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## **Control of adipogenesis by oxylipins, GPCRs and PPARs**

Valentin Barquissau<sup>1,2</sup>, Rayane AGhandour<sup>3</sup>, Gérard Ailhaud<sup>3</sup>, Martin Klingenspor<sup>4</sup>,  
Dominique Langin<sup>1,2,5</sup>, Ez-Zoubir Amri<sup>3</sup> and Didier F Pisani<sup>3\*</sup>

<sup>1</sup>Inserm, UMR1048, Obesity Research Laboratory, Institute of Metabolic and Cardiovascular Diseases, Toulouse, 31432, France

<sup>2</sup>University of Toulouse, UMR1048, Paul Sabatier University, Toulouse, 31432, France

<sup>3</sup>Université Côte d'Azur, CNRS, Inserm, iBV, France

<sup>4</sup>Technische Universität München, Chair of Molecular Nutritional Medicine, Else Kröner-Fresenius Center, 85350 Freising-Weihenstephan, Germany

<sup>5</sup>Toulouse University Hospitals, Department of Clinical Biochemistry, Toulouse, 31059, France

\*Corresponding author: Dr. Didier F Pisani, iBV; Université de Nice Sophia-Antipolis, UMR7277 CNRS - UMR1091 INSERM; Faculté de Médecine; 28 Avenue de Valombrose 06107 Nice cedex 2, France; Tel: +33 493 37 77 07; e-mail: [pisani@unice.fr](mailto:pisani@unice.fr)

**Abstract.** Oxylipins are bioactive metabolites derived from the oxygenation of  $\omega$ 3 and  $\omega$ 6 polyunsaturated fatty acids, triggered essentially by cyclooxygenase and lipoxygenase activities. Oxylipins are involved in the development and function of adipose tissue and their productions are strictly related to diet quality and quantity. Oxylipins signal *via* cell surface membrane (G Protein-coupled receptors) and nuclear receptors (peroxisome proliferator-activated receptors), two pathways playing a pivotal role in adipocyte biology. In this review, we made an attempt to cover the available knowledge about synthesis and molecular function of oxylipins known to modulate adipogenesis, adipocyte function and phenotype conversion, with a focus on their interaction with peroxisome proliferator-activated nuclear receptor family.

**Key words.** Eicosanoid; prostanoid; adipocyte; PPAR $\alpha$ ; PPAR $\beta/\delta$ ; PPAR $\gamma$ .

**Abbreviations.** PUFA, polyunsaturated fatty acids; LA, linoleic acid; ARA, arachidonic acid; LNA,  $\alpha$ -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DGLA, dihomo- $\gamma$ -linolenic acid; COX, cyclooxygenase; LOX, lipoxygenase; PG, prostaglandin; TX, thromboxane; LT, leukotriens; Rv, resolvin; WAT, white adipose tissue; BAT, brown adipose tissue; GPCR, G protein-coupled receptor; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; CRE, CREB response element; UCP, uncoupling protein.

## 1. Introduction: Metabolism, fatty acids and adipose tissue development.

Unbalanced energy homeostasis in mammals is generally due to reduction in energy expenditure aggravated by fat and carbohydrate overconsumption. High-fat diets are considered to be obesogenic as they produce a consistent increase in white fat mass that is related to the quality, quantity and the duration of the diet. However, the contribution of dietary fat quantity in increasing body weight remains controversial, as no clear correlation between this increase and the amount of ingested fat has been reported [1, 2]. This raises the possibility of a qualitative issue which is observed regarding the composition of dietary lipids at the disposal of world-wide populations, in particular those of industrialized countries. More specifically, dietary changes in the proportions of polyunsaturated fatty acids (PUFAs) of n-6 ( $\omega$ 6) (linoleic acid, LA: 18:2 n-6; arachidonic acid, ARA: 20:4 n-6) and n-3 ( $\omega$ 3) series ( $\alpha$ -linolenic acid, LNA: 18:3 n-3; eicosapentaenoic acid, EPA: 20:5 n-3; docosahexaenoic acid, DHA: 22:6 n-3) led to substantial increases in the  $\omega$ 6/ $\omega$ 3 PUFA ratio. PUFAs are “essential” fatty acids as they are required for normal development and their endogenous synthesis is extremely limited in humans [3]. In adult, both a high intake of  $\omega$ 6 LA and diets associated with a very high  $\omega$ 6/ $\omega$ 3 ratios have been associated with the development of several diseases, including cardiovascular, inflammatory and autoimmune diseases, as well as cancer [4-6]. Studies have shown that mice exposed to diets with high levels of  $\omega$ 6 PUFA, during the perinatal period, displayed a progressive accumulation of body fat across generations [7]. This observation is in agreement with human situation as overweight and obesity increased within a given population during the last decades, and emerged earlier in life [7]. At the same time, several studies demonstrated the pivotal role of PUFA metabolites, especially ARA-derived metabolites, in fat mass increase through combination of adipocyte hyperplasia and hypertrophy of adipose tissue, as well as in decrease of energy expenditure due to inhibition of thermogenic adipocyte activity [8-10]. In addition, a meta-analysis showed that a decrease in  $\omega$ 3 PUFA intake can be considered as risk factor for several pathologies in humans [11]. Altogether, these data demonstrated the crucial function of PUFA metabolites in all stages of life, from development to adult life.

