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# Prostaglandin $E_2$ signals white-to-brown adipogenic differentiation

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**Abbreviations:** 15d-$PGJ_2$, 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$; 20-HETE, 20-hydroxy eicosatetraenoic acid; Akt, protein kinase B; ATP, adenosine triphosphate; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; BMPs, bone morphogenic proteins; cAMP, cyclic adenosine 3′, 5′-monophosphate; C/EBP, CCAAT enhancer binding protein; CIDEA, cell death-inducing DFFA-like effector a; COX, cyclooxygenase; FLAP, 5-lipoxygenase activating protein; IL, interleukin; LOX, lipoxygenase; LT, leukotriene; MCP-1, monocyte chemotactic protein-1; mPGES-1, microsomal prostaglandin E (PGE) synthase-1; Myf-5, mesenchymal myogenic factor-5; $PGE_2$, prostaglandin E2; PAI-1, plasminogen activator inhibitor-1; PPARγ, peroxisome proliferator-activated receptor γ; PGC-1α, PPARγ co-activator-1 α; PRDM16, PR domain containing 16; TG, triglyceride; TGFβ, transforming growth factor β; UCP1, uncoupling protein 1; WAT, white adipose tissue; Wnt5b, wingless-type MMTV integration site family, member 5B; Zfp423, zinc-finger protein 423

The formation of new adipocytes from precursor cells is a crucial aspect of normal adipose tissue function. During the adipogenic process, adipocytes differentiated from mesenchymal stem cells give rise to two main types of fat: white adipose tissue (WAT) characterized by the presence of adipocytes containing large unilocular lipid droplets, and brown adipose tissue (BAT) composed by multilocular brown adipocytes packed with mitochondria. WAT is not only important for energy storage but also as an endocrine organ regulating whole body homeostasis by secreting adipokines and other mediators, which directly impact metabolic functions in obesity. By contrast, BAT is specialized in dissipating energy in form of heat and has salutary effects in combating obesity and associated disorders. Unfortunately, WAT is the predominant fat type, whereas BAT is scarce and located in discrete pockets in adult humans. Luckily, another type of brown adipocytes, called beige or brite (brown-in-white) adipocytes, with similar functions to those of "classical" brown adipocytes has recently been identified in WAT. In this review, a close look is given into the role of bioactive lipid mediators in the regulation of adipogenesis, with a special emphasis on the role of the microsomal prostaglandin E (PGE) synthase-1, a terminal enzyme in $PGE_2$ biosynthesis, as a key regulator of white-to-brown adipogenesis in WAT.

## White versus Brown Adipose Tissue

White adipose tissue (WAT) adipocytes are nucleated cells comprising a characteristic unilocular lipid droplet mainly composed of triglycerides (TGs) and cholesterol esters, which occupies most of the cell, and a thin rim of cytoplasm displaced to the periphery.[1] The major function of WAT is the formation (adipogenesis) and storage of lipids (i.e., fatty acids) in the form of TGs within periods of less energy expenditure.[1] Indeed, during times in which energy intake is higher than the metabolic demand, adipocytes would expand nearly 1000-fold in volume and 10-fold in diameter in order to store the excess of fuel as TGs.[1] In contrast, in periods of food restriction or in periods demanding more energy expenditure, adipose tissue serves, via lipolysis, as the major source of fatty acids. Under starving conditions, lipolysis is an essential mechanism whereby rate-limiting enzymes such as hormone-sensitive lipase, adipose triglyceride lipase (ATGL) and monoglyceride lipase catalyze hydrolysis of TGs to release free fatty acids (FFA) into the circulation.[1] Circulating FFA are subsequently taken up via fatty acid transporters present in metabolically active and insulin-sensitive tissues (primarily skeletal muscle and liver), which then use these lipids as energy substrates for generation of adenosine triphosphate (ATP) high-energy bonds for metabolic functions trough oxidative phosphorylation.[2] Extensive research has been conducted on this topic, especially on mechanisms regulating lipolysis, which is a highly regulated process under the control of the sympathetic nervous system, hormones and other paracrine/autocrine factors.[1,2] Most of these studies have focused on the pathogenic consequences of the disruption of lipolysis and lipogenesis equilibrium and their role on the onset of type 2 diabetes, insulin resistance, and non-alcoholic fatty liver disease.

