosis and CV risk (363). In the Bezafibrate Coronary  
1166 Atherosclerosis Intervention Trial (BECAIT), bezafibrate treatment decreased  
1167 angiographic evidence of coronary atherosclerosis (364,365). Moreover, in the HHS,  
1168 gemfibrozil decreased cardiovascular events, especially among patients with diabetes  
1169 (355). Data from the VA-HIT (366), and subgroup analyses from the Bezafibrate  
1170 Infarction Prevention Trial (BIPT) (367) have demonstrated that fibrate therapy  
1171 significantly reduces the risk of cardiovascular events in particular in patients with  
1172 diabetes with raised TGs and low HDL-C. However, large, well-powered  
1173 interventional studies resulted in disappointing outcomes. The FIELD trial saw no  
1174 benefit of fenofibrate, with the intervention group showing only non-significant  
1175 differences compared to the controls (330). One potential explanation for this failure  
1176 was the relatively high drop-in usage of statins in the placebo group and the lower  
1177 overall rates of cardiovascular events. The ACCORD study looked at addition of  
1178 fenofibrate to simvastatin, but enrolled patients with little dyslipidaemia (368-371).

1179 The currently running phase 3 trial PROMINENT will test the efficacy of pemafibrate  
1180 in diabetic patients with high baseline TG levels (372). Meanwhile, fibrates are  
1181 mainly prescribed for the management of raised TGs in patients at risk of pancreatitis.

1182 One clinical feature noted through many of the fenofibrate studies was reduction in  
1183 markers of systemic inflammation, including C-reactive protein (CRP) and IL-6.  
1184 These reductions occurred even in populations lacking a significant change in lipid  
1185 parameters, suggesting that the effect was direct, and not mediated through changes in

1186 lipid-mediated vascular damage (373). This phenomenon may be the physiological  
1187 consequence of immune cell PPAR activation and cross-talk/inhibition of NF- $\kappa$ B  
1188 signaling. The combination of anti-inflammatory efficacy along with promotion of  
1189 hepatic FAO suggested that PPAR $\alpha$  activation may prevent the progression of liver  
1190 steatosis to NASH and ultimately cirrhosis. The dual PPAR $\alpha/\delta$  agonist elafibranor  
1191 was tested for efficacy in patients with liver steatosis. A reduction in histological end-  
1192 points of acute NASH was seen, although the trial did not achieve its originally set  
1193 target of NASH resolution (206). Therapeutic efficacy of elafibranor is currently  
1194 assessed in the phase 3 trial RESOLVE-IT, enrolling patients with active NASH and  
1195 fibrosis stage F1-3 (374).

1196

#### 1197 **Selective PPAR $\alpha$ modulators (SPPARMs) in humans**

1198 Overall it is surprising that there are so few therapeutic applications for fibrates,  
1199 considering the important role that PPAR $\alpha$  has in regulating hepatic energy  
1200 metabolism in response to the fasted state. Furthermore, although we have good  
1201 evidence for cross-talk between PPAR $\alpha$  and inflammatory signaling pathways,  
1202 including NF- $\kappa$ B, the fibrates have not found a role in the management of chronic  
1203 inflammatory diseases.

1204 In an attempt to maximize the therapeutic potential of PPAR $\alpha$  as a drug target,  
1205 SPPARM $\alpha$  are being developed to gain greater pharmacological effect (375,376). A  
1206 restricted activation potential can be explained at the molecular level by a general  
1207 decrease in affinity for cofactors due to partial activation, binding to different target  
1208 gene promoters and, but not limited by, the induction of a differential receptor  
1209 conformation, which leads to a changed PTM status and the recruitment of a specific

1210 subset of PPAR cofactors, also according to their expression level in different organs  
1211 (26,375,377).

1212 Pemafibrate is an example of a high affinity PPAR $\alpha$  agonist with SPPARM properties  
1213 which is currently in phase 3 clinical development (203). Pemafibrate was designed to  
1214 possess higher PPAR $\alpha$  activity and selectivity over existing PPAR $\alpha$  agonists.  
1215 Pemafibrate monotherapy induced atheroprotective effects in several murine models  
1216 of atherosclerosis. As a multi-level strategy compound, pemafibrate acts on  
1217 inflammation, lipid metabolism and RCT (190,378). Promising results were achieved  
1218 with pemafibrate for the treatment of a severe complication of type 2 diabetes, i.e.  
1219 diabetic nephropathy, by targeting the pathways leading to renal oxidative stress  
1220 (379). The first-line treatment for hypercholesterolaemia, a common feature of  
1221 atherosclerotic cardiovascular disease (ASCVD), is statins, which lower the levels of  
1222 LDL-C. However, remaining residual risks, such as elevated TG levels and decreased  
1223 HDL-C, underscore the necessity for complementary therapies. Pemafibrate was  
1224 found superior to fenofibrate with regard to serum TG-lowering effect and hepatic and  
1225 renal safety, when used over 24 weeks in adults with dyslipidaemia (203).  
1226 Consequently, in recent randomized, double-blind, placebo-controlled clinical trials in  
1227 patients with dyslipidaemia, statins were combined with the SPPARM $\alpha$  pemafibrate,  
1228 which showed a favorable benefit-to-risk ratio during phase 2 clinical trials for the  
1229 treatment of combined dyslipidaemia (380,381). The compound was used as an add-  
1230 on therapy in combination with statin treatment to treat the residual CV risk. Next to  
1231 decreasing TG levels, this drug combination targets also several other atherogenic  
1232 lipoproteins, hereby improving apolipoprotein ratios and lowering LDL-C levels  
1233 (380,381). In patients with type 2 diabetes with hypertriglyceridaemia, pemafibrate  
1234 was also shown to ameliorate lipid abnormalities, and well tolerated (382).

1235 Next to SPPARMs, combining selective RXR agonists with statins can be an  
1236 alternative option for the treatment of atherosclerosis. Suboptimal doses of both  
1237 treatments not only minimized dose-related adverse effects, but also resulted in  
1238 synergistic beneficial effects on endothelial function, compared to each drug alone.  
1239 The anti-inflammatory effects were mediated in part by increased RXR $\alpha$ -PPAR $\alpha$  and  
1240 RXR $\alpha$ -PPAR $\gamma$  interactions (383).

1241 Finally, dual or pan-PPAR modulators with partial or selective activation profiles may  
1242 be good alternatives, depending on the clinical application.

1243 Metabolic disorders such as diabetes, atherosclerosis, hyperlipidaemia and obesity,  
1244 rarely occur in isolation, but usually arise in the same individuals. This complex  
1245 phenotype is called the metabolic syndrome or syndrome X (4,375,384). Treatment of  
1246 this aggregation of metabolic abnormalities, usually accompanied by an inflammatory  
1247 context, would benefit from a more holistic approach, such as the application of a  
1248 pan-PPAR agonist, to target the multiple facets of this complex disease phenotype.

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1259 **VII. Inflammation-priming and inflammation-resolving**  
1260 **molecular actions of PPAR $\alpha$**

1261 Evidence suggests that PPAR $\alpha$  can counter inflammation via multiple, distinct  
1262 mechanisms (23,385,386). Both *in vitro* and *in vivo* studies identified PPAR $\alpha$   
1263 influences on both acute and chronic inflammatory processes (154,165,387,388).

1264 The first indication of a beneficial role for PPAR $\alpha$  in suppressing inflammation came  
1265 from studies on LTB<sub>4</sub> action in PPAR $\alpha$ -deficient mice. Compared to WT mice, the  
1266 duration of LTB<sub>4</sub>-induced ear-swelling responses was prolonged. This anti-  
1267 inflammatory effect correlated with LTB<sub>4</sub> activation of PPAR $\alpha$ , thereby identifying a  
1268 feedback loop through PPAR $\alpha$  to limit LTB<sub>4</sub> action. These findings implied that  
1269 some of the anti-inflammatory actions of PPAR $\alpha$  occur through up regulation of  
1270 enzymes involved in  $\omega$  and  $\beta$ -oxidation pathways and thereby accelerated degradation  
1271 of signaling lipids including LTB<sub>4</sub> (165). Subsequently, Poynter *et al.* showed that  
1272 PPAR $\alpha$ -deficient murine splenocytes had NF- $\kappa$ B activity, and thereby produced more  
1273 IL-6 and IL-12 in response to lipopolysaccharide (LPS), supporting PPAR $\alpha$ -  
1274 dependent anti-inflammatory effects in non-metabolic tissues (131). Most recent  
1275 research has focused on the anti-inflammatory actions of PPAR $\alpha$ . Hence, the majority  
1276 of the subchapters below discuss the many and diverse molecular mechanisms  
1277 accounting for the anti-inflammatory effect of PPAR $\alpha$  in various models (**Fig. 4 and**  
1278 **Fig. 5**). However, under certain conditions PPAR $\alpha$  induces target genes that are more  
1279 consistent with a pro-inflammatory profile (see further below), suggestive of  
1280 inflammation-priming (**Fig. 6**) or -sensitization, the phenomenon of sequential signals  
1281 leading to cellular adaptation and affecting subsequent responses, specifically in a  
1282 context of inflammation. The augmented second response may well be gene-specific  
1283 and transient, as has been described for the response of synoviocytes to a repeated

1284 treatment with pro-inflammatory cytokines (389). These consecutive signals may  
1285 encompass pro-inflammatory mediators, as in the study above, but may also combine  
1286 signals of a different nature. For example, the anti-inflammatory glucocorticoid  
1287 hormones transcriptionally upregulate several components of the innate immune  
1288 system and thus prime pro-inflammatory responses (390-392), a circuit thought to  
1289 contribute to more effective resolution of inflammation. We hypothesize similar  
1290 mechanisms may also apply to PPAR $\alpha$  agonist action. Hence, an overall anti-  
1291 inflammatory network may also comprise a component which is “pro-inflammatory”  
1292 dependent on the specific inflammatory and cellular context. More work is needed to  
1293 substantiate these speculations. Data that may support this concept, however limited,  
1294 are discussed in the last subsection.

1295

#### 1296 **Effector cells mediating the anti-inflammatory activity of PPAR $\alpha$**

1297 The extent to which PPAR $\alpha$  plays a decisive role in various immune cells in humans  
1298 as compared to rodents remains a point of contention. Literature and public resources  
1299 such as the human immune genome project indicate that PPAR $\alpha$  expression is lower  
1300 in human immune cells than in hepatocytes. This originally supported the concept that  
1301 cell types like hepatocytes with high PPAR $\alpha$  are likely contributing to the immune  
1302 regulatory effects by providing secondary signals to the immune cells, but data is  
1303 accumulating on functional PPAR $\alpha$  effects in different immune cells. In addition, it is  
1304 not inconceivable that during an inflammatory response, a change in the PPAR $\alpha$   
1305 expression profile of immune cells may turn a dormant factor into an active  
1306 contributor to assist in blocking the inflammatory response. In support of that  
1307 assumption, the well-known adrenal stress hormones GCs enhance PPAR $\alpha$  transcript  
1308 levels in T and B lymphocytes (130), at least in the mouse.

1309 PPAR $\alpha$  is not only expressed in undifferentiated human monocytes but also in  
1310 differentiated human monocyte-derived macrophages and is transcriptionally active  
1311 upon ligand binding. In TNF and IFN- $\gamma$  activated macrophages PPAR $\alpha$  can induce  
1312 apoptosis (32). All three PPAR subtypes play important roles in human macrophages.  
1313 For example, the anti-inflammatory properties of PPAR $\gamma$  have been connected to its  
1314 ability as a driver of M2 polarization in human atherosclerosis (393) .

1315 Brocker *et al.* recently compared hepatocyte- to macrophage-specific PPAR $\alpha$  null  
1316 mice and found that the downregulation of the inflammatory cytokines IL-15 and IL-  
1317 18 depended on macrophage PPAR $\alpha$ , identifying K $\ddot{u}$ pffer cell PPAR $\alpha$  as an important  
1318 mediator of the anti-inflammatory effects of PPAR $\alpha$  agonists (264). Functional human  
1319 PPAR $\alpha$  was demonstrated following a global gene expression profiling experiment on  
1320 human primary macrophages treated with specific PPAR $\alpha$  agonists (263). The role of  
1321 PPARs, fatty acid signaling, and fatty acid oxidation within primary human immune  
1322 cell types in a variety of inflammatory conditions requires further experimental study.