## 2. Origin of oxylipins.

Fatty acids are key components of cell membranes as complex lipids, and of stored energy under the form of triglycerides. Lipase activities enable the release of stored PUFAs which are prone to undergoing oxidation reactions through both enzymatic and non-enzymatic activities to form oxygenated derivatives. Due to their potency and short half-life, these derivatives, termed oxylipins, are not stored but synthesized *de novo* in a tightly regulated and dynamic manner [12], keeping low the level of free PUFAs [13]. Synthesis of oxylipins takes place from developmental stages to adult life. In mammals, oxylipins can be divided into two main categories, the eicosanoids (synthesized from C20 PUFAs, *i.e.* ARA, EPA and dihomono- $\gamma$ -linolenic acid, DGLA) and the docosanoids (synthesized from C22 PUFAs, *i.e.* DHA). A third class of metabolites are derived directly from linoleic acid and composed of two members, 9- and 13-hydroxyoctadecadienoic acids (HODEs). Oxylipins, which differ by their length and double bond configuration, are synthesized by several alternative and consecutive reactions and display crucial signaling functions. The diversity of their functions is directly related to their structural diversity (review in [14-16]).

The two major enzymatic pathways of oxylipin precursors are triggered by cyclooxygenases (COX-1 and COX-2) and lipoxygenases (3-, 5-, 12- and 15-LOX). Depending on the PUFA and the pathway involved, they generate prostanoids (*i.e.* prostaglandins, PGs; thromboxanes, TXs; prostacyclin, PGI<sub>2</sub>), leukotrienes (LTs) or resolvins (Rvs). There is a third pathway involving the enzyme cytochrome P450 epoxygenase which generates epoxides (*i.e.* epoxyeicosatrienoic acids, EETs) and hydroperoxydes metabolites (*i.e.* hydroperoxyeicosatrienoic acids, HPETEs).

Prostanoids are oxygenated derivatives of ARA, EPA and DGLA synthesized upon COX activities which catalyze the formation of a cyclopentane ring. COX-1 is permanently activated and contributes to the maintenance of physiological functions by maintaining a basal synthesis of PGs [17]. COX-2 has been identified as inducible form, especially in response to inflammatory situation [18]. COX-1 and -2 metabolize ARA, DGLA and EPA into PGH<sub>2</sub>, PGH<sub>1</sub> and PGH<sub>3</sub>, respectively. These intermediate

metabolites act as a core from which diverge different enzymatic pathways leading to the synthesis of the various prostanoids (PGs, PGI<sub>2</sub> and TXs).

LTs, another eicosanoid member of the family, derive from ARA or EPA by the action of LOXs. These enzymes introduce a hydroperoxide function on positions 3, 5, 12 or 15 of the PUFAs. Their derivatives, obtained by the activity of one or several LOXs activities, include LTA, cysteinyl leukotrienes LTB, LTC and LTD, as well as lipoxins (LxA and LxB), oxo-ETE, hepoxilins (HxA and HxB) and RvE series, these latter compounds arise from EPA only. Finally, DHA is also converted by LOXs which leads to the formation of several docosanoids such as RvD, neuroprotectins and maresins [19].

Oxylipins can activate soluble intracellular receptors or diffuse out of the cell through the plasma membrane and signal through membrane receptors in a paracrine or autocrine manner [20, 21].

### **3. PPAR family: molecular view.**

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the ligand-activated nuclear hormone receptor superfamily. PPAR $\alpha$  (NR1C1) was the first subtype to be identified in the early 1990s when its ligands were found to induce peroxisome proliferation [22]. So far, two additional PPAR subtypes, PPAR $\beta/\delta$  (NR1C3) and PPAR $\gamma$  (NR1C2), have been discovered. At the organism level, PPARs have a wide distribution but display tissue isotype specificity [23, 24]. PPAR $\alpha$  is mainly expressed in tissues with elevated oxidative capacities such as liver, kidney, heart, skeletal muscle or BAT. PPAR $\beta/\delta$  (also named FAAR [25]) is widely expressed as it is found in tissues such as gastrointestinal tract, skin, heart, skeletal muscle or adipose tissues [26]. PPAR $\gamma$  displays several isoforms among which PPAR $\gamma$ 2 is exclusively and highly expressed in adipose tissues. PPAR natural ligands are fatty acids and their metabolites derived from diet or from signaling pathways, namely oxylipins (see below, section 4).