Although WAT has classically been considered a mere storage of fat and energy and with a limited role in thermal insulation, this tissue is now generally recognized as an important endocrine organ that secretes a number of hormones and signaling factors, collectively known as adipokines.[3] Adipokines released by WAT exert diverse biological functions such as regulation of appetite and satiety, glucose and lipid metabolism, blood pressure

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homeostasis, inflammation and immune functions.[3-5] More than 50 different molecular entities secreted from WAT have been described, including cytokines and related proteins (leptin, tumor necrosis factor [TNF] α, interleukin [IL]-6, IL-10, and monocyte chemotactic protein-1 [MCP-1]), proteins of the fibrinolytic cascade (plasminogen activator inhibitor-1 [PAI-1]), complement-related proteins (visfatin and adiponectin), and other biologically active peptides such as resistin and chemerin.[3-5] Detailed information on the role of these adipokines in whole body homeostasis can be found elsewhere.[3-5]

In addition to WAT, another type of adipose tissue exists in mammals: the brown adipose tissue (BAT). Compared with WAT, BAT contains smaller adipocytes and uses the chemical energy in lipids to produce heat through non-shivering thermogenesis.[6,7] BAT adipocytes are brown rather than white because they are packed with mitochrondia. Moreover BAT adipocytes contain uncoupling protein 1 (UCP1), an integral membrane protein that acts as a proton channel diverting the respiratory chain from ATP synthesis to the generation of heat.[6,7] In other words, the uncoupling of the oxidative phosphorylation in BAT adipocyte mitochrondia results in the combustion of the excess of lipids and the dissipation of heat by its distribution to the rest of the body through the circulation.[6,7] According to these unique properties, BAT is in the focus of scientific interest for the search of novel therapies aimed at combating metabolic diseases and complications associated with obesity, such as type 2 diabetes.[6,7] Unfortunately, BAT is difficult to find in adult humans, since most of BAT pads existing within the posterior neck in neonatal humans to provide cold adaptive thermogenesis for newborns are lost soon after birth.[6,7] Nevertheless, recent studies using non-invasive imaging technologies such as fluorolabeled 2-deoxy-glucose positron emission tomography ($^{18}$FDG-PET) scanning and MRI scanning have clearly demonstrated that significant amounts of BAT deposits are still active in adult humans.[8]

In addition to "classical" brown adipocytes found in BAT, a second type of brown adipocytes termed "beige" or "brite" cells has been identified in WAT. Beige cells are defined by their multilocular lipid droplet morphology, high mitochondrial content and the expression of a core set of brown fat-specific genes (UCP1, cell death-inducing DFFA-like effector A [CIDEA] and peroxisome proliferator activated receptor gamma (PPARγ) coactivator 1 α [PGC-1α]).[9] In contrast to "classical" brown adipocytes, beige cells do not derive from pluripotent mesenchymal myogenic factor-5 (Myf-5)-positive cells (see below).[10] In addition, these two cell types are differentially regulated since a number of quantitative trait loci are associated with the induced development of beige adipocytes but not "classical" brown adipocytes.[11] Furthermore, while "classical" brown adipocytes express high levels of UCP1 and other thermogenic genes under basal conditions, beige cells only express these genes in response to agonists of the β-adrenergic receptors and PPARγ.[12] The existence of this separate type of brown adipocytes in WAT has been recently reinforced by the cloning of beige-cell lines from mouse inguinal WAT.[13] Unexpectedly, recent studies have demonstrated that a number of beige cell-selective genes are abundantly expressed in human BAT depots, suggesting that human BAT may also be composed of beige/brite cells.[9,13] Together, the engagement of beige/brite adipocytes and the induction of a thermogenic program in WAT depots are relevant in terms of energy homestasis because they ultimately exert protection against obesity and obesity-related co-morbidities by wasting the surplus of energy through increased heat production. In this regard, increases in number of beige cells in WAT are closely associated with a protection against diet-induced obesity and metabolic diseases.[9]

