Roles of PPARs in health and disease

Sander Kersten, Béatrice Desvergne & Walter Wahli

Institut de Biologie Animale, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland

In developed societies, chronic diseases such as diabetes, obesity, atherosclerosis and cancer are responsible for most deaths. These ailments have complex causes involving genetic, environmental and nutritional factors. There is evidence that a group of closely related nuclear receptors, called peroxisome proliferator-activated receptors (PPARs), may be involved in these diseases. This, together with the fact that PPAR activity can be modulated by drugs such as thiazolidinediones and fibrates, has instigated a huge research effort into PPARs[1]. Here we present the latest developments in the PPAR field, with particular emphasis on the physiological function of PPARs during various nutritional states, and the possible role of PPARs in several chronic diseases.

The PPARs were first cloned as the nuclear receptors that mediate the effects of synthetic compounds called peroxisome proliferators on gene transcription. It soon became clear that eicosanoids and fatty acids can also regulate gene transcription through PPARs. At the molecular level, PPARs act in a similar manner to other nuclear hormone receptors. First, they bind a specific element in the promoter region of target genes. PPAR and some other nuclear hormone receptors bind the promoter only as a heterodimer with the receptor for 9-*cis* retinoic acid, RXR (retinoid X receptor). Second, they activate transcription in response to binding of the hormone (ligand) (Fig. 1a). For the PPAR:RXR heterodimer, binding of the ligand of either receptor can activate the complex, but binding of both ligands simultaneously is more potent[1].

Three PPAR isotypes have been identified: α, β (also called δ and NUC1) and γ. PPARα is expressed most in brown adipose tissue and liver, then kidney, heart and skeletal muscle. PPARγ is mainly expressed in adipose tissue, and to a lesser extent in colon, the immune system and the retina. PPARβ is found in many tissues but the highest expression is in the gut, kidney and heart[1].

PPARs are ligand-dependent transcription factors: activation of target gene transcription depends on the binding of the ligand to the receptor. Some ligands are shared by the three isotypes, such as polyunsaturated fatty acids and probably oxidized fatty acids. Several compounds bind with high affinity to PPARα, including long-chain unsaturated fatty acids such as linoleic acid, branched, conjugated and oxidized fatty acids such as phytanic acid and conjugated linolenic acid, and eicosanoids such as 8S-HETE and leukotriene (LT) B_4[1–4] (Fig. 1b). This last compound is particularly interesting, as a membrane receptor for LTB_4 has also been cloned. The functional relationship between these two types of receptor is not clear. The prostaglandin 15-deoxy-$D^{12,14}$-prostaglandin J_2 is the most potent natural ligand of PPARγ, but the extent to which its *in vivo* effects are mediated through PPARγ is not known.

For a review of PPARs, particularly their molecular mode of action, see ref. 1 and references therein (including many not cited here for reasons of space).

PPAR function at the cellular level

Much of the function of PPARs can be extrapolated from the identity of their target genes, which so far all belong to pathways of lipid transport and metabolism. PPARα has mostly been studied in the context of liver parenchymal cells, where it is highly expressed. The target genes of PPARα are a relatively homogenous group of genes that participate in aspects of lipid catabolism such as fatty acid uptake through membranes, fatty acid binding in cells, fatty acid oxidation (in microsomes, peroxisomes and mitochondria) and lipoprotein assembly and transport (Fig. 2).

Whereas PPARα operates in the catabolism of fatty acids in the liver, PPARγ influences the storage of fatty acids in the adipose tissue (Fig. 2). With the C/EBP transcription factors, PPARγ is part of the adipocyte differentiation program that induces the maturation of pre-adipocytes into fat cells[5]. Most of the PPARγ target genes in adipose tissue are directly implicated in lipogenic pathways, including lipoprotein lipase (LPL), adipocyte fatty acid binding protein (A-FABP or aP2), acyl-CoA synthase and fatty acid transport protein (FATP).

PPARβ has received little attention, probably because of the lack of a connection with important clinical manifestations. However, PPARβ is linked to colon cancer (see below), among other functions. PPARβ regulates the expression of acyl-CoA synthetase 2 in the brain, linking PPARβ to basic lipid metabolism[6]. Moreover, it probably participates in embryo implantation and decidualization[7]. These data will spur new interest in the study of PPARβ function.

PPARs in whole body physiology

The role of these transcription factors in whole body human physiology and metabolism can best be illustrated by comparing two opposite nutritional states: early absorptive period or fed state and late post-absorptive period or fasting state. In the fed state, which in humans is up to 4 h after a large meal, carbohydrates and fat enter the circulation in the form of glucose and chylomicrons, respectively (Fig. 3a). Most glucose is taken up by the liver and, if

Box 1

Gene targeting and human molecular genetics

Use of genetic approaches such as gene targeting and human molecular genetics has led to major advances in our knowledge about the physiological role and medical significance of PPARγ. Unlike *PPARα* null mice, which have no obvious phenotype unless fasted, null mice for *PPARγ* die as embryos[13,26], *PPARγ* null embryos that are rescued to term have no visible white adipose tissue and a fatty liver[26]. Also, embryonic stem cells lacking PPARγ are unable to differentiate into adipocytes *in vitro*[5]. Both results show the contribution of PPARγ to adipogenesis.