PPARs exert their transcriptional activity after heterodimerization with retinoic X receptor (RXR), by binding DNA through interactions between their DNA binding domain and target sequences called PPAR response elements (PPREs). PPAR-RXR

heterodimer activity represents a permissive transcriptional model where ligands of either receptor activate transcription of target genes, with a synergistic effect arising from binding of ligands to both receptors [23, 24]. Furthermore, PPAR transcriptional activity is also regulated by several co-activators, such as PGC-1 $\alpha$ [27], and co-repressors, such as NCoR[28]. Finally, post-translational modifications such as phosphorylation, ubiquitylation and sumoylation affect PPAR activity [28, 29]. Thus, PPAR activity is highly dependent on the complex interplay between expression level of PPAR isotypes, affinity for the promoter, ligand and cofactor availability and inputs from upstream signaling pathways [23].

In addition to direct genomic actions, PPARs also mediate indirect repressive transcriptional effects, called “transrepression”, either through protein-protein interactions with transcription factors preventing them from binding their target genes or by blocking release of corepressor complexes. These mechanisms are of importance for anti-inflammatory PPAR properties [30].

PPARs are involved in crucial cellular processes ranging from cell proliferation and differentiation to inflammation and energy metabolism [23]. However, as sensors of lipid metabolites, they have a special role in regulating adipose tissue homeostasis.

PPAR $\gamma$  is the master regulator of white and brown adipose tissue formation and function expressed at similar levels in both tissues. It is active early during adipogenesis [31] and is required for adipocyte differentiation and survival [32, 33]. Adipose tissue-specific knockout of PPAR $\gamma$  triggers severe lipoatrophy leading to absence of white (WAT) and brown (BAT) adipose tissue in mice [34], which is consistent with its role in stimulating expression of genes required for adipocyte functions: fatty acid uptake, transport and esterification, lipogenesis, lipolysis, thermogenesis, adipokine synthesis and secretion. Beyond adipogenesis, PPAR $\gamma$  activation in mature white adipocytes promotes white-to-brown adipocyte conversion at both molecular and functional levels [35-38].

As a major factor regulating oxidative capacities, PPAR $\alpha$  is much more abundant in BAT compared to WAT and is expressed later in the adipogenic process compared to PPAR $\beta/\delta$  and PPAR $\gamma$ [31]. In murine brown adipocytes, PPAR $\alpha$  controls the expression of genes involved in fatty acid oxidation as well as in the thermogenic

programmethrough induction of PGC1 $\alpha$  and PRDM16, two coactivators driving brown adipogenesis [39-41]. However, recent findings suggest that PPAR $\alpha$  may also have important functions in WAT as PPAR $\alpha$  agonist treatment promotes WAT browning in wild-type mice [42]. Conversely, PPAR $\alpha$  knockout-mice are resistant to  $\beta$ 3-adrenergic receptor-agonist induced browning [35]. Accordingly, in human adipocytes, PPAR $\alpha$  activation induces expression of oxidative and brown adipocyte-specific genes, including UCP1 [35, 43, 44].

PPAR $\beta/\delta$  is expressed at similar levels in WAT and BAT [31]. Its importance in early stages of white adipogenesis relies on its capacity to regulate mitotic clonal expansion of adipocyte precursors and to induce PPAR $\gamma$  expression [26, 45-48]. Furthermore, it has recently been shown that PPAR $\beta/\delta$  was required in brown adipocytes to reach full expression of UCP1 and thermogenic genes and to endow mice with cold protection [40, 49] and similarly, UCP1 expression was increased in WAT of mice overexpressing PPAR $\beta/\delta$ [50].