## Adipogenesis

Adipocytes, like other mesenchymal cells, are generally described to derive from the mesodermal layer of the embryo. White adipocytes are believed to derive from the lateral plate mesoderm, whereas brown adipocytes would originate from paraxial mesoderm.[14] The potential of paraxial mesoderm to differentiate into BAT was initially suggested by Loncar et al.,[15] who performed pioneer transplantation of mesoderm from a 9-day-old rat embryo under the renal capsule of a recipient, resulting in the exclusive generation of BAT. The mesodermal origin of interscapular BAT was later confirmed through an in vivo lineage tracing strategy, in which myogenin-expressing dermomyotome, a paraxial mesoderm-derived structure, gave rise to dermis and muscle as expected, but also to BAT.[16] The expression of Myf-5 is an established marker used to distinguish white (Myf-5-negative) from brown (Myf-5-positive) adipocytes.[7] Similar to white adipocytes, beige/brite cells are apparently derived from cell precursors originated in the lateral plate mesoderm, although the origin of these cells is still controversial.[7] Approximately 300 genes were identified by microarray analysis to be differentially expressed in undifferentiated primary brown as compared with white adipocyte progenitors.[17] Subsequent studies using muscle-selective Myf-5-Cre knock-in mice confirmed that skeletal muscle and "classical" brown adipocytes, but not white adipocytes, arise from common Myf-5-expressing progenitors.[18]

Adipogenesis takes place in two different phases: determination of preadipocytes from multipotent stem cells and terminal differentiation of preadipocytes into mature adipocytes. The first phase involves the commitment of pluripotent stem cells to the adipocyte lineage (preadipocyte), which cannot be distinguished morphologically from its cell precursors but has lost the potential to differentiate into osteoblasts, myocytes, and chondrocytes.[19] Several genes have been involved in this process, including the wingless-type MMTV integration site family, member 5B (Wnt5b), transforming growth factor (TGF) β, and bone morphogenic proteins (BMPs). The β-catenin-independent Wnt5b ligand has been shown to promote adipogenesis by targeting alternative cell surface receptors and inhibiting β-catenin nuclear translocation.[20] In turn, TGF-β has been shown to exert either pro- or anti-adipogenic actions through stimulation of the transcription factor mothers against decapentaplegic homolog (also known as SMAD) in different in vitro and ex vivo models.[21] Finally, several BMPs also promote preadipocyte commitment via SMAD/p38 signaling.[22] Specifically, BMP2 and BMP4

promote preadipocyte determination, whereas BMP7 promotes brown adipogenesis.[23] The zinc-finger protein 423 (Zfp423) also induces adipose lineage commitment in part through amplification of the BMP signaling pathway, an effect that depends on its SMAD-binding capacity.[24]

In the second phase (i.e., terminal differentiation), preadipocytes take on the characteristics of mature adipocytes. In the case of mature white adipocytes, cells acquire the machinery that is necessary for lipid transport and synthesis, insulin sensitivity and the secretion of adipocyte-specific proteins.[25] Several factors have been described to be involved in the terminal differentiation of preadipocytes, once these cells have committed to the adipogenic lineage. The most relevant is PPARγ, which is defined as the "master regulator" of fat cell formation, since PPARγ is not only necessary but also sufficient for the adipogenic process. Indeed, most of the transcription factors identified as promoters or inhibitors of adipogenesis (i.e., PR domain containing 16 [PRDM16], CCAAT enhancer binding protein [C/EBP] α and β, and PGC-1α) exert their actions by either inducing or repressing PPARγ.[22,25] For example, PRDM16 is a BAT-specific transcription factor that interacts with PGC-1α (a PPARγ co-activator) and C/EBPβ to trigger a brown adipocyte differentiation program.[18] On the other hand, C/EBPα induces PPARγ in a positive feedback loop, thereby maintaining the differentiated cell state of adipocytes.[22] Finally, PGC-1α is a PPARγ coactivator that regulates the expression of mitochrondial genes involved in adaptive thermogenesis, like UCP1, in brown adipocytes.[22]