1323 Linking inflammation to metabolism, murine macrophage LXR is critical for PPAR $\alpha$   
1324 to promote cholesterol efflux and reverse cholesterol transport (RCT) *in vivo*,  
1325 contributing to anti-atherogenic effects and hence indirectly resolving inflammatory  
1326 responses within the blood vessels. In support, PPAR $\alpha$  activation was demonstrated to  
1327 inhibit the development of atherosclerosis by promoting macrophage RCT in  
1328 humanized hypercholesterolemic mice expressing the human APOA1 gene (394).

1329 Connecting metabolic and immune responses to cancer, in murine and human  
1330 melanoma models, an increase in free FA species during tumor progression was  
1331 shown to activate PPAR $\alpha$  signaling in CD8<sup>+</sup> tumor-infiltrating lymphocytes.  
1332 Consequently, CD8<sup>+</sup> tumor-infiltrating lymphocytes switched toward FA catabolism  
1333 to preserve their effector functions. Promoting FA catabolism with fenofibrate



1334 markedly improved the capacity of CD8<sup>+</sup> tumor-infiltrating lymphocytes to delay  
1335 tumor growth, via a synergistic mechanism involving a Programmed cell Death  
1336 protein-1 (PD-1) blockade to efficiently boost the efficacy of melanoma  
1337 immunotherapy (395). Together, these data highlight the interplay between the  
1338 metabolic programs of T lymphocytes and tumor cells, with potential impact on  
1339 melanoma immunotherapy (396). Ligand activation of PPAR $\alpha$  also antagonizes NF-  
1340  $\kappa$ B in murine T and B lymphocytes, and conversely, T cell activation results in  
1341 reduced PPAR $\alpha$  expression. Of note, treatment of T lymphocytes with specific  
1342 PPAR $\alpha$  activators can upregulate the expression of endogenous PPAR $\alpha$ -controlled  
1343 genes in combination with histone deacetylase inhibitors. The presence of functional  
1344 PPAR $\alpha$  within T and B lymphocytes again emphasizes a role for this nuclear receptor  
1345 in cells of the immune system (130). In addition to targeting NF- $\kappa$ B, PPAR $\alpha$  also  
1346 counters proinflammatory signaling in T lymphocytes at the level of IL-2 and c-Jun  
1347 activation (397). In evidence, genetic ablation of the PPAR $\alpha$  gene resulted in the loss  
1348 of antagonism of NF- $\kappa$ B, increased production of Th1 and decreased production of  
1349 Th2 cytokines by T cells (397). In another study, the PPAR $\alpha$  agonist Wy-14,643 was  
1350 shown to increase IL-4 secretion and to inhibit IFN- $\gamma$  expression in murine mitogen-  
1351 activated splenocytes, and to reduce the proliferation of human T cell lines (398).  
1352 Similarly, stimulation of isolated CD4-positive human T cells with PPAR $\alpha$  agonists at  
1353 clinically relevant concentrations decreased IFN- $\gamma$ , TNF $\alpha$  and IL-2 production.  
1354 However, in this study no effect on viability was noted (399).

1355 Epidermal Langerhans cells (LCs) play a pivotal role in initiating and maintaining  
1356 primary immune responses in the skin. PPAR $\alpha$  is expressed in immature LC but  
1357 downregulated in mature LC. Pharmacological PPAR $\alpha$  activation inhibits LC  
1358 maturation, migratory capacity, cytokine expression, and the ability to drive T-cell

1359 proliferation. Moreover, PPAR $\alpha$  in LC also inhibits NF- $\kappa$ B activity. Taken together,  
1360 PPAR $\alpha$  activation by endogenous ligands may provide a molecular signal that allows  
1361 LC to remain in an immature state (400). The activation of PPARs by drugs, and  
1362 products of intermediary energy metabolism within the diverse cells of the immune  
1363 system may reveal new opportunities to target inflammatory process or to advance  
1364 adjunctive therapies to enhance anti-cancer treatment.

1365

### 1366 **Interactions between PPAR $\alpha$ and other transcription factors**

1367 The best-known mechanism by which PPAR $\alpha$  (as well as the other PPAR subtypes)  
1368 inhibits many inflammatory genes is transrepression. In this tethering mechanism,  
1369 PPAR $\alpha$  acts directly to negatively regulate the expression of pro-inflammatory genes  
1370 by antagonizing the activities of other regulators of inflammation such as members of  
1371 the NF- $\kappa$ B, AP-1 and STAT families, as also already alluded to in the section above  
1372 (27) (Fig. 4A).

1373 In this regard, it was found that fibrate-activated PPAR $\alpha$  inhibits IL-1-stimulated  
1374 secretion of IL-6 and 6-keto-prostaglandin by decreasing IL-6 and COX-2 gene  
1375 transcription in smooth-muscle cells (SMCs). Inhibition of COX-2 induction occurred  
1376 transcriptionally as a result of PPAR $\alpha$  interference with NF- $\kappa$ B signaling (23). This  
1377 effect of PPAR $\alpha$  in the vascular wall may influence the progress of atherosclerosis.  
1378 Accordingly, Delerive *et al.* have demonstrated that aortas from PPAR $\alpha$ -null mice  
1379 display an exacerbated inflammatory response to LPS, as assessed by enhanced IL-6  
1380 secretion, and that fibrate treatment represses IL-6 mRNA levels in LPS-stimulated  
1381 aortas of PPAR $\alpha$  wild-type, but not of PPAR $\alpha$ -null mice, confirming that the observed  
1382 anti-inflammatory properties of fibrates are PPAR $\alpha$ -dependent. This vascular anti-  
1383 inflammatory response is a result of PPAR $\alpha$  interference with NF- $\kappa$ B and AP-1

1384 transactivation capacity involving direct protein-protein interaction with p65 and c-  
1385 Jun. Of note, a bidirectional antagonism between the PPAR $\alpha$  and NF- $\kappa$ B signaling  
1386 pathway exists (387).

1387 In human vascular endothelial cells, PPAR $\alpha$  inhibits the thrombin-mediated activation  
1388 of endothelin-1, a vasoactive peptide. This inhibition occurs at the transcriptional  
1389 level and occurs via interference with the AP-1 signaling pathway by preventing the  
1390 binding of AP-1 proteins to their target sequence (401). Consistently, PPAR $\alpha$   
1391 activators prevent TNF $\alpha$ -induced vascular cell adhesion molecule 1 (VCAM-1)  
1392 expression in human endothelial cells, partly through inhibition of the NF- $\kappa$ B  
1393 pathway. This PPAR $\alpha$  action, with a consequent decrease in monocyte adherence to  
1394 endothelial cells, may have important implications on early stage of atherosclerosis  
1395 (402). PPAR $\alpha$  agonists decrease cytokine-induced tissue factor in monocytes most  
1396 likely by interfering negatively with AP-1 and/or NF- $\kappa$ B activity (403,404). Because  
1397 these pathways are involved in the activation of inflammatory response genes  
1398 implicated in atherogenesis, PPAR $\alpha$  activation may be beneficial in combatting the  
1399 pathogenesis of atherosclerosis. In addition, oxidized eicosapentaenoic acid (EPA)  
1400 attenuates monocyte chemotactic protein 1 (MCP-1) and IL-8 expression in  
1401 endothelial cells, by inhibiting NF- $\kappa$ B in a PPAR $\alpha$ -dependent manner (405).

1402 The transcription factor, specificity protein 1 (Sp1), is also involved in the anti-  
1403 inflammatory mechanism of PPAR $\alpha$ . PPAR $\alpha$  activation inhibits vascular endothelial  
1404 growth factor receptor-2 (VEGFR-2) in endothelial cells, which occur at the  
1405 transcriptional level via decreasing Sp1 site-dependent binding to the promoter  
1406 sequence through an increased interaction with the Sp1 transcription factor (406).

1407 Thus, PPAR $\alpha$  activators may control inflammatory responses in part by targeting

1408 endothelial VEGFR2 signaling, which is critical for angiogenic responses during  
1409 chronic inflammation.

1410 Further work has demonstrated that the mechanism of PPAR interference with NF- $\kappa$ B  
1411 action additionally involves a PPAR $\alpha$ -mediated induction of the inhibitory protein  
1412 I $\kappa$ B $\alpha$  expression. Interestingly, fibrates increase I $\kappa$ B $\alpha$  expression in primary smooth  
1413 muscle cells and hepatocytes, thus preventing nuclear p50/p65 NF- $\kappa$ B translocation  
1414 and thereby arresting p65-mediated gene activation, to oppose development of the  
1415 inflammatory response. PPAR $\alpha$ -mediated I $\kappa$ B $\alpha$  upregulation did not require a  
1416 functional PPRE in the I $\kappa$ B $\alpha$  promoter, but rather involved functional Sp1 and NF- $\kappa$ B  
1417 sites, in a cross-talk mechanism (407). Cytosolic retention of p50/p65 NF- $\kappa$ B extends  
1418 the list of candidate anti-inflammatory PPAR $\alpha$  mechanisms from those mainly  
1419 characterized by direct binding and inactivation of transcription factors, such as p65/  
1420 NF- $\kappa$ B and c-Jun (23,387). In further, detailed molecular studies fibrates were found  
1421 to decrease basal and IL-6-stimulated expression of the human fibrinogen gene in  
1422 human primary hepatocytes and hepatoma HepG2 cells by interfering with the pro-  
1423 inflammatory transcription factor CAATT/enhancer-binding protein  $\beta$  (C/EBP- $\beta$ ) via  
1424 sequestration of the coactivator glucocorticoid receptor-interacting protein  
1425 1/transcriptional intermediary factor 2 (GRIP1/TIF2) (408) (**Fig. 4A**). This anti-  
1426 inflammatory action of PPAR $\alpha$  is not restricted to fibrinogen but also applies to other  
1427 acute phase genes containing a C/EBP response element in their promoters (408).

1428 In addition, fibrate-activated PPAR $\alpha$  negatively regulates IL-1-stimulated CRP gene  
1429 expression in isolated primary human hepatocytes by two mechanisms, the inhibition  
1430 of p50-NF- $\kappa$ B translocation and the down-regulation of basal p50-NF- $\kappa$ B and C/EBP-  
1431  $\beta$  expression in a PPAR $\alpha$ -dependent fashion (409). Such observations reveal a direct  
1432 contribution of PPAR $\alpha$  in controlling the inflammatory gene response in the liver.

1433 Furthermore, chronic PPAR $\alpha$  agonist treatment inhibited the expression of IL-6  
1434 induced acute phase response (APR) gene expression *in vivo*. This suppression occurs  
1435 transcriptionally due to the down-regulation of the IL-6 receptor components gp80  
1436 and gp130 in the liver, thereby reducing the phosphorylation and activation of the  
1437 downstream transcription factors STAT3 and c-Jun that transduce the IL-6 signal; and  
1438 to the reduction of the basal expression of the transcription factors CCAAT enhancer-  
1439 binding protein- $\alpha$ , - $\beta$ , - $\delta$  (410). Related to STAT signaling, Van der Meer *et al.*  
1440 proposed that activated PPAR $\alpha$  may downregulate gene expression in HepG2 cells by  
1441 causing the loss of STAT1 and STAT3 binding to DNA. Therefore, interfering with  
1442 STAT-dependent transcriptional activity, which has been previously well documented  
1443 for other PPAR subtypes, may also contribute to the anti-inflammatory action of  
1444 PPAR $\alpha$  activation (72) (**Fig. 4A**). Inhibitory effects of PPAR $\alpha$  agonists on IL-1- and  
1445 IL-6-induced expression of APR proteins including CRP, serum amyloid A (SAA),  
1446 fibrinogen and haptoglobin, were also observed in mice displaying liver-restricted  
1447 expression of PPAR $\alpha$  (411).

1448 PPAR $\alpha$  reduced the stimulation of the APR mediated by LPS and prevented the LPS-  
1449 induced increase of proinflammatory mediators IL-1, IL-6 and TNF in plasma via a  
1450 liver-specific transcriptional mechanism. This resulted in decreased expression of the  
1451 adhesion molecules, intercellular adhesion molecule 1 (ICAM-1) and VCAM-1 in the  
1452 murine aorta, suggesting that PPAR $\alpha$  can control the systemic and vascular  
1453 inflammatory response via a liver-specific action (411). Importantly, this implies that  
1454 the spectrum of PPAR $\alpha$ -controlled genes may be vastly larger in a more physiological  
1455 context than in isolated cells (412). Further evidence of this comes from analysis in  
1456 human liver slices where there was suppression of inflammatory genes, but not in

1457 primary hepatocytes. The most highly repressed genes included chemokines, IFN- $\gamma$ -  
1458 induced genes and other immune-related genes (412).