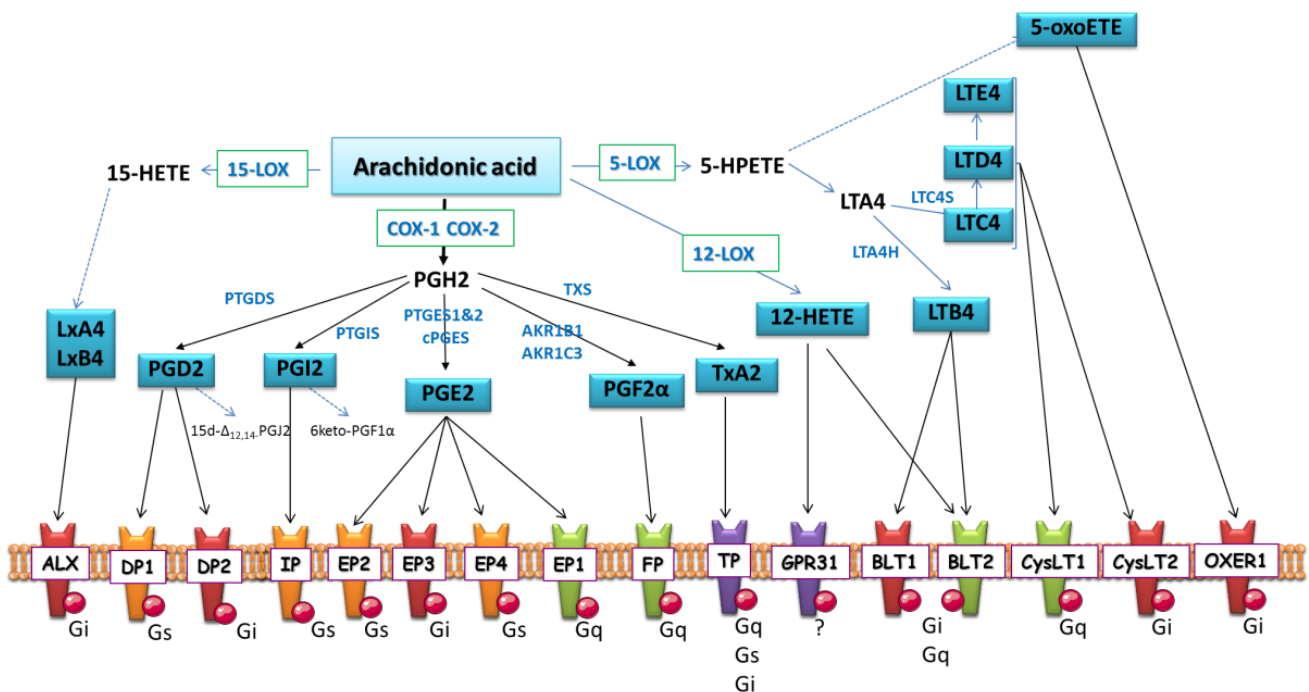
#### **4. Indirect and direct modulations of PPARs by oxylipins.**

The diversity, concentration and fate of the produced oxylipins as well as the variety of PPAR equipment of targeted cells will determine the nature of subsequent events which may explain the consistent failure to identify “true” ligands of a given PPAR. Moreover, for a given ligand acting intracellularly or from outside once secreted leads to various events. For example, prostacyclin is classically synthesized and secreted in order to activate its cognate plasma membrane receptor IP. Interestingly, prostacyclin can also signal through nuclear receptors of the producing cell, under conditions where COX-2 and the prostacyclin synthase (PGIS) co-localize in the periphery of nuclear membrane [51]. Another hypothesis, not completely proven so far, is the nuclear production of PUFA metabolites and/or their trafficking to the nucleus *via* cytosolicfatty acid binding proteins (FABPs). FABPs are intracellular lipid binding proteins, able to carry fatty acids from one point to another. There is some evidence that FABPs can translocate to the nucleus, where they deliver fatty acids or other hydrophobic compounds and thus favor their interaction with nuclear receptors[52, 53].



Oxylipins act mainly in an autocrine or paracrine manner and exert their effects through appropriate membrane receptors. These receptors are coupled to G protein characterized by the associated  $\alpha$  subunit allowing to activate or inhibit the production of the second messengers,  $Ca^{2+}$  and cAMP. Besides mediation through classic  $G\alpha_q$  (induces calcium signaling),  $G\alpha_s$  (activates adenylate cyclase) and  $G\alpha_i$  (inhibits adenylate cyclase) subunits, it has been shown that some of the oxylipin effects could be mediated by  $G\beta\gamma$  subunits, which are able to negatively control adenylate cyclase or activate some ion channels and MAPK pathways (reviewed in [54]). The receptors for ARA-derived metabolites are well known and have been extensively studied (**Figure 1**). Interestingly, prostanoids and leukotrienes derived from EPA are able to activate the same receptors as ARA metabolites but with lower affinity [55]. Resolvin, protectin and maresin receptors are less known, excepted for ChemR23, a  $G\alpha_i$ -coupled receptor displaying a high affinity for RvE1[56].

**Figure 1.** Eicosanoid synthesis pathways derived from ARA and their targeted receptors.



Oxylipins are able to indirectly mediate PPARs activity through the activation of these pathways. Indeed, when oxylipins activate cAMP/PKA pathway, they lead to the phosphorylation of CREB which in turn is able to 1) transactivate expression of numerous PPAR partners, such as PGC1 and C/EBP family members, or 2) synergize

the transcriptional activity of PPARs on promoters harboring PPREs and CREs (reviewed in [57]). Moreover, cAMP mediated pathway can induce the phosphorylation of PPAR cofactors such as PGC1 $\alpha$ . In an opposite manner, the inhibition of adenylate cyclase activity by Gai subunit disrupts these cAMP dependent pathways. Another key pathway linking PPAR and oxylipins is the Ca<sup>++</sup> pathway. PGF2 $\alpha$  has been reported to activate Ca<sup>++</sup> signaling and to induce PPAR $\gamma$  phosphorylation and thus its inactivation [58]. ERK1/2 signaling pathway appeared to be involved in this phenomenon in adenocarcinoma cells in which PGF2 $\alpha$ , via IP/Ca<sup>++</sup> signaling, allowed phosphorylation of ERK1/2 which in turn led to PPAR $\gamma$  degradation through the ubiquitin/proteasome pathway [59, 60]. Other data showed that Ca<sup>++</sup>/calcineurin mediated pathway inhibits PPAR $\gamma$  expression [61-64]. Altogether, these observations demonstrate that oxylipin-mediated activation of Ca<sup>++</sup> signaling *via* G $\alpha$ q-coupled receptors inhibits PPARs expression and activity, while oxylipin-mediated activation of cAMP signaling *via* G $\alpha$ s-coupled receptors promotes PPARs expression and activity.