## Local Production of Bioactive Lipid Mediators in WAT

The ability of WAT to generate bioactive lipid mediators was first described in the late 1960s when Shaw and Ramwell identified a group of hydroxyl C20 carboxylic acids known as prostaglandins (PGs) derived from the oxygenation of the polyunsaturated fatty acid arachidonic acid in rat epipidymal fat pads.[26] A schematic diagram of the PG biosynthetic pathway is given in **Figure 1**. Among the different cyclooxygenase (COX)-derived products in WAT, $PGE_2$ was recognized as one of the most abundant PGs.[26] This finding was consistent with previous studies pointing to $PGE_2$ as a negative regulator of hormone-stimulated lypolysis.[27] Along these lines, pre-incubation of adipocytes with COX inhibitors was reported to enhance lipolysis.[28] Given that the lipolytic actions of catecholamines are mediated by cyclic adenosine 3′,5′-monophosphate (cAMP), which in turn activates TG lipase, the anti-lipolytic actions of $PGE_2$ are likely associated with the modulation of adipocyte cAMP levels.[29] In addition to $PGE_2$, the COX-derived product 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (15d-$PGJ_2$) has been postulated to play a physiologically relevant role in adipose tissue. 15d-$PGJ_2$ is a cyclopentenone metabolite produced by dehydration of $PGD_2$.[30] The predominant enzymatic source of 15d-$PGJ_2$ formation in vivo is COX-2 and unlike other PGs, no specific membrane receptor has been identified for this lipid mediator.[30] Instead, 15d-$PGJ_2$ exerts its anti-inflammatory and adipogenic actions through binding and activation of the nuclear receptor PPARγ (**Fig. 1**).[31]

Apart from COX products, WAT also has the ability to produce and release lipoxygenase (LOX) products, especially leukotriene (LT) $B_4$, which apparently is the predominant LOX metabolite in this tissue.[32] In this regard, WAT expresses all enzymes necessary for the formation of 5-LOX products (5-LOX, 5-LOX activating protein [FLAP], $LTA_4$ hydrolase, and $LTC_4$ synthase).[32] $LTB_4$ has been identified as an inflammatory factor in WAT and FLAP overexpression and excessive generation of 5-LOX products are common findings in WAT of obese patients and animals with insulin resistance.[32,33] Moreover, a direct relationship between $LTB_4$ and enhanced release of inflammatory adipokines (i.e., MCP-1 and IL-6) has been reported in obese WAT.[32] Consistent with this finding, a selective FLAP has been shown to alleviate adipose tissue inflammation and insulin resistance in obesity.[32] Neither a role in adipogenesis nor a role in WAT lipolysis has been demonstrated for $LTB_4$.[34]

In addition to heightened production of pro-inflammatory COX and LOX-derived lipid mediators, obese WAT also has the capacity to generate anti-inflammatory lipid mediators. Indeed, WAT expresses all enzymes necessary for the biosynthesis of resolvins, protectins, and maresins derived from omega-3 polyunsaturated fatty acids, as well as all receptors necessary for their signaling (reviewed in Spite et al. 2014).[35] Moreover, a deficit in the levels of these endogenous anti-inflammatory and pro-resolving lipid mediators has been characterized by means of LC-MS/MS-based metabolo-lipidomic analyses in inflamed visceral and subcutaneous fat compartments from *ob/ob* obese mice and *db/db* obese/diabetic mice.[35] In humans, a remarkable deficit in these mediators has been reported in subcutaneous fat from patients with peripheral vascular disease, in whom the inflammatory status of WAT is profoundly exacerbated.[35] In patients with vascular disease, unique signature profiles and a heterogeneous capacity to generate pro-resolving lipid mediators were identified among WAT from different anatomic locations, being the perivascular fat the depot with higher biosynthetic capacity. Consistent with these observations, administration of exogenous pro-resolving mediators successfully rescued the impaired resolution capacity of obese WAT, enhanced the expression and secretion of adiponectin in parallel with decreased secretion of pro-inflammatory adipokines/cytokines including leptin, TNFα, IL-6, and IL-1β, improved glucose tolerance, decreased fasting blood glucose, and increased insulin-stimulated Akt (also known as protein kinase B) phosphorylation in this tissue.[35] In human monocyte-adipocyte assays, these pro-resolving mediators reduced MCP-1 and $LTB_4$-stimulated monocyte adhesion to adipocytes as well as monocyte transadipose migration, which are likely events in the progression of inflamed adipose tissue.[35] Interestingly, proresolving mediators skewed adipose tissue macrophages toward an M2 phenotype, which is anti-inflammatory in nature.[35] Collectively, these findings are consistent with the notion that unresolved chronic "low grade" inflammation in obese adipose tissue is the result of an inappropriate resolution capacity allowing the inflammatory response to proceed uncontrolled. At present, a