1459 Fibrate activation of PPAR $\alpha$  inhibits hepatic inflammatory responses, which  
1460 unimpeded lead to liver injury and progression to fibrosis, by a mechanism relying on  
1461 transrepressive activity, rather than binding to cognate PPREs and activation of lipid  
1462 metabolism genes (388). These findings indicate that PPAR $\alpha$  agonists with  
1463 dissociated activity, selectively modulating PPAR $\alpha$  transrepression, might have the  
1464 potential to prevent the progression of NASH to liver fibrosis through a direct  
1465 counteraction of inflammatory responses independent of effects on intrahepatic lipid  
1466 accumulation. In the same line, a recent report has shown that PPAR $\alpha$  gene and  
1467 protein expression are decreased in NASH (413). The PPAR $\alpha$  levels were restored by  
1468 pharmacological inhibition or genetic suppression of miRNA-21, a micro-RNA  
1469 upregulated in the liver of patients with NASH, decreasing thereby liver injury,  
1470 inflammation and fibrosis (413). These findings suggest that inhibition of the miRNA-  
1471 21 pathway might be an interesting new therapeutic strategy to combine with some of  
1472 the current PPAR $\alpha$  pharmacological agonists in NASH (413).

1473 A PPRE-dependent mechanism of PPAR $\alpha$ -NF- $\kappa$ B interference in the regulation of  
1474 complement C3 transcription has been observed in a human hepatoma cell line,  
1475 identifying transactivation as another mechanism for the anti-inflammatory effects of  
1476 PPAR $\alpha$  (**Fig. 2B**). Ligand-dependent activation of PPAR $\alpha$  abolishes TNF $\alpha$ -mediated  
1477 upregulation of complement C3 through a physical interaction of PPRE-bound  
1478 PPAR $\alpha$  with p65 within the C3 promoter (414).

1479 Finally, the transcription factor Sp1 is also involved in the anti-inflammatory  
1480 mechanism of PPAR $\alpha$ . PPAR $\alpha$  activation inhibits vascular endothelial growth factor  
1481 receptor-2 (VEGFR-2) in endothelial cells, which occur at the transcriptional level via

1482 decreasing Sp1 site-dependent binding to the promoter sequence through an increased  
1483 interaction with the Sp1 transcription factor (406).

1484

#### 1485 **Cooperation between PPAR $\alpha$ and other anti-inflammatory nuclear receptors**

1486 Simultaneous activation of PPAR $\alpha$  and GR $\alpha$ , a well-characterized NF- $\kappa$ B repressor,  
1487 enhances transrepression of NF- $\kappa$ B-driven gene expression and additively represses  
1488 proinflammatory cytokine production, including MCP-1 and IL-6 in various cell lines  
1489 (385) (**Fig. 4B**). This finding offers a rationale for the control of inflammation by  
1490 GR $\alpha$  and PPAR $\alpha$  by inflammatory gene repression and suggests new approaches for  
1491 the treatment of inflammatory diseases where combinations of PPAR $\alpha$  and GR $\alpha$   
1492 activation could offer a more effective anti-inflammatory intervention.

1493 This phenomenon is not restricted to PPAR $\alpha$  since a similar proof of concept was  
1494 recently also shown for PPAR $\gamma$  agonists in skin and lung. Indeed, house-dust mite  
1495 induced skin sensitization, which aggravates asthmatic responses in mice via a mixed  
1496 Th2/Th17 response, was prevented by treatment combining glucocorticoids with  
1497 rosiglitazone (415). Similarly, the role of PPAR $\alpha$  in estrogen-mediated anti-  
1498 inflammatory activity in lungs was shown by the demonstration of a weakened  
1499 response to estrogen in PPAR $\alpha$ -deficient mice (416).

1500

#### 1501 **Regulation of MAPK and PKC kinase activities**

1502 Given that inflammatory responses are driven in part by the activation of MAPKs,  
1503 PPAR $\alpha$  ligands were also shown to modulate the degree of macrophage inflammation  
1504 not only by reducing NF- $\kappa$ B activation, but also by reducing MAPK phosphorylation,  
1505 hence effectively decreasing the expression of the proinflammatory mediator COX-2  
1506 and inducible nitric oxide synthase (163,417).

1507 PPAR $\alpha$  was further shown to polarize T-cell differentiation processes, also via a  
1508 modulation of MAPK activities. In CD4(+) T lymphocytes, unliganded PPAR $\alpha$   
1509 suppressed p38 MAPK phosphorylation. Ligand activation reversed this inhibition,  
1510 augmenting the expression of the transcription factor of T cells (T-bet), a lineage-  
1511 determinant of Th1 inflammatory responses (418) which drives IFN- $\gamma$  expression.  
1512 PPAR $\alpha$  thus reduced the level of activation-induced IFN- $\gamma$  produced by CD4<sup>+</sup> T cells.  
1513 Mechanistically, the control of PPAR $\alpha$  over T-bet expression occurs via a DNA-  
1514 binding independent mechanism, mediated through the ability of PPAR $\alpha$  to repress  
1515 the phosphorylation of p38-MAP kinase following T cell activation. These data  
1516 suggested a novel and important function for PPAR $\alpha$  within T cells. Both in  
1517 transformed T and B lymphocytes, PPAR $\alpha$  ligands effectively repress NF- $\kappa$ B activity  
1518 (130). Additionally, the anti-inflammatory effects of PPAR $\alpha$  in macrophages involve  
1519 an inhibition of the PKC signaling pathway (52,154). Little is known about the exact  
1520 molecular mechanisms underlying the PPAR $\alpha$ -mediated modulation of (potentially  
1521 other) kinase activities and to what extent this may contribute to an overall anti-  
1522 inflammatory action.

1523

#### 1524 **Anti-inflammatory activity of PPAR $\alpha$ in a PPRE-dependent manner**

1525 In addition to down-regulating the expression of pro-inflammatory genes, PPAR $\alpha$  also  
1526 suppresses the inflammatory response by direct up-regulation of genes with anti-  
1527 inflammatory properties, suggesting a possible cooperation between PPAR $\alpha$ -  
1528 dependent transactivation and transrepression to govern anti-inflammatory pathways.  
1529 Besides the upregulation of I $\kappa$ B $\alpha$ , discussed above, as a mechanism contributing to its  
1530 anti-inflammatory actions (409,419), activated PPAR $\alpha$  strongly up-regulated the  
1531 hepatic expression of IL-1 receptor antagonist (IL-1ra), an inhibitor of cytokine



1532 signaling, during LPS-induced inflammation, while numerous inflammatory genes  
1533 including STAT3, SAA and IL-18 were found to be down-regulated (420).  
1534 Transactivation and chromatin immunoprecipitation studies identified IL-1ra as a  
1535 direct positive target gene of PPAR $\alpha$  with a functional PPRE present in the promoter.  
1536 Moreover, up-regulation of IL-1ra by PPAR $\alpha$  was conserved in human HepG2  
1537 hepatoma cells and the human monocyte/macrophage THP-1 cell line (420).  
1538 In the same context, Heme Oxygenase-1 (HO-1), a PPAR $\alpha$  target gene with a  
1539 functional PPRE, is also directly upregulated by PPAR $\alpha$  ligands and contributes to the  
1540 anti-inflammatory effects in human vascular cells (421).  
1541 Interestingly, the gene coding for vanin-1 (Vnn1), a protein with anti-inflammatory  
1542 potential, is robustly activated in liver by PPAR $\alpha$  agonists, such as Wy-14,643,  
1543 fenofibrate, and clofibrate (211), and functional PPRE sites have been identified in the  
1544 Vnn1 promoter (422). Vanin activity prevents steatosis development in response to  
1545 fasting, while its deficiency affects multiple genes involved in  
1546 inflammation/immunity (423).  
1547 Considering the above studies, it is clear that PPAR $\alpha$  may affect markers of  
1548 inflammation in a direct or indirect manner. Below, we continue the discussion on  
1549 recent findings regarding the role of PPARs in some specific examples of  
1550 inflammatory disease models.

1551

### 1552 **PPAR $\alpha$ in the metabolic syndrome as an inflammatory disease**

1553 Inflammation is a critical component of the metabolic syndrome, which represents a  
1554 cluster of risk factors that include dyslipidemia, insulin resistance, hypertension,  
1555 inflammation and coagulation disorders. Consequently, PPAR $\alpha$ , being on the  
1556 crossroad between inflammation and metabolism, is an important regulator of the

1557 metabolic syndrome (27,424). It is now well-accepted that pro-inflammatory  
1558 cytokines and specific immune cell populations promote metabolic disease  
1559 progression. Accordingly, a chronic low-grade inflammatory condition, which is  
1560 characterized by increased circulating inflammatory cytokines and acute phase  
1561 proteins and which often accompanies the metabolic syndrome, has been implicated  
1562 as a major factor both in the establishment of the metabolic syndrome and its  
1563 associated pathophysiological consequences (207). PPAR $\alpha$ , and other PPAR  
1564 isoforms, have all demonstrated anti-inflammatory effects in these conditions (425).  
1565 In hyperlipidemic patients and patients with metabolic syndrome PPAR $\alpha$  activation  
1566 using fenofibrate decreases plasma levels of several acute phase and inflammatory  
1567 proteins that are normally increased during inflammatory conditions, such as high-  
1568 sensitivity CRP and IL-6 (23,373,410). In addition, fenofibrate treatment also reduces  
1569 plasma concentrations of the cytokines interferon- $\gamma$  (IFN- $\gamma$ ) and TNF $\alpha$  in  
1570 hyperlipoproteinemia type IIb patients (426). Addition of fenofibrate to simvastatin-  
1571 treated patients with atherosclerosis and early glucose metabolism disturbances  
1572 further reduces lymphocyte release of IL-2, IFN- $\gamma$  and TNF- $\alpha$ , accompanied by a  
1573 reduction in plasma CRP levels, compared to statin alone (427). It was also found that  
1574 fibrates have an anti-inflammatory effect in the interaction between adipocytes and  
1575 macrophages in obese adipose tissue, thereby improving the dysregulated  
1576 adipocytokine production and obesity-related metabolic syndrome (428).

#### 1578 **PPAR $\alpha$ in age-related inflammation**

1579 Enhancing PPAR $\alpha$  levels and activity was shown to ameliorate age-related  
1580 inflammatory processes. Increased inflammatory marker levels during aging correlate  
1581 with a decreased PPAR $\alpha$  activity. Administration of PPAR $\alpha$  activators improved the  
1582 splenocyte cellular redox balance and reduced the age-associated elevations in NF- $\kappa$ B

1583 activity and constitutive pro-inflammatory cytokine production (131,429). The PPAR  
1584 pan agonist MHY2013 alleviated age-related hepatic lipid accumulation, in part by  
1585 upregulating  $\beta$ -oxidation signaling and suppressing inflammation in the liver (430).

1586 In a similar context, a recent study has demonstrated that LPS-induced liver  
1587 inflammation and lipid accumulation were associated with a chronically overactive  
1588 inflammasome/IL-1 $\beta$  pathway in aged rat livers and that these effects were due to the  
1589 dysregulation of PPAR $\alpha$  and SREBP1c. Indeed, in these aged rat livers, PPAR $\alpha$  was  
1590 consistently inactivated and the levels of SREBP1c were chronically increased (132).  
1591 Therefore, targeting PPAR $\alpha$  signaling might reduce the age-related sensitivity to  
1592 endotoxin-induced liver inflammation.

1593 These data, positioning PPAR $\alpha$  at the crossroad of aerobic metabolism and  
1594 inflammation, fit the observations that link non-aerobic glycolytic ATP generation to  
1595 the production of pro-inflammatory cytokines (80). To illustrate, complex host  
1596 responses to the TLR9 agonist CpGA were found to be mediated by plasmacytoid  
1597 dendritic cell (pDC) production of IFNs, operating in an autocrine fashion. FAO was  
1598 necessary for full pDC activation, pointing to PPAR $\alpha$  as a potential target to modulate  
1599 downstream effects of type I IFNs (431).