ligands	PPAR $\alpha$	PPAR $\beta/\delta$	PPAR $\gamma$
<b>PGA1</b>	Yu et al. 1995 [65]	Yu et al. 1995 [65]	Yu et al. 1995 [65]
<b>PGA2</b>	Yu et al. 1995 [65]	Yu et al. 1995 [65]	Yu et al. 1995 [65]
<b>PGD1</b>	Yu et al. 1995 [65]	Yu et al. 1995 [65]	Yu et al. 1995 [65]
<b>PGD2</b>	Yu et al. 1995 [65]	Yu et al. 1995 [65]	Yu et al. 1995 [65]
<b>LTB4</b>	Devchand et al. 1996 [66]		
<b>8-HETE</b>	Yu et al. 1995 [65]		
<b>PGJ2</b>	Brun et al. 1996 [67]		Forman et al. 1995 [71]
<b>PGI2</b>	Brun et al. 1996 [67]	Brun et al. 1996 [67]	Brun et al. 1996 [67]
<b>15d-<math>\Delta^{12,14}</math>-PGJ2</b>	Brun et al. 1996 [67]	Brun et al. 1996 [67]	Forman et al. 1995 [71] Klierer et al. 1995 [72]
<b><math>\Delta^{12}</math>-PGJ2</b>			Fujimori et al. 2012 [73]
<b>15-HETE</b>			Huang et al. 1999 [74]
<b>12-HETE</b>		Naruhn et al. 2010 [70]	Sun et al. 2015 [75]
<b>9-HODE</b>			Nagy et al. 1998 [76]
<b>13-HODE</b>	Delerive et al. 2000 [68]		Huang et al. 1999 [74]
<b>Hepoxilin</b>	Yu et al. 2007 [69]		Hallenborg et al. 2010 [77]
<b>5-oxoETE</b>			O'Flaherty et al. 2005 [78]

**Table 1.** List of the oxylipins able to bind PPARs and corresponding references.

In addition to these indirect relationships between oxylipins and PPARs, it is well accepted that numerous PUFAs metabolites are PPAR ligands. Several studies have demonstrated the ability of these metabolites to promote the transactivation effects of PPAR $\alpha$ , PPAR $\beta/\delta$  and PPAR $\gamma$  using *in vitro* approaches based on PPRE-reporter assays. A non-exhaustive list of these oxylipins is proposed in **Table 1**.

It is important to note that some of these ligands have been essentially characterized *in vitro*, and *in vivo* function remains questionable, especially with regard to the high doses used in these approaches.

### **5. Interaction between PPARs, GPCRs and oxylipins in commitment and differentiation of preadipocytes.**

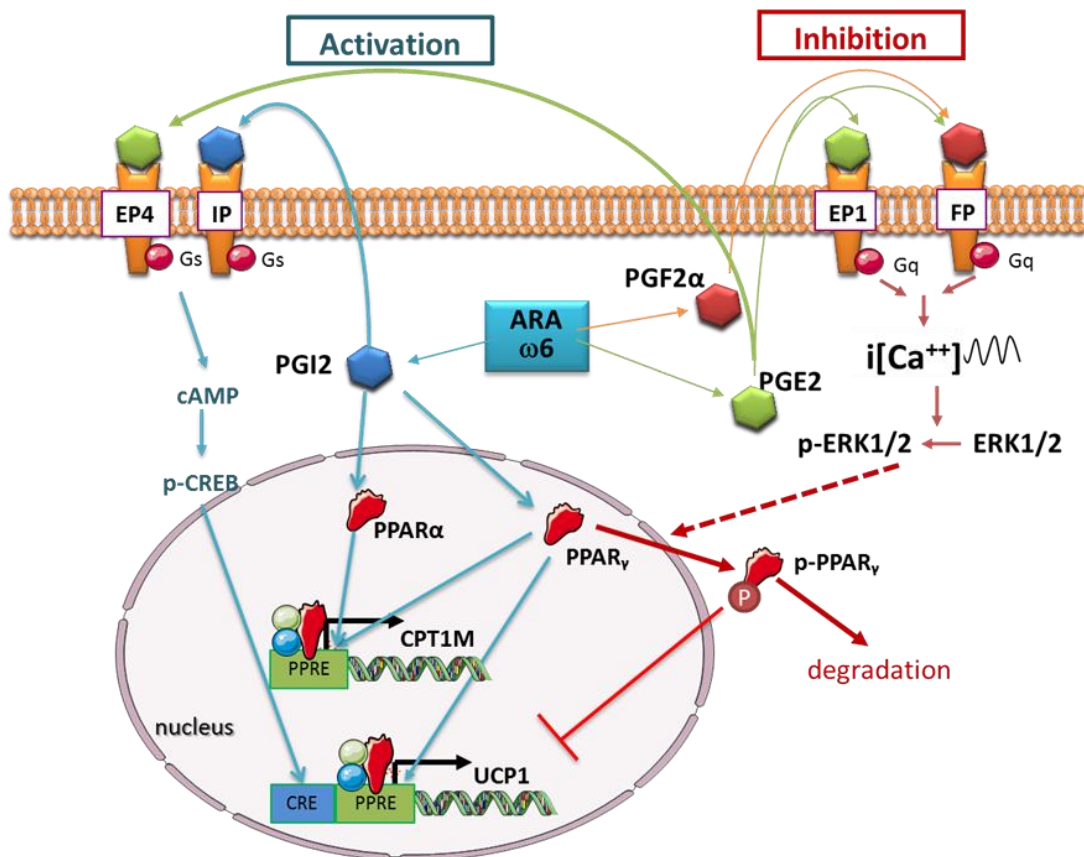
Earlier, it has been demonstrated that, among long chain fatty acids, ARA was the main adipogenic component of fetal bovine serum required for differentiation of cultured mouse white preadipocytes [9, 79]. These findings were obtained owing to the establishment and characterization of Ob17 preadipocyte cell line able to convert to mature adipocytes [80, 81]. Treatment of these cells with indomethacin [82], a cyclooxygenase inhibitor, as well as co-treatment with ARA [9] demonstrated the involvement of oxygenated metabolites in differentiation mechanisms.