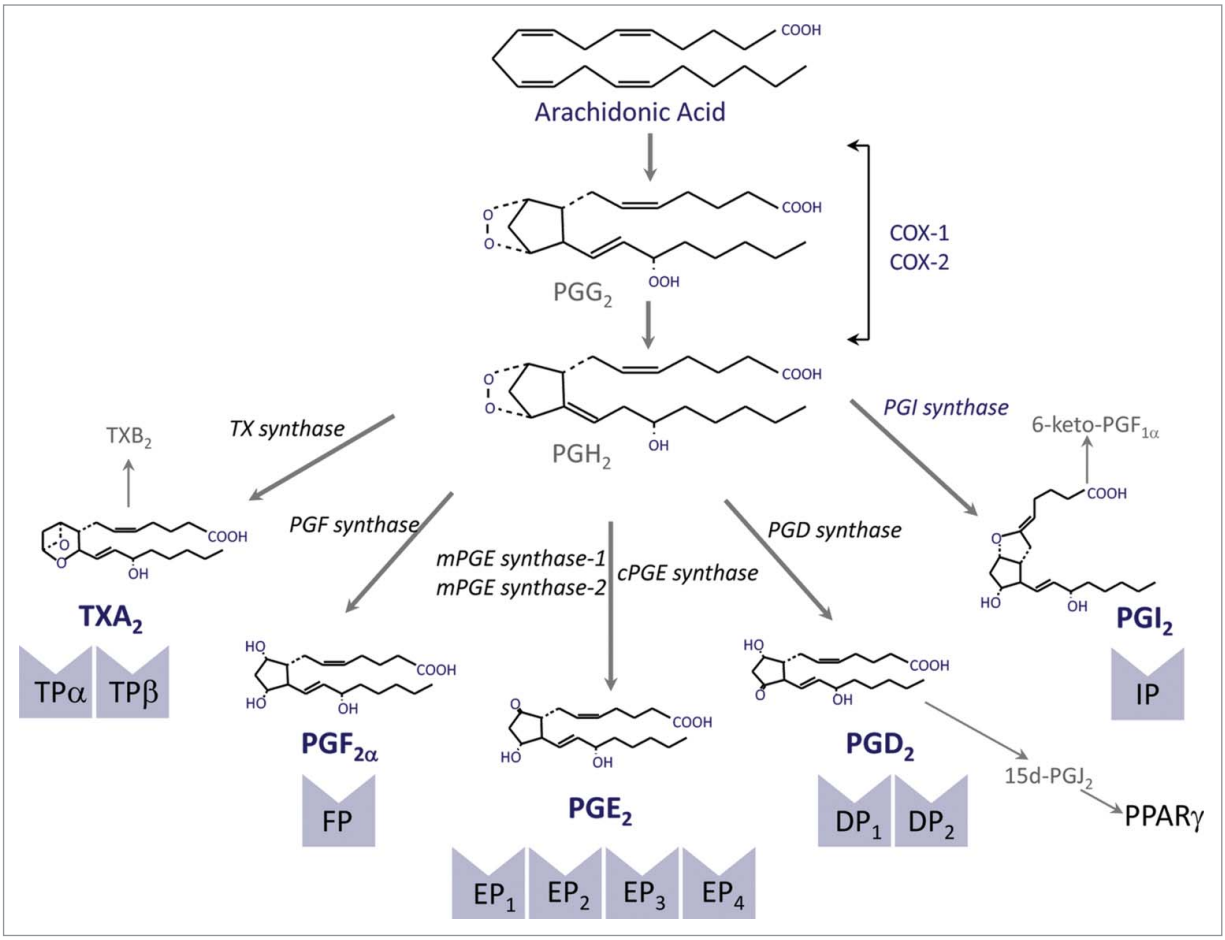


**Figure 1.** The cyclooxygenase (COX) pathway. COX exists in two different isoforms (COX-1 and COX-2) and oxygenates arachidonic acid to form prostaglandin (PG) $G_2$ that is further reduced to $PGH_2$. $PGH_2$ is a highly unstable endoperoxide that is rapidly converted by specific synthases to PGs of the E, F and D series and also to $PGI_2$ (prostacyclin) and thromboxane (TX) $A_2$. Both $PGI_2$ and $TXA_2$ have very short half-lifes and are rapidly hydrolyzed to the inactive compounds 6-keto-$PGF_{1\alpha}$ and $TXB_2$, respectively. $PGD_2$ undergoes nonenzymatic dehydration, losing water to form the cyclopentenone 15-deoxy-$\Delta^{12\text{-}14}$-$PGJ_2$ (15d-$PGJ_2$). The biological effects of PGs are mediated by ten different types and subtypes of receptors, which belong to the G protein-coupled rhodopsin-type receptor superfamily of seven transmembrane domains. Four of the receptor subtypes bind $PGE_2$ (EP1, EP2, EP3 and EP4), two bind $PGD_2$ (DP1 and DP2), two bind $TXA_2$ (TPα and TPβ) and the rest are single receptors for $PGF_{2\alpha}$ and $PGI_2$ (FP and IP, respectively). 15d-$PGJ_2$ is a natural ligand of PPARγ.

role for these omega-3-derived anti-inflammatory lipid mediators in WAT adipogenesis and lipolysis still remains elusive.

## Signaling Pathways Involved in the Biological Actions of $PGE_2$ and Other Lipid Mediators

$PGE_2$ binds four different receptor subtypes designated EP1, EP2, EP3, and EP4 (**Fig. 1**).[36] These receptor subtypes differ in their signal transduction pathways: EP1 is coupled to the mobilization of intracellular $Ca^{2+}$ stores; EP2 and EP4 are coupled to the stimulation of adenylate cyclase whereas EP3 is coupled to the inhibition of adenylate cyclase.[36] Early work on adipose tissue demonstrated that $PGE_2$ increases oxygen consumption in rat brown adipose tissue via EP1 receptor.[37] More recent work has demonstrated that $PGE_2$ may suppress 3T3-L1 adipocyte differentiation by binding to EP4 and eliciting an increase in intracellular cAMP levels in preadipocytes.[38] On the other hand, addition of the $PGD_2$ metabolite 15d-$PGJ_2$, which is a natural PPARγ ligand, to human adipocytes inhibits the secretion of pro-inflammatory adipokines and more importantly stimulates adipogenesis.[39] 15d-$PGJ_2$ also exerts proadipogenic actions in fibroblasts, although in this case lymphocytes are the source of this cyclopentenone PG.[40] Surprisingly, impaired adipogenic program has been identified in 3T3-L1 cells with stable transfection of PGD synthase and appreciably higher levels of endogenous $PGD_2$-derived metabolites, suggesting a complex regulatory interaction between PPARγ and proadipogenic lipid mediators.[41] Finally, 5-LOX-derived LTs signal through two receptors for $LTB_4$ (BLT-1 and BLT-2) and two receptors for cys-LTs (CysLT1 and CysLT2).[32] Of interest, mice deficient for the $LTB_4$ receptor BLT-1 show reduced monocyte recruitment to hypertrophied adipose tissue and decreased adipose tissue inflammation and insulin resistance.[42]