1600

#### 1601 **PPAR $\alpha$ in inflammatory bowel disease**

1602 Considering the intestine as an organ contributing to immune system, a link between  
1603 PPAR $\alpha$  signaling and the control of colitis was found upon studying the IL-10 gene  
1604 deficient mouse as a model for human inflammatory bowel disease. In this model  
1605 anti-inflammatory IL-10 gene expression changes were related to PPAR $\alpha$  activation  
1606 although the mechanisms responsible remain undefined (432). Infectious disease  
1607 control in intestine also involves PPAR $\alpha$  activation. As such, clearance of a *Giardia*

1608 muris infection was found to coincide not only with IL-17 production, contributing to  
1609 protection, but also with an early PPAR $\alpha$  induction (433). Along similar lines,  
1610 fenofibrate represses IL-17 and IFN- $\gamma$  expression and improves colitis in IL-10-  
1611 deficient animals, suggesting a possible therapeutic potential in Crohn's disease (434).  
1612 Of note, PPAR $\alpha$  protects the intestine from colitis-induced permeability (435)

1613

#### 1614 **PPAR $\alpha$ in inflammatory skin disease**

1615 PPAR $\alpha$  activation impaired epidermal Langerhans Cell (LCs) maturation and  
1616 migratory capacity and alters cytokine production through the inhibition of NF- $\kappa$ B  
1617 phosphorylation (400), suggesting a role for PPAR $\alpha$  in combatting inflammatory skin  
1618 diseases (436). In this regard, PPAR $\alpha$ -deficient mice exhibit impaired recruitment of  
1619 neutrophils and monocytes/macrophages to the wound bed and present a transient  
1620 delay in healing, which coincides with the inflammatory phase (437). These data  
1621 support the concept that PPAR $\alpha$  favors skin healing via a modulation of the  
1622 inflammatory phase (437). In addition, PPAR $\alpha$  activation is beneficial in mouse  
1623 models with a hyperproliferative epidermis (438) and in models of irritant and allergic  
1624 dermatitis (439). The anti-inflammatory properties of PPAR $\alpha$  agonists, coupled with  
1625 their anti-proliferative and pro-differentiating effects, suggest that they could be  
1626 beneficial for the treatment of a variety of cutaneous diseases (439). In a model of  
1627 atopic dermatitis, topical application of the PPAR $\alpha$  agonist WY14643 significantly  
1628 decreased antigen-induced skin inflammation, providing *in vivo* evidence for the role  
1629 of PPAR $\alpha$  in controlling skin inflammation and pointing to a potential benefit in  
1630 combating systemic inflammatory responses in patients with atopic dermatitis, a  
1631 disease characterized by inflammatory infiltrates and barrier dysfunction (440).  
1632 Interestingly, PPAR $\alpha$  may be the molecular mediator of the anti-allergic and anti-

1633 inflammatory effects of palmitoylethanolamide, a natural FA derivative present in  
1634 murine skin (441). Taken together, the many observations above underscore the  
1635 implications of PPAR $\alpha$  in inflammatory skin disorders and paves the way for trials of  
1636 topical PPAR $\alpha$  agonist application.

1637

### 1638 **PPAR $\alpha$ in other inflammatory diseases**

1639 Expanding the applicability of PPAR $\alpha$  agonists to other inflammatory diseases, anti-  
1640 inflammatory properties of activated PPAR $\alpha$  were observed in primary murine  
1641 astrocytes, a cell type implicated in the pathology of experimental autoimmune  
1642 encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Fibrates  
1643 decreased the LPS-induced secretion of pro-inflammatory cytokines TNF $\alpha$ , IL-1 $\beta$  and  
1644 IL-6 and NO production, likely via interference with the DNA binding of NF- $\kappa$ B  
1645 (442), suggesting a putative function of PPAR $\alpha$  in controlling neurodegenerative  
1646 diseases. In the same line, PPAR $\alpha$  activation significantly inhibited radiation-induced  
1647 microglial production of pro-inflammatory cytokines such as TNF $\alpha$ , IL-1 $\beta$ , Cox-2 and  
1648 iNOS by inhibiting nuclear translocation of the NF- $\kappa$ B/p65 subunit and reducing  
1649 phosphorylation of nuclear c-Jun/AP-1 (443). Genetic ablation of PPAR $\alpha$  in an  
1650 experimental model of autoimmune encephalomyelitis increased clinical symptoms in  
1651 male but not female SV.129 H<sup>2b</sup> mice, consistent with the finding that male mice of  
1652 this strain express higher levels of PPAR $\alpha$  than female mice (397). Recently, PPAR $\alpha$   
1653 was reported to contribute to the anti-inflammatory activity of melatonin in Spinal  
1654 Cord Injury (SCI), again by modulating the NF- $\kappa$ B pathway. It was demonstrated that  
1655 melatonin was less effective in PPAR $\alpha$  null, compared to WT mice, as evaluated by  
1656 inhibition of the degree of spinal cord inflammation and tissue injury, neutrophil

1657 infiltration, pro-inflammatory cytokine expression, NF- $\kappa$ B activation and iNOS  
1658 expression (444).

1659

### 1660 **The paradox of PPAR $\alpha$ as a pro-inflammatory mediator**

1661 The above studies (**Fig. 5**) are in line with the well-accepted notion that PPAR $\alpha$  is  
1662 involved primarily in anti-inflammatory signaling. As stated in the introduction to this  
1663 chapter, PPAR $\alpha$  has also been ascribed potential “pro-inflammatory” effects (**Fig. 6**).

1664 Lee *et al.* suggested that oxidized phospholipids induce MCP-1 and IL-8 expression  
1665 through a PPAR $\alpha$ -dependent signaling cascade in human aortic endothelial cells  
1666 (445). These data demonstrate that PPAR $\alpha$  can mediate the activation of endothelial  
1667 cells by stimulating the production of monocyte chemotactic activity in response to  
1668 oxidized phospholipids and lipoproteins (445)

1669 Additionally, PPAR $\alpha$  activation by Wy-14,643 enhances IL-1 $\beta$ -induced pro-  
1670 inflammatory cytokine release, including IL-6, IL-8 and TNF- $\alpha$ , in ocular cells and  
1671 induces proangiogenic responses (446). In contrast, in the FIELD and ACCORD  
1672 studies (330,333) fibrates showed a protection against diabetic retinopathy. In line, the  
1673 antiangiogenic activity of PPAR $\alpha$  ligands specifically depended on the activation of  
1674 PPAR $\alpha$  since PPAR $\alpha$  ligands inhibited FGF2-corneal neovascularization in wild-type  
1675 but not in PPAR $\alpha$  deficient mice (447). Consistent with these data fenofibrate was  
1676 shown to improve diabetic retinopathy in type 1 diabetes animal models and  
1677 ischemia-induced retinal neovascularization. Herewith, PPAR $\alpha$ -dependency also  
1678 formed the mechanistic basis for the therapeutic effects of fenofibrate (448). The anti-  
1679 inflammatory effects of fenofibrate-activated PPAR $\alpha$  were ascribed to the inhibition  
1680 of NF- $\kappa$ B signaling in the diabetic retina (448). The basis for these differences

1681 remains unclear but might reflect a reduced complexity of *in vitro* studies versus *in*  
1682 *vivo* studies.

1683 Then again, in a mouse model of endotoxemia, fenofibrate administration strongly  
1684 increased circulating LPS-induced TNF levels (449). In a rat hypertensive model,  
1685 bezafibrate was shown to sensitize for endotoxin effects, concomitant with low levels  
1686 of HDL and increased levels of TG (450). In murine macrophage lines and in contrast  
1687 to Wy-14,643, the natural PPAR $\alpha$  ligands LTB<sub>4</sub> and 8(S)-HETE showed a pro-  
1688 inflammatory potential by increasing NOS activity (348). These observations are  
1689 again not restricted to PPAR $\alpha$ , since PPAR $\gamma$  activity in T-cells and dendritic cells  
1690 promotes Th2 immunity (451,452). These are interesting findings suggesting that  
1691 PPAR $\alpha$  activation may result in different responses depending on the inflammatory  
1692 settings, an effect which may involve combinatorial signaling. Although the  
1693 mechanisms by which such potentially “pro-inflammatory” effects occur is not  
1694 understood, these data point to the need for a careful evaluation of the effects of  
1695 PPAR $\alpha$  agonists in the relevant cell types, and in different physiological contexts.  
1696 How to reconcile these seemingly contradictory findings with the wealth of data on  
1697 anti-inflammatory PPAR actions is as of yet unclear. For instance, reduced PPAR $\alpha$   
1698 expression has also been linked to a lower overall survival and an enhanced tissue  
1699 bacterial load in sepsis (453). The timing and nature of the pro-inflammatory stimulus  
1700 and/or treatment may play a decisive role. It may well be that PPAR $\alpha$  exerts a  
1701 context-dependent immune-system priming role, as has been documented recently  
1702 also for glucocorticoids (391). Given that GR and PPAR $\alpha$  are able to physically  
1703 interact and able to co-control genes involved in both inflammatory and metabolic  
1704 pathways (160,385), increases the likelihood of this assumption. More research is  
1705 clearly necessary to disentangle and explain these paradoxical observations. This

1706 hitherto hidden role of PPAR $\alpha$  not only merits further mechanistic attention but also  
1707 requires a better understanding at the level of physiological relevance.



## 1708 **VIII. Future perspectives**

1709 On account of their pleiotropic effects, PPARs may be targets in a number of disease  
1710 conditions. It is clear there is still a lot of ground to cover, foremost by studying novel  
1711 generations of more potent and better tolerated single, dual or pan PPAR agonists in  
1712 the clinic. More basic research involving novel PPAR $\alpha$  agonists is important, as many  
1713 mechanistic aspects differ from the first generation of PPAR $\alpha$  agonists. Plus, novel  
1714 fields of application may still arise, for which the first generation of drugs did not  
1715 show therapeutic benefit.

1716 Fibrates might be beneficial in the prevention of coronary heart disease. The highest  
1717 benefit is achieved in cardiovascular disease patients with high TG and low HDL-C  
1718 (454). A clinical benefit of PPAR $\alpha$  activation with novel agonists remains to be  
1719 demonstrated in patients with high TG/low HDL and residual cardiovascular risk.  
1720 Fibrates might be useful in the treatment and prevention of atherosclerosis, especially  
1721 the new fibrates with greater potency open new perspectives. However, it remains  
1722 unclear whether fibrates in general are useful in the treatment or prevention of heart  
1723 failure.

1724 It is now evident that the effects of PPAR $\alpha$  are much more widespread and cover  
1725 numerous aspects of inflammation and inflammatory related diseases, however many  
1726 mechanistic fundamental details of its function still remain to be unraveled. For  
1727 instance, nuclear receptor regulation of circadian rhythms in inflammatory metabolic  
1728 diseases deserves more clinical investigation. There is newfound appreciation for the  
1729 timing of treatment interventions. For example, a recent study shows a link between  
1730 the circadian clock and perioperative myocardial injury in patients undergoing aortic  
1731 valve replacement, whereby antagonism of Rev-erb $\alpha$ , another nuclear receptor family  
1732 member and PPAR $\alpha$  target gene (260) seems to provide a benefit for cardioprotection

1733 (455). Moreover, the circadian clock regulates the expression and activity of genes  
1734 and proteins involved in the progression of several autoimmune diseases (456,457).  
1735 Future therapeutics relying on PPARs in a context of inflammation will therefore  
1736 most probably have to take “the clock” into consideration.

1737 The role of PPAR $\alpha$  needs to be further evaluated in various pathophysiological  
1738 conditions. For instance, it is unclear how PPAR $\alpha$  may influence immune cell  
1739 (metabolic) control in immune/inflammatory diseases that are glucocorticoid resistant,  
1740 such as sepsis.

1741 Defining the molecular mechanisms of PPAR $\alpha$  function that govern inflammatory  
1742 programs will improve our understanding of its roles and will allow us to further  
1743 exploit their potential in the therapeutic field.

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3524 **Figure 1: Schematic presentation of the human PPAR $\alpha$  gene and protein domain**  
3525 **structure-function relationship.** (A) The human PPAR $\alpha$  gene contains at least 8  
3526 exons. Exons are shown in boxes with translated regions shaded. 5' UTR encoded by  
3527 exons 1, 2 and the 5'-end of exon 3. The coding DNA sequence spans the 3' region of  
3528 exon 3, exons 4–7 and the 5' extremity of exon 8. The 3' UTR consists of the last 232  
3529 base pairs of exon 8. The splicing events S<sub>wt</sub> and S<sub>tr</sub> generating hPPAR $\alpha$ <sub>wt</sub> and  
3530 hPPAR $\alpha$ <sub>tr</sub> transcripts respectively are depicted. The variant transcript lacks exon 6  
3531 resulting in a frame shift generating a premature TGA termination codon. The  
3532 corresponding protein products of the two splice isoforms are underlined. (B) PPAR $\alpha$   
3533 contains four distinct domains. The amino-terminal A/B domain contains the ligand-  
3534 independent activation function-1 (AF-1), the C domain consists of a highly  
3535 conserved DNA-binding domain (DBD), the D domain consists of a variable hinge  
3536 region and the C terminal E domain includes ligand-binding domain (LBD) and a  
3537 ligand-dependent activation function-2 (AF-2). Per domain, main functions are listed.  
3538 Hsp90, Heat shock protein 90; PPRE, PPAR response element; UTR, untranslated  
3539 region; wt, wild type; tr, truncated.