Shortly thereafter, using Ob17 cells, the inhibitory effect of PGF $2\alpha$  on the differentiation process was demonstrated [83]. Thirty years later, the molecular signaling driving the anti-adipogenic effect of PGF $2\alpha$  has been deciphered. PGF $2\alpha$  binds and activates the FP membrane receptor coupled to Gq subunit (**Figure 2**). This receptor activation triggers an intracellular Ca $^{2+}$  flux and activation of calcineurin, a phosphatase known to inhibit the expression of PPAR $\gamma$  [63].

A second pathway has recently been described in murine 3T3-L1 preadipocytes [84]. In these cells the activation of FP receptor leads to activation of Ca $^{2+}$ /PKC/MAPK signaling pathway. The resulting phosphorylation of ERK1/2 induces an overexpression of Fos-related antigen-1 (Fra-1) which blocked adipogenesis through the inhibition of PPAR $\gamma$  activity [84]. This inhibitory effect of PG $2\alpha$  did not reflect the positive effect of ARA on adipogenesis and thus might involve other metabolites.

Among prostanoids, some candidates may explain the pro-adipogenic effect of ARA. The  $15d\text{-}\Delta^{12,14}\text{-PGJ}_2$ , a PGD<sub>2</sub>-derived metabolite, has been characterized as a strong PPAR $\gamma$  ligand and displayed a pro-adipogenic effect when it is used at high concentration [71][72]. Prostacyclin (PGI<sub>2</sub>) is synthesized and secreted by adipocytes and is able to modulate PPAR $\gamma$  activity directly and indirectly. Upon secretion from preadipocytes, prostacyclin was found to be active *via* the prostacyclin receptor (IP) present at the cell surface of preadipocytes. ARA, acting through the IP/prostacyclin pathway, triggered cAMP production and activated the pro-adipogenic protein kinase A pathway [85, 86] (Figure 2).

**Figure 2.** Schematic representation of pathways activated by ARA-derived metabolites and their impact on PPAR and thermogenic adipocyte gene expression. PPRE: PPAR response element; CRE: CREB response element; ARA: arachidonic acid; PG: prostaglandins; PGI<sub>2</sub>: prostacyclin.



In rodents, both *ex vivo* and *in vivo* exposure of white adipose tissue to carbaprostacyclin, a stable analogue of PGI<sub>2</sub>, was able to stimulate the formation of

adipocytes within a few hours[87]. Similar results were obtained with human preadipocytes [88]. To gain further insights into the contribution of prostacyclin signaling pathway in mouse adipose tissue development, wild-type mice and mice invalidated for IP (*ip*<sup>-/-</sup>) were used. In contrast to wild-type mice, pups from *ip*<sup>-/-</sup> mice showed no gain in body weight or in fat mass when fed a LA-enriched diet (LA/LNA ratio of 30) compared to the balanced LA/LNA diet (ratio of 4)[9]. Last but not least, carbaprostacyclin (cPGI<sub>2</sub>), a stable analog of prostacyclin, is able to bind to PPARs. When cPGI<sub>2</sub> activated PPAR $\gamma$  and PPAR $\alpha$ , but not PPAR $\beta/\gamma$ , it promoted adipogenesis [67]. Like for 15d- $\Delta$ <sup>12,14</sup>.PGJ<sub>2</sub>, the data about cPGI<sub>2</sub> are convincing but did not demonstrate that the ARA positive effect on adipogenesis is triggered by this prostanoid. Other PUFAs derived metabolites such as 9- and 13-HODE, first identified in macrophages, have been described to bind to PPAR $\gamma$  in adipocytes [76] and to be involved in adipogenesis [89]. Nevertheless, none of these fatty acid metabolites has been demonstrated to accumulate strikingly during the early steps of adipocyte differentiation and thus might not be considered as physiological ligands.