## Microsomal PGE Synthase-1 (mPGES-1) is Involved in a White-to-Brown Transition of the Adipogenic Program

A coordinate interaction between mPGES-1 and PPARγ in controlling the process of preadipocyte differentiation in WAT has recently been identified.[34] mPGES-1 is an inducible enzyme that cooperates with COX-2, the first upstream enzyme of the PG biosynthetic cascade, in the biosynthesis of $PGE_2$.[43] Several studies have previously linked the COX pathway and $PGE_2$ to the adipogenic program, although their results have yielded controversial views. For example, preadipocytes stably transfected with either COX-1 or COX-2 were shown to have a lower PPARγ expression and to exhibit a suppression of the adipogenic program.[44] This finding is consistent with the observation that mice genetically deficient for mPGES-1 show basal elevations in PPARγ expression and transcriptional activity of this nuclear receptor.[45] In contrast, PPARγ appears to be downregulated in adipose tissue from COX-2 deficient mice, which show an attenuation in adipocyte differentiation.[46] Also, Kim and collaborators recently described that a COX-2 dependent metabolism of 20-hydroxy-eicosatetraenoic acid (20-HETE) induces PPARγ and the adipogenic program in mesenchymal stem cell-derived adipocytes.[47] On the other hand, COX-2-deficient mice provided the first proof of concept that UCP1 expression in WAT is dependent on COX activity.[48] A recent study by Vegiopoulos and collaborators has demonstrated that transgenic mice overexpressing COX-2 did not exhibit changes in PPARγ expression in adipose tissues.[49] An interesting finding of this study was that overexpression of COX-2 was associated with de novo recruitment of brown adipocytes in WAT, suggesting that PGs may be involved in the browning of WAT.[49] However, the study by Vegiopoulos and collaborators poses the limitation that in addition to $PGE_2$, COX-2 activity gives rise to other PGs, including $PGI_2$, $PGF_{2\alpha}$, and $PGD_2$, and therefore, this study left unanswered the relative role of each individual PG in the development of brown adipocytes in WAT. Moreover, this study did not provide a plausible hypothesis whether WAT beige cells come from progenitor cells (preadipocytes) pushed to develop into beige/brown adipocytes by PGs or whether PGs induce the direct conversion of differentiated white adipocytes into beige cells.