3540 **Figure 2: PPRE-dependent transcription regulation.** (A) PPRE-dependent  
3541 activation. In the absence of specific ligands, PPAR $\alpha$ /RXR heterodimers bind to the  
3542 PPRE located in the promoter of target genes, or elsewhere in the genome, and recruit  
3543 corepressor complexes thereby maintaining PPAR $\alpha$  target genes in a silent state  
3544 (ligand-independent repression). Upon ligand activation, the nuclear corepressors are  
3545 released and the coactivator complexes are then recruited and bound to PPAR $\alpha$ /RXR $\alpha$   
3546 heterodimers, hence inducing expression of target genes involved in several biological  
3547 processes including glucose and lipid metabolism. (B) PPRE-dependent inhibition of  
3548 NF- $\kappa$ B transcriptional activity. Upon ligand activation, PPRE-bound PPAR $\alpha$  directly

3549 interferes with the activity of p65 antagonizing thereby its binding to an NF- $\kappa$ B-RE in  
3550 the complement promoter. Sirt1; Silent information regulator 1; PPRE, PPAR-  
3551 response element; RXR, Retinoid X receptor; DR1, Direct repeat1; NF- $\kappa$ B-RE, NF-  
3552  $\kappa$ B response element.

3553 **Figure 3: Levels of modulating PPAR $\alpha$  activity.** PPAR $\alpha$  activity is controlled at  
3554 several levels including gene expression, protein translation, ligand selectivity,  
3555 posttranslational modifications affecting receptor stability and cofactor recruitment.  
3556 Ub, Ubiquitinated; S, SUMOylated; P, Phosphorylated; PPRE, PPAR response  
3557 element; GSK3, glycogen synthase kinase 3; AMPK, AMP kinase; PKC, protein  
3558 kinase A; PKA, protein kinase A; PIASy, Protein inhibitor of activated STATy; RXR,  
3559 retinoid X receptor.

3560 **Figure 4: Models of PPAR $\alpha$  transrepression activity.** (A) PPAR $\alpha$  directly interacts  
3561 with pro-inflammatory transcription factors such as NF- $\kappa$ B, AP-1 and STAT to  
3562 negatively regulate their targets leading to anti-inflammatory effects through a DNA-  
3563 independent mechanism. (B) Simultaneous ligand-activation of PPAR $\alpha$  and GR leads  
3564 to the enhanced repression of TNF-induced IL-6 transcriptional activity, likely via a  
3565 mechanism involving a direct physical interference between PPAR $\alpha$  and GR.

3566 Ub, Ubiquitinated; S, SUMOylated; P, Phosphorylated; PPRE, PPAR response  
3567 element; GSK3, glycogen synthase kinase 3; AMPK, AMP kinase; PKC, protein  
3568 kinase A; PKA, protein kinase A; PIASy, Protein inhibitor of activated STATy; RXR,  
3569 retinoid X receptor; C/EBP- $\beta$ , CAATT/enhancer-binding protein  $\beta$ ; STAT, signal  
3570 transducer and activator of transcription; AP-1, activator protein-1; NF- $\kappa$ B, nuclear  
3571 factor- $\kappa$ B.

3572 **Figure 5: Anti-inflammatory effects of PPAR $\alpha$  activation.** (A) Effect of human  
3573 PPAR $\alpha$  activation, by various synthetical ligands on inflammatory mediators. (B)



3574 Effect of rodent PPAR $\alpha$  on inflammatory mediators. Note that for some of these  
3575 effects, conclusions on an involvement of PPAR $\alpha$  were reached by using PPAR $\alpha$  -/  
3576 mice, in which an aggravated inflammatory effect was observed. hASMCs, human  
3577 aortic smooth muscle cells; hUVEC, human vascular endothelial cells; COX-2,  
3578 cyclooxygenase; PG, prostaglandin; VCAM, vascular cell adhesion molecule; MCP-  
3579 1, Monocyte chemotactic protein-1; VEGFR2, vascular endothelial growth factor  
3580 receptor-2; CRP, C-reactive protein; IFN- $\gamma$ , interferon- $\gamma$ ; APP, acute phase response  
3581 proteins; LPS, lipopolysaccharide; SAA, serum amyloid A; IL-1ra, IL-1 receptor  
3582 antagonist; LTB<sub>4</sub>, Leukotriene B<sub>4</sub>; iNOS, inducible nitric oxide synthase.

3583 ***Figure 6: Stimulatory effects of human and murine PPAR $\alpha$  activation on***  
3584 ***inflammatory mediators.*** Note here that mostly natural ligands seem able to trigger  
3585 PPAR $\alpha$ -dependent pro- inflammatory effects. MCP-1, Monocyte chemotactic protein-  
3586 1; LPS, lipopolysaccharide; 8(*S*)-HETE, 8(*S*)- hydroxyeicosatetraenoic acid; LTB<sub>4</sub>,  
3587 Leukotriene B<sub>4</sub>; NOS, nitric oxide synthase.

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<b>Table 1: PPAR<math>\alpha</math> functional domains</b>		
<i>Domain</i>	<i>Functions</i>	<i>Ref</i>
N-Terminal (A/B domain)	Transactivation function AF-1 Phosphorylation sites Subtype-selective target gene expression	(35,40) (41-43) (38,44)
DBD (C domain)	Zinc-fingers binding motifs Phosphorylation sites Recognition PPRE Dimerization	(45) (46) (47,48) (49)
Hinge (D domain)	Nuclear localization signals Docking for Coactivators Phosphorylation sites SUMOylation sites Interacts with HSP90	(33,50) (51) (46,52) (53) (54)
C-Terminal LDB (E/F domain)	Transactivation function AF-2 / Cofactor recruitments SUMOylation sites Dimerization Interacts with HSP90	(55) (56) (51,57) (54)

**Table 1: PPAR $\alpha$  functional domains and their relative functions.** PPAR $\alpha$  displays a classical nuclear receptor canonical structure. Per domain, main PPAR $\alpha$  functions are listed (details in the text). Hsp90, Heat shock protein 90; PPRE, PPAR response element.

<b>Table 2: Phosphorylation modifications affecting PPAR<math>\alpha</math> activity</b>				
<i>Kinase involved</i>	<i>Residues phosphorylated</i>	<i>Effect on transcriptional activity</i>	<i>Physiological outcome</i>	<i>Ref</i>
ERK1/2 MAPK	Ser12 and Ser21 in A/B domain in hPPAR $\alpha$	Increased ligand-dependent transactivation	Cross-talk between insulin signaling and PPAR $\alpha$ activity	(58,149)
P38 MAPK and JNK	Ser6, Ser12 and Ser21 in A/B domain in mPPAR $\alpha$	Increased ligand-dependent transactivation	Increased cardiac mitochondrial fatty acid $\beta$ -oxidation during periods of stress	(150)
ERK-MAPK	Ser6, Ser12 and Ser21 in A/B domain in mPPAR $\alpha$	Decreased transactivation	Down-regulation of PPAR $\alpha$ and its target genes during cardiac myocyte hypertrophic growth.	(151)
PKC	Ser179, Ser230 in D domain in hPPAR $\alpha$	Increased transactivation Decreased transrepression	Molecular switch dissociating the transactivation and transrepression functions of PPAR $\alpha$	(52)
PKA	Mainly C domain	Increased ligand-dependent transactivation	Enhanced $\beta$ -oxidation, production of glucose and ketone bodies under stress, fasting or exercise	(155)
AMPK	n.d.	Increased transactivation	Increased fatty acid oxidation and PPAR $\alpha$ gene expression in skeletal muscle	(113,156, 157)
GSK3	Predominantly at serine 73 in the A/B domain in rPPAR $\alpha$	Decreased PPAR $\alpha$ stability	Links GSK3 signaling and PPAR $\alpha$ activity to metabolic regulation	(42)

**Table 2: Phosphorylation modifications affecting PPAR $\alpha$  activity.** PPAR $\alpha$  undergoes several phosphorylation modifications that markedly impact receptor function (details in the text).

**Table 3: SUMOylation modifications affecting PPAR $\alpha$  activity**

<i>SUMO Ligase (E3) identified</i>	<i>Residues SUMOylated</i>	<i>Effect on transcriptional activity</i>	<i>Physiological outcome</i>	<i>Ref</i>
PIASy	SUMO-1, Lys358 in the LBD region in mPPAR $\alpha$	Transcriptional repression	Sex-specific gene repression and protective effect in estrogen-related liver diseases, including inflammation and cholestasis	(56)
	SUMO-1, Lys185 in the hinge region in hPPAR $\alpha$	Downregulation of transactivation	Decrease in NCoR-specific hPPAR $\alpha$ target gene expression	(53)

**Table 3: SUMOylation modifications affecting PPAR $\alpha$  activity.** PPAR $\alpha$  undergoes several SUMOylation modifications that markedly impact receptor function (details in the text).

**Table 4: PPAR $\alpha$  Natural and Synthetic Ligands**

<b>Natural Ligands</b>				
<i>Type</i>	<i>Ligand</i>		<i>Biological &amp; therapeutic outcome</i>	<i>Ref</i>
Fatty acids	Arachidonic Acid		PPAR $\alpha$ binding and activation.	(19,191)
Eicosanoids	8(S)-HETE		PPAR $\alpha$ -mediated transcription activation and induction of adipogenic differentiation of 3T3-L1 preadipocytes	(19,171)
	20-HETE		PPAR $\alpha$ target gene expression (APOA1) activation	(172)
	Leukotriene B4		LTB <sub>4</sub> breakdown stimulation which may contribute to the resolution of inflammation.	(19,165,174,175)
Phospholipids	Oxidized phospholipids		PPAR $\alpha$ activation in a phospholipase A2-dependent manner in endothelial cells.	(176)
	Phosphatidylcholine (16:0/18:1-GPC)		Interaction with PPAR $\alpha$ -LBD and coactivator peptide motifs. PPAR $\alpha$ -dependent gene induction and hepatic steatosis decreased.	(179)
Polyphenols	Resveratrol		PPAR $\alpha$ activation in cell-based reporter assays. Brain protection against ischemic stroke in mice.	(180-183)
<b>Synthetic Ligands</b>				
<i>Ligand</i>	<i>EC50</i>	<i>Status of Clinical development</i>	<i>Biological &amp; therapeutic outcome</i>	<i>Ref</i>
Clofibrate	50 (m)-55(h) $\mu$ M	In clinical use	Decreased the lipid levels in hypercholesterolemic patients, mainly as the results of a reduction in VLDL, and less in LDL fraction.	(192-194)
Fenofibrate	18(m)-30(h) $\mu$ M	In clinical use	Improved the plasma lipid profiles and lipoprotein profiles. Decreased atypical sphingolipids in plasma of dyslipidaemic patients.	(194-196)
Bezafibrate	20(m)-80(h) $\mu$ M	In clinical use	Decreased plasma TG levels and increased HDL-C levels in dyslipidaemic and diabetic patients.	(197)
Gemfibrozil	61(h) $\mu$ M	In clinical use	Increased HDL-C levels and lowered TG levels. Coronary heart disease and stroke prevention.	(196,198,199)