PGE<sub>2</sub> controls many aspects of adipocyte differentiation. This prostaglandin activates one or more of its cognate receptors (EP1, EP2, EP3, and EP4) in a dose-dependent manner [90]. This allows the induction of different intracellular signals; EP2 and EP4 have the highest affinity for PGE<sub>2</sub> and are coupled to G $\alpha$ s protein, while EP3 receptor is coupled to G $\alpha$ i and EP1, which has the lowest affinity for PGE<sub>2</sub>, is coupled to G $\alpha$ q proteins. Thus the effects of PGE<sub>2</sub> on adipogenesis remain various and opposed as they are strictly related to its concentration and the expression of its receptors [91]. For example, in preadipocytes, PGE<sub>2</sub> activates the EP2 and EP4 receptors and induces PKA pathway to initiate adipogenesis [92]. Conversely, a crucial involvement of mPGES1 (microsomal PGE<sub>2</sub> synthase 1) is observed in adipogenesis. PGE<sub>2</sub> suppressed PPAR $\gamma$  expression and blocked rosiglitazone-induced preadipocyte differentiation. Consistently, pharmacological mPGES-1 inhibition promotes this differentiation[93].

Among leukotriene family, LTB<sub>4</sub> has been characterized as a PPAR $\alpha$  ligand promoting lipid accumulation in differentiating adipocytes[94]. It is interesting to note that

LTB4 signals through two membrane receptors, BLT1 and BLT2 that are linked to Gαq and Gαi proteins, two well-known pathways endowed with anti-adipogenic activity.

Finally, hepxilin A3 and B3 (HxA3 and HxB3), a family of metabolites generated from ARA by the sequential activities of ALOX12 and ALOX3 (or epidermis LOX3), represent potential candidates to explain ARA effect on adipogenesis. Indeed, HxA3 and HxB3 are PPARγ ligands that accumulate in the cells in the beginning of adipocyte differentiation, and they induce a strong pro-adipogenic effect [77]. Interestingly, ALOX3 is co-localized with PPARγ in the nucleus of the cells, which is in accordance with a nuclear function of its metabolites. Finally, ALOX3 inhibition disrupted adipogenesis and PPARγ activity. Further studies are requested to validate these results especially in human models, as they are only described in mouse 3T3-L1 preadipocytes [77, 95].

PUFAs metabolites can be either pro- or anti-adipogenic depending on the activation level of the cAMP pathway [96]. In 3T3-L1 preadipocytes, ARA produced a pro-adipogenic effect by a transient raise of cAMP levels. By contrast, if the cAMP pathway is already activated, there is an increased synthesis of anti-adipogenic PGs, such as PGE2 and PGF2α [96]. This situation can be observed *in vivo* in case of a high protein diet or in a situation of chronic inflammation [96, 97]. In conclusion, the control of adipocyte differentiation by PUFAs metabolites is complex and depends on several environmental factors.

## **6. Interaction between PPARs, GPCRs and oxylipins in adipocyte function.**

In addition to the control in early steps of adipogenesis, oxylipins are known to modulate adipocyte function (lipogenesis, lipolysis, secretion...) and, more recently, phenotypic conversion such as browning or whitening processes. As adipocyte functions are sensitive to cAMP- and Ca<sup>++</sup>-mediated pathways, modulations are mainly due to activation of membrane GPCRs by oxylipins. Differently, phenotypic conversion is transcriptionally controlled and thus involves preferentially oxylipins that are able to activate nuclear receptors such as PPARs.

Early, the DGLA derived prostanoid PGE<sub>1</sub> has been described as an anti-lipolytic agent in various species [98]. PGE<sub>2</sub> displayed the same properties on lipolysis [99], most likely through the same receptor EP<sub>3</sub> known to inhibit cAMP mediated pathways induced by lipolytic agents [100]. Thus, inhibition of lipolysis seems to be specific to G<sub>ai</sub>-coupled receptor as PGF<sub>2</sub> $\alpha$ , which activates G<sub>aq</sub>-coupled receptor, is unable to inhibit lipolysis induced by norepinephrine [101]. Lipolysis is under the control of cAMP levels and downstream events, as well as cGMP levels in some situations [102], thus oxylipins able to bind to G<sub>as</sub>-coupled receptors shall be considered as potential lipolytic agents. This observation has been validated for prostacyclin [92], which potentiates the effect of norepinephrine on lipolysis [103]. The oxylipin-mediated restriction of lipolysis seems to be a preponderant event *in vivo*, as recent data demonstrate that inhibition of COX activity by indomethacin favored lipolysis under fasting condition [8, 104].