In our study, we obviated these limitations by focusing our interest on mPGES-1, a COX-2 down-stream terminal synthase responsible for the biosynthesis of $PGE_2$, and by exploring the direct actions of this lipid mediator on preadipocytes isolated from WAT.[34] Using this approach, we were able to gather evidence that mice lacking PPARγ specifically in the adipose tissue are resistant to gain body weight and to increase WAT volume by mechanisms linked to $PGE_2$ formation.[34] Indeed, the results from our study support a coordinated negative regulation between PPARγ and $PGE_2$. On one hand, mice deficient in PPARγ showed increased expression of COX-2 and mPGES-1 and augmented $PGE_2$ levels. On the other hand, exogenous $PGE_2$ was able to suppress PPARγ expression whereas opposite effects were seen after inhibition of endogenous $PGE_2$ biosynthesis in adipose tissue.[34] Moreover, this mPGES-1-derived product was able to divert preadipocyte differentiation in WAT to beige/brite mature adipocytes accompanied by upregulation of UCP1. A proof of concept of the role of mPGES-1 was obtained by inhibiting either the expression or the activity of this terminal enzyme. In particular, the addition of a selective pharmacological mPGES-1 inhibitor as well as a siRNA directed against mPGES-1 to preadipocytes resulted in the reduction of browning markers (i.e., UCP1, CIDEA, and PGC-1α) and browning determination factors (i.e.,