WY14643	0.63(m)-5(h) $\mu$ M	Used in basic research only	Agonist activity in functional assays. Inhibition of NF- $\kappa$ B transcriptional activity and decreased inflammatory responses <i>in vitro</i> and <i>in vivo</i> .	(19,23,191,192,194)
GW9578	0.005(m)-0.05(h) $\mu$ M	Used in basic research only	Potent lipid lowering Activity. Decreased LDL-C in Sprague-Dawley rats. Improved insulin sensitivity in obese Zucker rats.	(194,200,201)
GW7647	0.001(m)-0.006(h) $\mu$ M	Used in basic research only	Agonist activity in GAL4-PPAR binding assay. Decreased TGs in fat-fed hamsters and rats.	(202)
Pemafibrate (K-877)	1 nM (h)	Phase 3 trials ongoing for treatment of dyslipidaemia.	Improved lipid profile in patients with dyslipidaemia. Enhances RCT and decreased inflammation and atherosclerosis.	(188,190,203)
<i>Dual PPAR<math>\alpha</math>/<math>\beta</math>(<math>\delta</math>) agonists</i>				
Elafibranor (GFT505)	10-20 nM	Phase 3 trial ongoing for treatment of NASH	Improved hepatic steatosis, inflammation and fibrosis in NASH rodent models and in patients with NASH. Improved lipid profile and insulin sensitivity in patients with dyslipidaemia or prediabetes and in obese individuals.	(189,204-206)

**Table 4: The natural and synthetic PPAR $\alpha$  ligands and their physiological roles.**

(h), EC50 for human PPAR $\alpha$  ; (m), EC50 for murine PPAR $\alpha$

**Table 5: List of selected PPAR $\alpha$  target genes**

Target gene	Official gene symbol	Gene functions	Comments	Fibrates		Ref
				WT	KO	
<b>LIVER</b>						
FA Transport Protein (FATP)	SLC27A1	Facilitates the uptake of long chain fatty acids by the liver Fatty acid transport	Direct PPAR $\alpha$ target with a functional PPRE in its promoter.	↑	=	(268-270)
Fatty acid translocase (FAT/CD36)	CD36	Uptake of long-chain fatty acids and oxidized LDL	Transcriptional mechanism remains unclear. Induction goes through the distal promoter region of the gene even though no PPRE could be identified.	↑	=	(270-272)
Acyl-coA synthetase	ACS	Catalyzes fatty acid metabolism by converting inactive fatty acids into active acyl CoA derivatives	Direct PPAR $\alpha$ target with a functional PPRE in its promoter.	↑	=	(208,223,269, 273,274)
Fatty acid binding protein	FABP	Intracellular lipid trafficking	Functional PPRE was identified. FABP2 and FABP1 have different effects on the ability of different agonists to promote PPAR $\alpha$ activation ligand-dependently.	↑	=	(275-277)
Acyl-CoA binding protein (ACBP)	DBI	Fatty acyl CoA esters transport	Functional PPRE was identified. ACBP expression is regulated by fasting/refeeding in rodents. A dual regulation by PPAR $\alpha$ and SREBP-1c might be considered.	↑	=	(278,279)
Carnitine palmitoyl-transferase 1	CPT1A	Catalyzes the transfer of a long-chain fatty acyl group from coenzyme A to carnitine.	Functional PPRE was identified.	↑	=	(280)
Carnitine palmitoyl-transferase 2	CPT2	Conjugates the fatty acid <i>back</i> to Coenzyme A for subsequent $\beta$ -oxidation.	Functional PPRE was identified.	↑	=	(208,281)
Medium-chain acyl-CoA dehydrogenase (MCAD)	ACADM	Mitochondrial fatty acid $\beta$ -oxidation	Functional PPRE was identified.	↑	=	(210,282-284)

Long-chain acyl-CoA dehydrogenase (LCAD)	ACADL	Mitochondrial fatty acid $\beta$ -oxidation	No PPRE / unknown mechanism.	↑	=	(208,285)
Very long chain acyl-CoA dehydrogenase (VLCAD)	ACADVL	Mitochondrial fatty acid $\beta$ -oxidation	No PPRE reported	↑	=	(208,286,287)
Dodecenoyl-CoA $\delta$ -isomerase	ECI1	Mitochondrial $\beta$ -oxidation of unsaturated and saturated fatty acids	Using microarray, expression of ECI1 was found to be decreased in PPAR $\alpha$ -deficient mice but no PPRE has yet been identified.	↑	?	(288,289)
Acyl-CoA oxidase	ACOX	Peroxisomal $\beta$ -oxidation	Functional PPRE was identified. The fasting-induced activation of ACOX expression was markedly blunted in PPAR $\alpha$ deficient mice.	↑	=	(71,165,210,288,290)
Bifunctional enzyme (BIEN)	EHHADH	Peroxisomal $\beta$ -oxidation	Three different PPRES have been identified in the promoter sequence of the BIEN gene, yet only one allows for efficient transactivation of the BIEN gene by PPAR $\alpha$ .	↑	=	(276,288,290,291)
Peroxisomal 3-ketoacyl-CoA thiolase.	ACAA1	Peroxisomal $\beta$ -oxidation	It is unclear whether the DR-1 sequence identified within its promoter mediates PPAR $\alpha$ transactivation. Differential gene regulation in human vs rodent hepatocytes.	↑	=	(71,291-293)
Peroxisomal membrane protein 11A PEX11 $\alpha$	PEX11A	Peroxisome proliferation	A functional PPRES 8.4 kb downstream of the PEX11 $\alpha$ was identified by a gene transfection study.	↑	?	(294-296)
Cytochrome P450 4A	CYP4A	Microsomal FA $\omega$ -hydroxylation	In rodents, CYP4A1 upregulation through a functional PPRES. In humans, no PPRES detected in the 5'-flanking region of the CYP4A11 gene. Induction upon fasting or fenofibrate	↑	=	(288,297-299)



			treatment in transgenic mice.			
Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mHMG-CoAS)	HMGCS2	Ketogenesis/condensation of acetyl-CoA and acetoacetyl-CoA to generate HMG-CoA	Functional PPRE identified in the rat and pig mHMG-CoAS gene. mHMG-CoAS proteins interact physically with PPAR $\alpha$ and act as a coactivator to up-regulate transcription from the PPRE of its own gene.	↑	=	(215,288)
Fibroblast growth factor 21	FGF21	Metabolic fuel homeostasis during ketosis	Functional PPRE identified. Induction upon fasting and PPAR $\alpha$ agonists treatment.	↑	=	(217,300,301)
FA desaturase 2 (Fads2)	FADS	Lipogenesis	DR-1 that functions in the regulation of human FADS2 gene transcription identified. An indirect regulatory mechanism involving SREBP-1c might be considered.	↑	=	(220,302-306)
Stearoyl-CoA desaturase (Scd1)	SCD	Lipogenesis	Functional PPRE identified. An indirect regulatory mechanism involving SREBP-1c might be considered.	↑	=	(302-304)
Malic enzyme (Mod1)	ME1	Lipogenesis	Functional PPRE identified. Decrease of ME1 expression in livers of PPAR $\alpha$ deficient mice.	↑	=	(272,288,307)
Phosphatidate phosphatase (Lpin2)	LPIN2	Lipogenesis	Dual target of PPAR $\alpha$ and PPAR $\beta/\delta$ . Induction by fasting and dietary FA maintained in PPAR $\alpha$ deficient mice.	↑	=	(232,308)
Acetyl-CoA carboxylase (ACC)	ACACA	Lipogenesis	PPAR $\alpha$ might indirectly influence ACC and FAS expression by controlling metabolic processes. In human primary	↑	?	(220-222)

Fatty acid synthase (FAS)	FASN	Lipogenesis	hepatocytes, PPAR $\alpha$ agonists, cooperatively with insulin and LXR agonists, induce FAS and ACC.	↑	?	
Liver X receptor alpha (LXR $\alpha$ )	NR1H3	Cholesterol metabolism	Induction in macrophages upon PPAR $\alpha$ treatment Decreased in livers upon fasting in PPAR $\alpha$ deficient mice.	↑	?	(309,310)
Cholesterol 7 $\alpha$ -hydroxylase (Cyp7a1)	CYP7A	Bile acid metabolism	Functional PPRE was identified. Murine Cyp7A1 species, harbors an additional PPRE. Indirect mechanism involving HNF4 $\alpha$ transactivation activity was also reported.	#	?	(228,231,311,312)
Cholesterol 27 $\alpha$ -hydroxylase (Cyp27a1)	CYP27A1	Bile acid metabolism	PPAR $\alpha$ affects the stability of Cyp27a1 mRNA. In rodents, fibrates suppress bile acid biosynthesis via PPAR $\alpha$ -mediated downregulation of Cyp27a.	↓	=	(231,310)
Sterol-12 $\alpha$ -hydroxylase	CYP8B1	Bile acid metabolism	Functional PPRE identified.	↑	=	(229)
Phosphoenolpyruvate carboxykinase (Pck1)	PKC1	Gluconeogenesis	Elusive regulation mechanism, although a functional PPRE was shown to be functional in adipocytes. PPAR $\alpha$ -mediated Pck1 regulation only observed in human hepatocytes. Induction during fasting reported.	?	?	(232,233,313)
Glycerol-3-phosphate dehydrogenase (GPDH)	GDP	Metabolic conversion of glycerol into glucose	PPAR $\alpha$ directly governs glycerol metabolic by directly upregulating expression of these genes. Upregulation by fasting only in wild-type mice.	↑	=	(233)
Glycerol kinase,	GK					
Glycerol transporters aquaporins 3&9	AQA3&9					
Pyruvate dehydrogenase kinase isoform 4 (Pdk4)	PDK4	Glucose oxidation	No PPRE yet identified. In wild type PPAR $\alpha$ mice, PDK4 expression	?	?	(314,315)

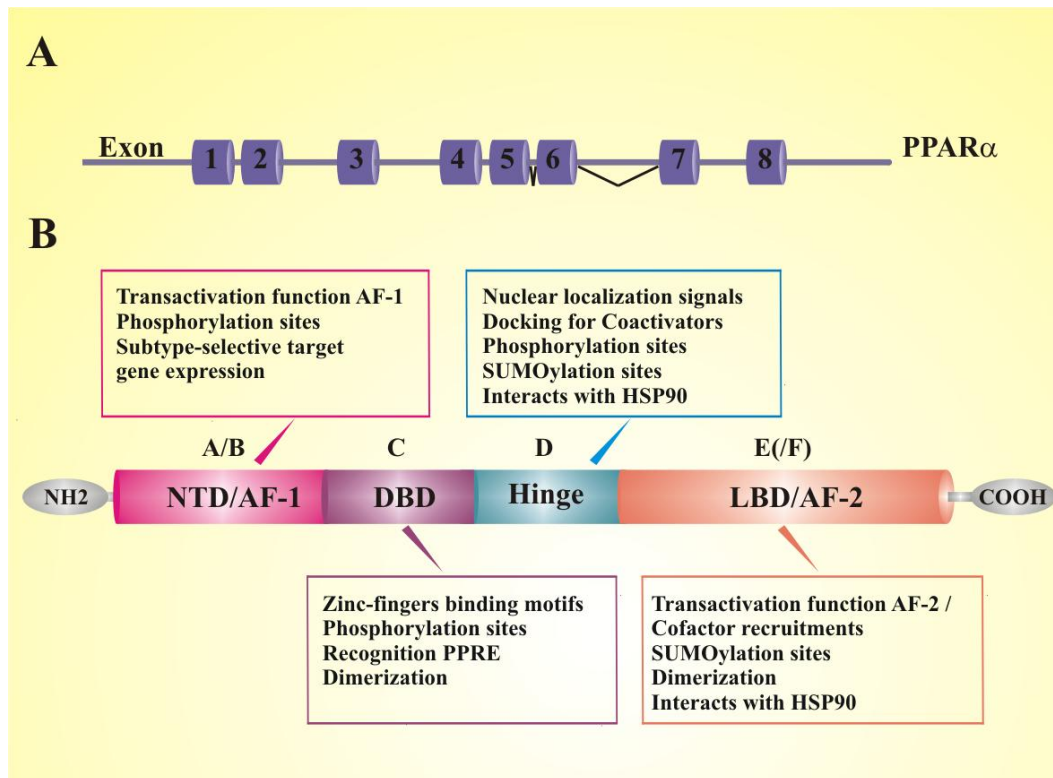
			increased in response to fasting with suppression by refeeding.			
Glycogen synthase 2 (Gys-2)	GYS2	Glycogen synthesis	Functional PPRE identified. Regulation mechanism involving competition between HNF4 $\alpha$ and PPAR $\alpha$ reported. Gys-2 mRNA reduction is PPAR $\alpha$ deficient mice and in fasted and refed state	↑	=	(238)
Lipoprotein lipase	LPL	Hydrolysis of TGs	Functional PPRE identified	↑	=	(48,249,270,289)
Angiopoietin-like protein 4	ANGPTL4	Inhibitor of LPL activity	Increased upon fasting.	↑	=	(256,316)
Rev-erba	NR1D1	Repressor of gene transcription	Functional PPRE identified. Negative interference with the activation of APOC3 gene promoter by HNF4	↑	?	(47,249,260)
Apolipoprotein AI	APOA1	Plasma HDL Metabolism	In humans, a functional PPRE was identified. In rodents, an indirect mechanism involving the PPAR $\alpha$ -dependent induction of the Rev-erba was reported.	↓	=	(259,260,317)
Apolipoprotein AII	APOA2	Plasma HDL Metabolism	Functional PPRE identified. Induced APOA2 plasma levels and hepatic mRNA expression in human. Both a marginal reduction and induction upon fibrate treatment observed in rodents.	↑	=	(258,318-320)
Apolipoprotein A-V	APOA5	Plasma TG Metabolism	Functional PPRE identified.	↑	?	(254,255)
Apolipoprotein C-III	APOC3	Plasma HDL Metabolism	Transcriptional suppression by fibrates involves HNF4.	↓	=	(250,253,321)