The involvement of oxylipins specifically in lipogenesis and fatty acid storage is difficult to assess, as these anabolic processes are tightly associated to adipocyte differentiation. Nevertheless, acute treatment of 3T3-L1 adipocytes demonstrated that PGE<sub>2</sub> and PGF<sub>2</sub> $\alpha$  might be considered as anti-lipogenic agents, likely via the activation of EP<sub>1</sub> and FP G<sub>aq</sub>-coupled receptors [105]. Differently, it has been shown that the dehydrated metabolites of PGJ<sub>2</sub>,  $\Delta^{12}$ -PGJ<sub>2</sub> and 15d- $\Delta^{12,14}$ -PGJ<sub>2</sub> were able to favor lipid accumulation in adipocytes through direct interaction with PPAR $\gamma$  [106, 107].

In adipocytes, the relationships between oxylipins and inflammation are very important. In this way, oxylipins are able to control and in return to be controlled by inflammatory cytokines. It is commonly accepted that  $\omega$ 6-PUFA-derived metabolites induce and sustain inflammation and that  $\omega$ 3-PUFA-derived metabolites are protective or resolve inflammation (reviewed in [108]), especially via their PPAR ligand function (reviewed in [109]). For example, LOX-derived metabolites are involved in chemoattraction of macrophages by the autocrine stimulation of MCP-1 secretion by adipocytes [110, 111]. In addition to the control of inflammatory cytokine secretion, oxylipins are able to modulate secretion of adipokines. Indeed, it has been reported that PGD<sub>2</sub>-derived metabolites are able to inhibit leptin secretion [112], whereas PGE<sub>2</sub> stimulates its secretion [113].

## 7. Interactions between oxylipins and the different types of adipocytes

White adipocytes are storing energy under the form of triglycerides whereas brown adipocytes dissipate energy from triglycerides by producing heat. These adipocytes are able to adapt their phenotype to specific situations. It is now accepted that white adipocytes can be phenotypically converted into thermogenic adipocytes (brite/beige adipocyte conversion) or in milk-producing epithelial cells (pink adipocyte conversion) (reviewed in [114]). These conversions are reversible and occur in situations where the genuine functional cells cannot assume their functions (brown adipocyte in chronic cold exposure) or along specific physiological situations (after pregnancy). So far, no data are available about involvement of oxygenated metabolites in white to pink adipocyte conversion. Otherwise, several studies demonstrated the involvement of oxylipins in white to brite adipocyte conversion. Eicosanoids derived from  $\omega$ 6 PUFA inhibit brite adipocyte formation, *in vitro* and *in vivo* [10]. ARA inhibited the expression and function of UCP1 and these effects were mediated via cyclooxygenase activities leading to increased synthesis and release of PGE2 and PGF2 $\alpha$ . Thorough analysis of the role of PGE2 and PGF2 $\alpha$  demonstrated that an oscillatory calcium pathway, due respectively to EP1 and FP receptor activation, was responsible of impairing the browning process. The sustained  $i[Ca^{++}]$  oscillations led in turn to the inhibition of the expression of PPAR $\gamma$  target genes, including UCP1 gene, and suggest a broader inhibitory effect of prostaglandin-mediated pathways *via* the modulation of PPAR $\gamma$  activity [10]. Moreover, it has been demonstrated that cPGI2 was able to induce recruitment of brite adipocytes by promoting the differentiation of progenitor cells and the conversion of white into brite adipocytes [115, 116]. The differentiation into brite adipocytes is dependent on the IP receptor and might involve reorganization of the cytoskeleton and/or chromatin [116, 117]. Similarly, PGE2 is able, through the activation of EP4, to promote white to brite adipocyte conversion [10, 118].

Interestingly, *in vivo*, COX pathway has been shown to be crucial for the induction of brite adipocytes formation in 129Sv mice (a strain resistant to obesity due to a high content of brown and brite adipocytes) [116, 118], but an opposite role of the same



pathway has been recently described in C57BL/6 mice (a strain prone to develop obesity through high fat diets) [8]. In this latter case, when mice were fed a high fat diet, the inhibition of COX activities with indomethacin prevented body weight gain, partly due to enhanced recruitment of brite adipocytes in subcutaneous WAT. These results demonstrate, once again, the complexity of the oxylipin effects depending on the physiological situation.

## **8. Conclusion**

In aggregate, oxylipins display a multiplicity of effects in adipocyte from the control of its differentiation to its function through the activation of two families of receptors: namely GPCR and PPAR families. In mammals, as oxylipins govern a large part of adipose tissue biology, any dysregulation in their levels may disrupt tissue homeostasis. Thus the nutritional control of their production, especially by an optimal qualitative and quantitative dietary PUFA supply, is of utmost interest in the prevention of metabolic disorders.

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