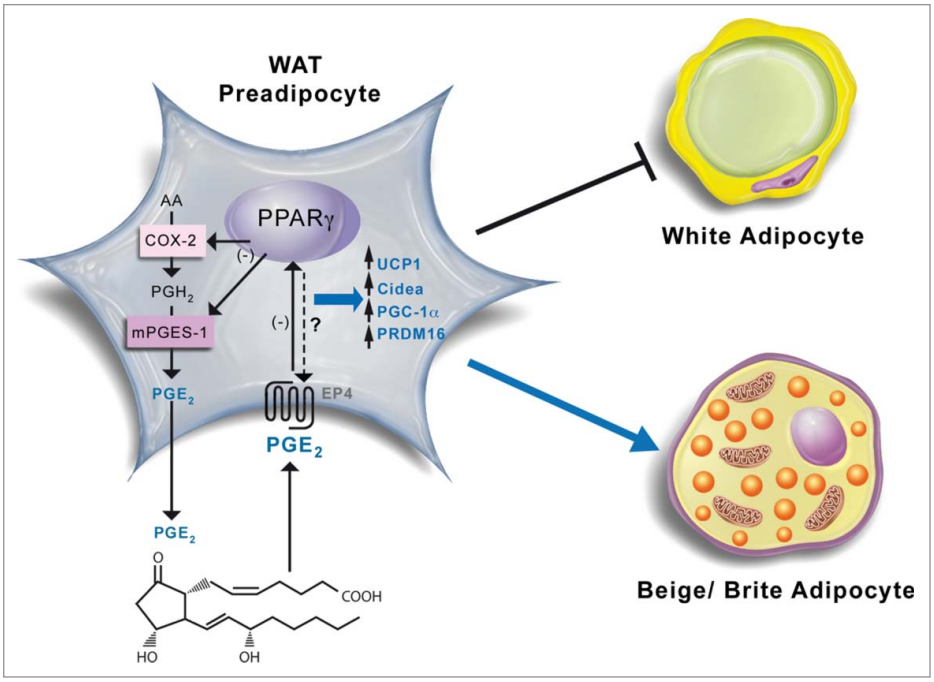


**Figure 2.** Schematic diagram of the proposed coordinated functional regulation of microsomal prostaglandin E (PGE) synthase-1 (mPGES-1) and peroxisome proliferator-activated receptor γ (PPARγ) in beige/brite adipogenesis in white adipose tissue (WAT). In WAT preadipocytes, mPGES-1 cooperates with cyclooxygenase-2 (COX-2) in the biosynthesis and release of prostaglandin (PG) $E_2$ from arachidonic acid. By binding to its receptors (presumably $PGE_2$ receptor EP4 subtype), $PGE_2$ is able to downregulate PPARγ expression, which in turn suppresses COX-2 and mPGES-1 expression in an autocrine fashion. Furthermore, the interaction between $PGE_2$ and PPARγ has the ability to induce brown adipogenic genes such as uncoupling protein 1 (UCP1), cell death-inducing DFFA-like effector a (CIDEA), PPARγ coactivator-1α (PGC-1α), and PR domain containing 16 (PRDM16), which divert pre-adipocyte differentiation into beige/brite adipocytes instead of white adipocytes.

PRDM16).[34] A schematic diagram of the hypothetical coordinated functional regulation between mPGES-1 and PPARγ in beige/brite adipogenesis is shown in **Figure 2**.

The mechanisms by which the COX-2/mPGES-1/$PGE_2$ axis and the nuclear receptor PPARγ interact during the process of adipogenesis are not completely delineated but might be related to the ability of $PGE_2$ to increase intracellular cAMP, which is a well-known mediator of the induction of "brown fat-like" cells residing in the WAT.[50] Another mechanism potentially implicated is the stabilization of several dominant transcriptional regulators of white-to-brown adipocyte development and function, including PRDM16.[51] In fact, a reduction in PRDM16 was observed in adipocytes incubated with an inhibitor of mPGES-1 activity or transfected with a siRNA that induce gene silencing of this terminal PG synthase.[34] Apparently, this process requires the integrity of both systems because $PGE_2$ was not able to inhibit white adipocyte differentiation in the absence of a PPARγ agonist.[34] Moreover, $PGE_2$ was able to directly induce the browning of WAT (i.e., upregulating UCP1) in wild-type mice but not in mice lacking PPARγ specifically in adipocytes.[34] Together, these findings are relevant in terms of energy homestasis because the engagement of beige/brite adipocytes and the induction of a thermogenic program in WAT depots are able to waste the surplus of energy through increased heat production, which ultimately exerts protection against obesity and obesity-related comorbidities.

## Summary and Future Perspectives

A wealth of new evidence supports the concept that browning of WAT has a therapeutic potential in fighting the metabolic complications associated with obesity. It is interesting to mention that even small amounts of active brown adipose tissue lead to an enhanced energy expenditure and to the promotion of weight loss accompanied by reductions in the incidence of obesity comorbidities such as type 2 diabetes and cardiovascular disease.[52] A plethora of novel factors carrying capacity to induce brown adipocyte differentiation has been recently identified, although the understanding of the mechanisms underlying their actions is still elusive. The observation that exposure of preadipocytes from WAT origin to the bioactive lipid mediator $PGE_2$ results in a browning effect during the adipocyte differentiation process adds value to the fact that COX-2-activity induces de novo recruitment of brown adipocytes in WAT. In summation, these studies point into the COX-2/mPGES-1/$PGE_2$ axis as a key regulator of the browning process in WAT. Considering that in addition to promote the browning and heat dissipating function in WAT, the COX-2/mPGES-1/$PGE_2$ pathway also has a direct responsibility in mounting inflammatory responses, further studies are needed to fully integrate the duality of $PGE_2$ actions in obese conditions. This is of special interest in the context of the "low-grade" inflammatory state present in obese WAT, which is directly linked to the development of the obesity-associated metabolic complications. Further studies are also needed to understand whether the approach of inducing the browning of WAT by bioactive lipid mediators described in animal models is also efficacious in humans. Finally, an important aspect that also needs further investigation is whether $PGE_2$-induced activation of beige/brite adipocytes takes place not only in lean individuals but also in WAT from obese patients.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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