## Heart

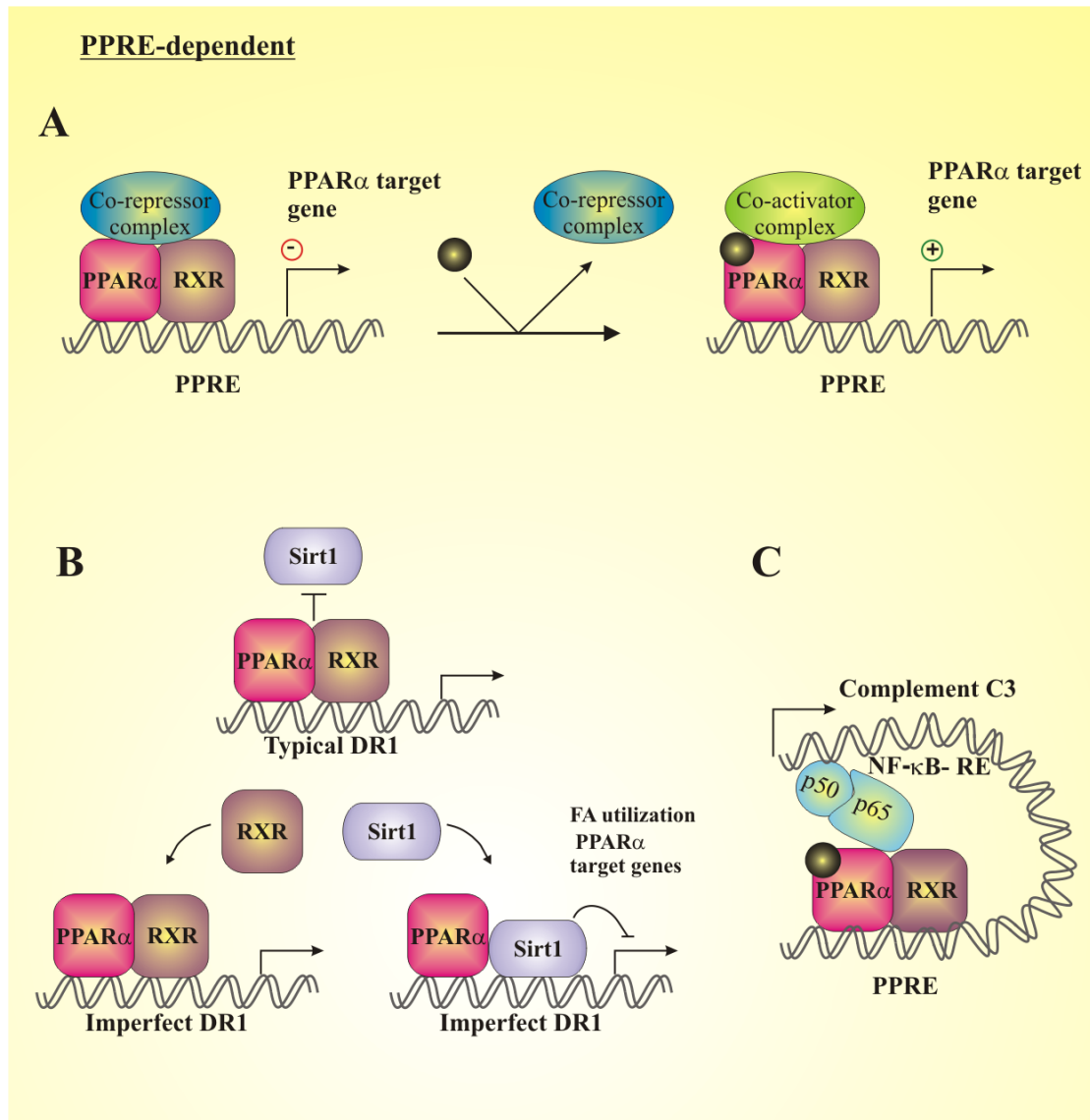
Acyl-CoA oxidase	ACOX	Peroxisomal $\beta$ -oxidation	Reduced fasting-induced ACOX expression in PPAR $\alpha$ -deficient mice.	?	?	(71,210)
Carnitine palmitoyl-transferase 1	CPT1	Fatty acid transport/oxidation	Functional PPRE identified. Reduced fasting-induced CPT1 expression in PPAR $\alpha$ deficient mice.	↑	?	(210,322,323)
Carnitine palmitoyl-transferase 2 (CPT2)	CPT2	Fatty acid oxidation	Functional PPRE identified.	?	?	(281,324)
Long-chain acyl-CoA synthetase (LCAS)	ACADL	Fatty acid activation	Functional PPRE identified.	?	?	(273,324)
Medium-chain acyl-CoA dehydrogenase (MCAD)	ACADM	Mitochondrial fatty acid $\beta$ -oxidation	The fasting-induced activation ACADM expression was abolished in PPAR $\alpha$ deficient mice.	?	?	(210,283,324)
<b>Muscle</b>						
Carnitine palmitoyl-transferase 1	CPT1	Fatty acid transport/oxidation	Functional PPRE identified	↑	?	(323,325)
Pyruvate dehydrogenase kinase isoform 4 (Pdk4) (soleus)	PDK4	Glucose oxidation	No PPRE has yet been identified	↑	?	(323,326)
<b>White adipose tissue</b>						
Fatty acid translocase (FAT/CD36)	CD36	Uptake of long-chain fatty acids and oxidized LDL	Transcriptional mechanism remains unclear.	↑	↑	(327)
Acyl-CoA oxidase	ACOX	Peroxisomal $\beta$ -oxidation	Functional PPRE identified.	↑	↑	(71,327)
Lipoprotein lipase	LPL	Hydrolysis of TGs	Functional PPRE identified. Basal levels of LPL mRNA were higher in PPAR $\alpha$ deficient mice as compared to WT mice.	↑	=	(249,327)
Glycogen synthase 2 (Gys-2)	GYS2	Glycogen synthesis	Gys-2 mRNA is reduced in PPAR $\alpha$ deficient mice.	?	?	(238)
<b>Small intestine</b>						
Pyruvate dehydrogenase kinase isoform 4 (Pdk4)	PDK4	Glucose oxidation	Intestine-specific regulation of PDK gene transcription by LXR.	?	?	(328)
Carnitine palmitoyl-transferase 1	CPT1	FA transport/oxidation	Intestine-specific regulation of CPT1 gene transcription	?	?	(328)

			by LXR.			
Apolipoprotein C-III)	APOC3	Plasma HDL Metabolism	PPAR $\alpha$ -dependent gene repression by fibrates <i>in vivo</i> .	↓	?	(251,327)
Fatty acid binding protein	FABP	Intracellular lipid trafficking	Reduced FABP mRNA in PPAR $\alpha$ deficient mice.	↑	?	(275,327)

**Table 5: Selected PPAR $\alpha$  target gene.** PPAR $\alpha$  regulates the expression of its target genes involved in different biological process via distinct mechanisms (more details for some examples are discussed in the text). ↑, represents an increase upon fibrate treatment; ↓, represents a decrease upon fibrate treatment; =, represents no change upon fibrate treatment; ?, no information available on a change; #, represents conflicting results; WT, wild-type mouse; KO, knockout PPAR $\alpha$  mouse.



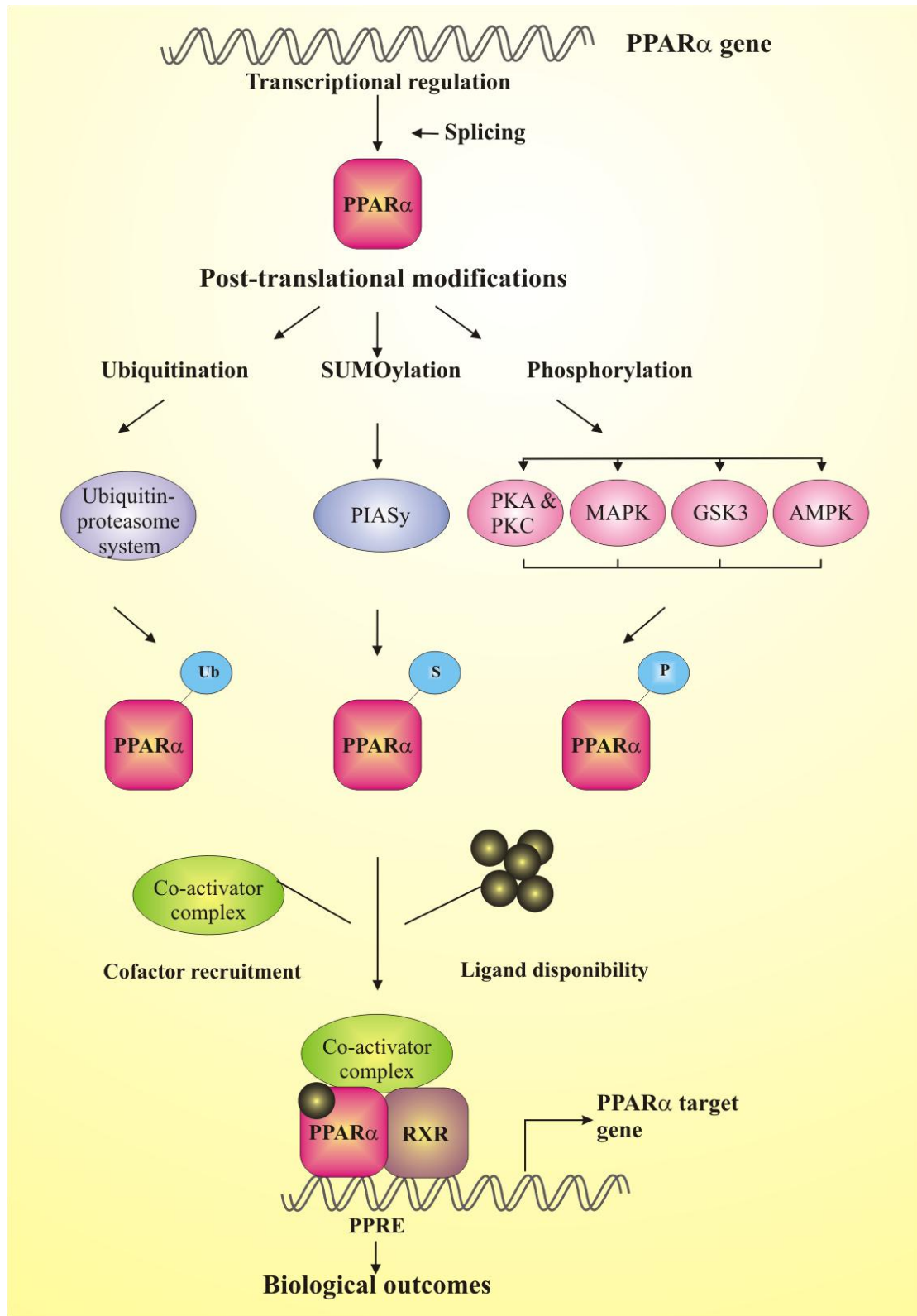
**Figure 1: Schematic of PPAR $\alpha$  gene and protein domain structure-function relationship.** (A) Schematic of the PPAR $\alpha$  gene showing 8 exons. (B) PPAR $\alpha$  contains four distinct domains. The amino-terminal A/B domain contains the ligand-independent activation function-1 (AF-1), the C domain consists of a highly conserved DNA-binding domain (DBD), the D domain consists of a variable hinge region and the C terminal E domain includes ligand-binding domain (LBD) and a ligand-dependent activation function-2 (AF-2). Per domain, main functions are listed. Hsp90, Heat shock protein 90; PPRE, PPAR response element.



**Figure 2: PPRE-dependent transcription regulation.** (A) PPRE-dependent activation. In the absence of specific ligands, PPAR $\alpha$ /RXR heterodimers bind to the PPRE located in the promoter of target genes, or elsewhere in the genome, and recruit corepressor complexes thereby maintaining PPAR $\alpha$  target genes in a silent state (ligand-independent repression). Upon ligand activation, the nuclear corepressors are released and the coactivator complexes are then recruited and bound to PPAR $\alpha$ /RXR $\alpha$  heterodimers, hence inducing expression of target genes involved in several biological processes including glucose and lipid metabolism. (B) The typical DR1 sequence favors PPAR $\alpha$ /RXR $\alpha$  heterodimerization, whereas the switch from PPAR $\alpha$ /RXR to

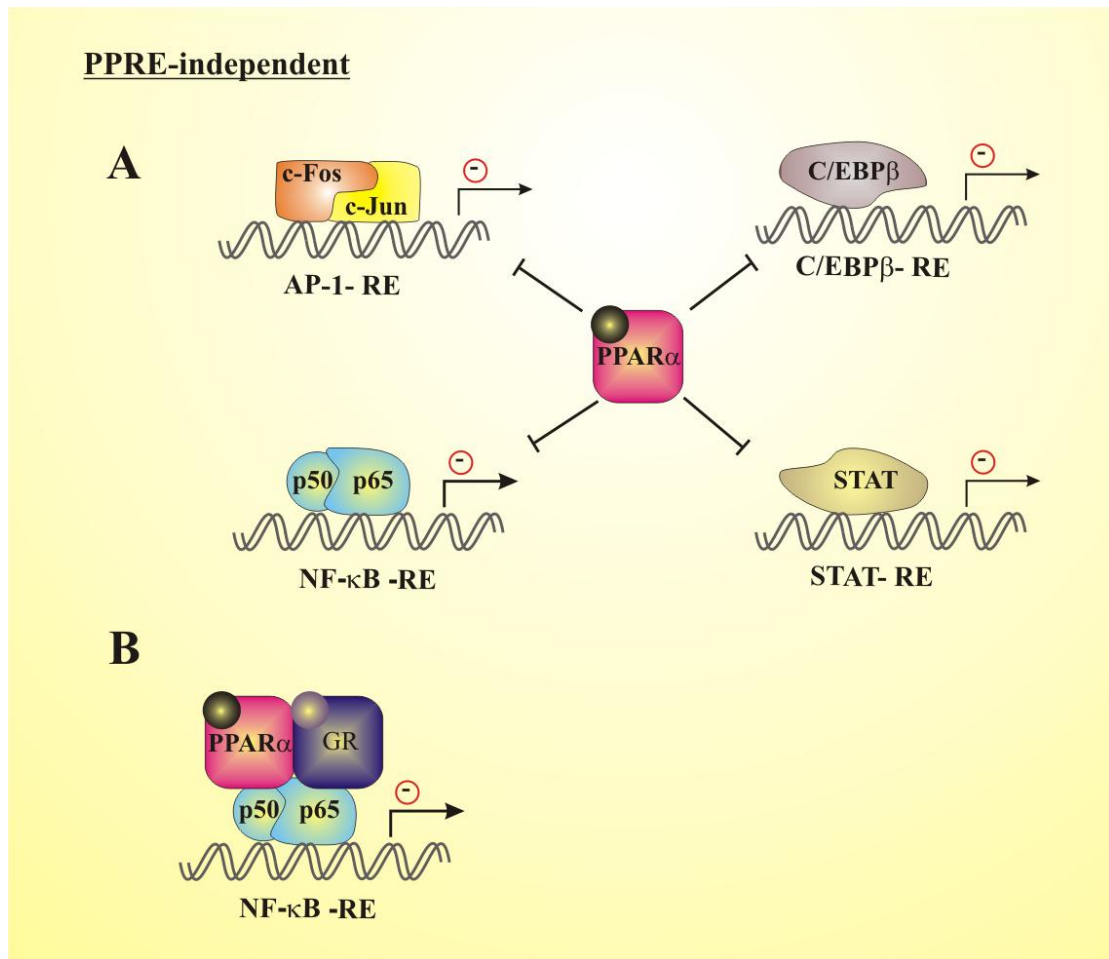
PPAR $\alpha$ /Sirt1 takes place on imperfect DR1s, thereby causing downregulation of many known PPAR $\alpha$  target genes mediating FA utilization (56). (C) PPRE-dependent inhibition of NF- $\kappa$ B transcriptional activity. Upon ligand activation, PPRE-bound PPAR $\alpha$  directly interferes with the activity of p65 antagonizing thereby its binding to an NF- $\kappa$ B-RE in the complement promoter (56). Abbreviations used in the figure: Sirt1; Silent information regulator 1; PPRE, PPAR-response element; RXR, Retinoid X receptor; DR1, Direct repeat1; NF- $\kappa$ B-RE, NF- $\kappa$ B response element.





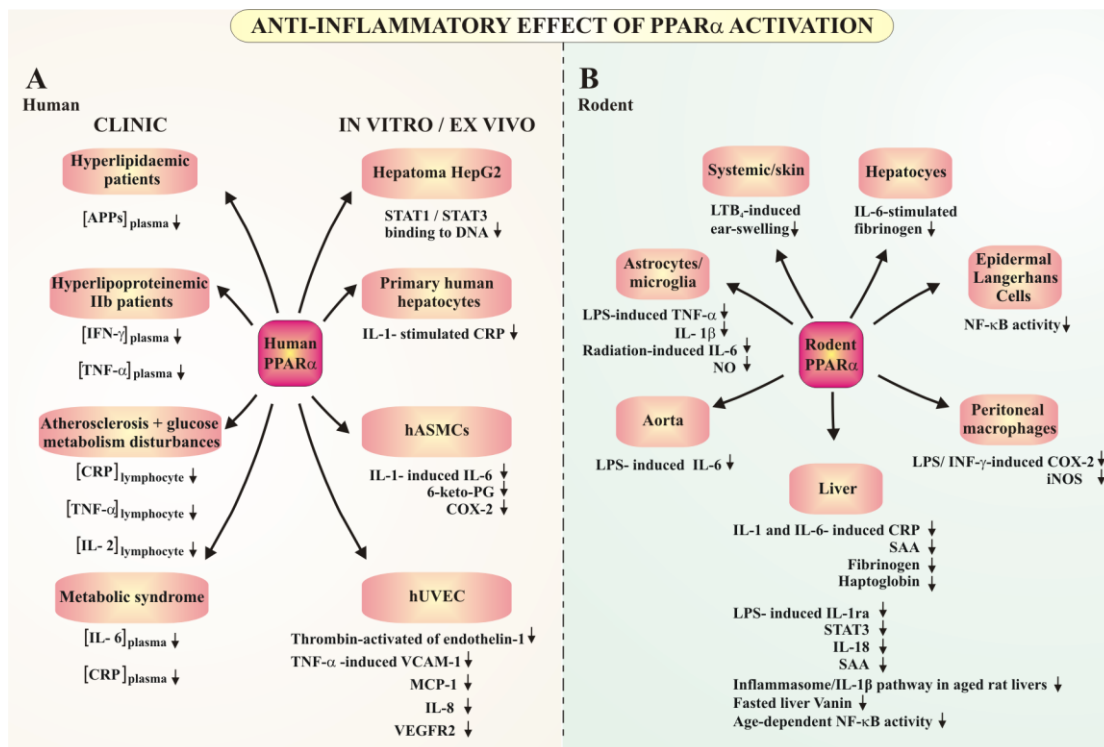
**Figure 3: Levels of modulating PPAR $\alpha$  activity.** PPAR $\alpha$  activity is controlled at several levels including gene expression, protein translation, ligand selectivity, posttranslational modifications affecting receptor stability and cofactor recruitment. Abbreviations used in the figure; Ub, Ubiquitinated; S, SUMOylated; P, Phosphorylated; PPRE, PPAR response element; GSK3, glycogen synthase kinase 3;

AMPK, adenosine monophosphate -activated protein kinase; PKC, protein kinase C;  
PKA, protein kinase A; PIASy, Protein inhibitor of activated STATy; RXR, retinoid  
X receptor.

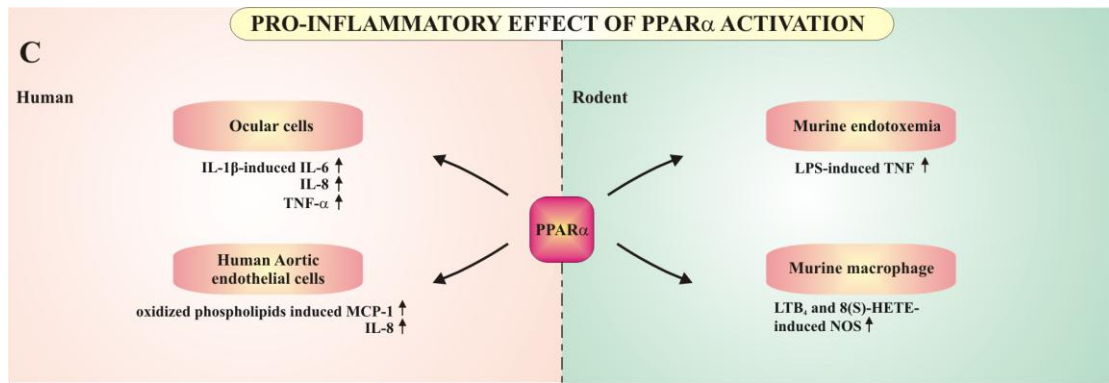


**Figure 4: Models of PPAR $\alpha$  transrepression activity.** (A) PPAR $\alpha$  directly interacts with pro-inflammatory transcription factors such as NF- $\kappa$ B, AP-1 and STAT to negatively regulate their targets leading to anti-inflammatory effects through a DNA-independent mechanism. (B) Simultaneous ligand-activation of PPAR $\alpha$  and GR leads to the enhanced repression of TNF-induced IL-6 transcriptional activity, likely via a mechanism involving a direct physical interference between PPAR $\alpha$  and GR.

Abbreviations used in the figure; Ub, Ubiquitinated; S, SUMOylated; P, Phosphorylated; PPRE, PPAR response element; GSK3, glycogen synthase kinase 3; AMPK, adenosine monophosphate -activated protein kinase; PKC, protein kinase C; PKA, protein kinase A; PIASy, Protein inhibitor of activated STATy; RXR, retinoid X receptor; C/EBP- $\beta$ , CAATT/enhancer-binding protein  $\beta$ ; STAT, signal transducer and activator of transcription; AP-1, activator protein-1; NF- $\kappa$ B, nuclear factor- $\kappa$ B.



**Figure 5: Anti-inflammatory effects of PPAR $\alpha$  activation.** (A) Effect of human PPAR $\alpha$  activation, by various synthetical ligands on inflammatory mediators. (B) Effect of rodent PPAR $\alpha$  on inflammatory mediators. Note that for some of these effects, conclusions on an involvement of PPAR $\alpha$  were reached by using PPAR $\alpha$   $-/-$  mice, in which an aggravated inflammatory effect was observed. Abbreviations used in the figure; hASMCs, human aortic smooth muscle cells; hUVEC, human vascular endothelial cells; COX-2, cyclooxygenase; PG, prostaglandin; VCAM, vascular cell adhesion molecule; MCP-1, Monocyte chemotactic protein-1; VEGFR2, vascular endothelial growth factor receptor-2; CRP, C-reactive protein; IFN- $\gamma$ , interferon- $\gamma$ ; APP, acute phase response proteins; LPS, lipopolysaccharide; SAA, serum amyloid A; IL-1ra, IL-1 receptor antagonist; LTB<sub>4</sub>, Leukotriene B<sub>4</sub>; iNOS, inducible nitric oxide synthase.



**Figure 6: Stimulatory effects of human and murine PPAR $\alpha$  activation on inflammatory mediators.** Note here that mostly natural ligands are able to trigger PPAR $\alpha$ -dependent pro-inflammatory effects. Abbreviations used in the figure; MCP-1, Monocyte chemotactic protein-1; LPS, lipopolysaccharide; 8(S)-HETE, 8(S)-hydroxyeicosatetraenoic acid; LTB<sub>4</sub>, Leukotriene B<sub>4</sub>; NOS, nitric oxide synthase.