The function of PPARγ in improving insulin sensitivity is controversial. In disagreement with the purported anti-diabetic effect of PPARγ, heterozygous PPARγ mice, which are viable, are less prone to insulin resistance on a high fat diet[13]. Remarkably, this protection is lost when these animals are treated with pioglitazone, a synthetic PPARγ ligand belonging to the TZD class. Tissue-specific gene targeting should soon clarify some of the discrepancies.

Human genetic studies give important clues about the function of PPARγ in mammalian metabolism and its link to certain chronic diseases. Four single amino-acid substitutions within human PPARγ have been described. A P12A mutation at the extreme amino-terminus of PPARγ is most common, but its effect on weight gain and insulin sensitivity is unclear[27–29]. A P115Q mutation was identified in four extremely obese patients with surprisingly little defect in insulin sensitivity. Very recently, two new mutations were found in three patients suffering from severe insulin resistance but not obesity[30]. Overall the data suggest that PPARγ promotes fat storage and insulin sensitivity, but additional studies are needed to confirm these findings.

glycogen stores are already filled, used for lipogenesis. The amount of the transcription factor sterol response element binding protein 1 (SREBP1, also called ADD1) rises in the fed state, which promotes the glycolytic conversion of glucose into acetyl-CoA and subsequently the synthesis of fatty acids from acetyl-CoA[8,9]. Fatty acids are converted to triglycerides and packaged into very low density lipoproteins (VLDL).

In adipose tissue, the amounts of SREBP and PPARγ are elevated, probably because of regulation by insulin[10]. PPARγ is a direct target gene of SREBP[11], which emphasizes the cooperative and additive functions between these two types of receptor. In addition, SREBP1 may be involved in producing an endogenous ligand (probably fatty acid) for PPARγ. The overall effect is stimulation of the uptake of glucose and fatty acids, and their subsequent conversion to triglycerides.

Triglyceride storage causes increased production of the hormone leptin in the adipose tissue. Leptin is the protein product of the *ob* gene, whose deletion leads to severe obesity in mice. Its expression is increased by long-term overfeeding as part of a feedback mechanism to limit further food intake and weight gain. Consistent with the role of PPARγ in promoting lipogenesis, production of leptin in adipose tissue is under negative control by PPARγ. Paradoxically, expression of both PPARγ and leptin is reduced by fasting and increased by feeding. In the latter case, PPARγ may attenuate the increase in leptin expression to limit wasteful lipolysis and fatty acid oxidation, processes which are stimulated by leptin[12]. If the above scenario is correct, decreased PPARγ expression may lead to increased leptin levels and, as a result, to lower food intake and weight gain. Studies with *PPARγ*+/– mice indicate that this is the case[13].

A different situation exists in the late post-absorptive or fasting state (Fig. 3b). In the liver, fatty acids are oxidized to acetyl-CoA and subsequently to ketone bodies, such as acetoacetate and β-hydroxybutyrate. Both processes are strongly stimulated by PPARα, expression of which is elevated upon fasting[14]. Fatty acids are ligands for PPARs, so it is possible that the large amounts of fatty acids liberated from the adipose tissue can stimulate their own metabolism by activating PPARα. Experiments with *PPARα* null mice show that PPARα is important in the hepatic response to fasting. When fasting, these mice suffer from a defect in fatty acid oxidation and ketogenesis, resulting in elevated plasma free fatty acids, hypoketonaemia, hypothermia and hypoglycaemia[14,15]. The hypoglycaemia emphasizes the important interplay between fatty acid and glucose metabolism in energy homeostasis.

In adipose tissue, expression of SREBP and PPARγ is low under fasting conditions. Under a strong adrenergic stimulus, triglycerides are hydrolysed to fatty acids and glycerol, but some of the released fatty acids are re-esterified to triglycerides, in a reaction that requires synthesis of glycerol from gluconeogenic precursors. The rate-limiting step for glyceroneogenesis is catalysed by phosphoenolpyruvate carboxykinase, whose transcription is positively controlled by PPARγ. Thus, even under highly catabolic conditions such as fasting, lipogenesis continues and is dependent upon PPARγ.

Therapeutic potential of PPAR ligands

In developed societies, metabolic disorders such as hyperlipidaemia, atherosclerosis, diabetes and obesity rarely occur in isolation, but are usually part of a complex phenotype of metabolic abnormalities called syndrome X. Synthetic agonists for both PPARα (fibrates) and PPARγ (thiazolidinediones; TZDs) are useful in the treatment of the diseases that are part of this syndrome.

Synthetic PPARγ ligands are used for their potent antidiabetic effects. In the United States, three TZDs, troglitazone (Rezulin), rosiglitazone (Avandia) and pioglitazone (Actos), are approved for use in type II diabetic patients. They bind PPARγ with moderate (troglitazone) to high (rosiglitazone) affinity, so it is believed that their hypoglycaemic effect is exerted by activating PPARγ. However,

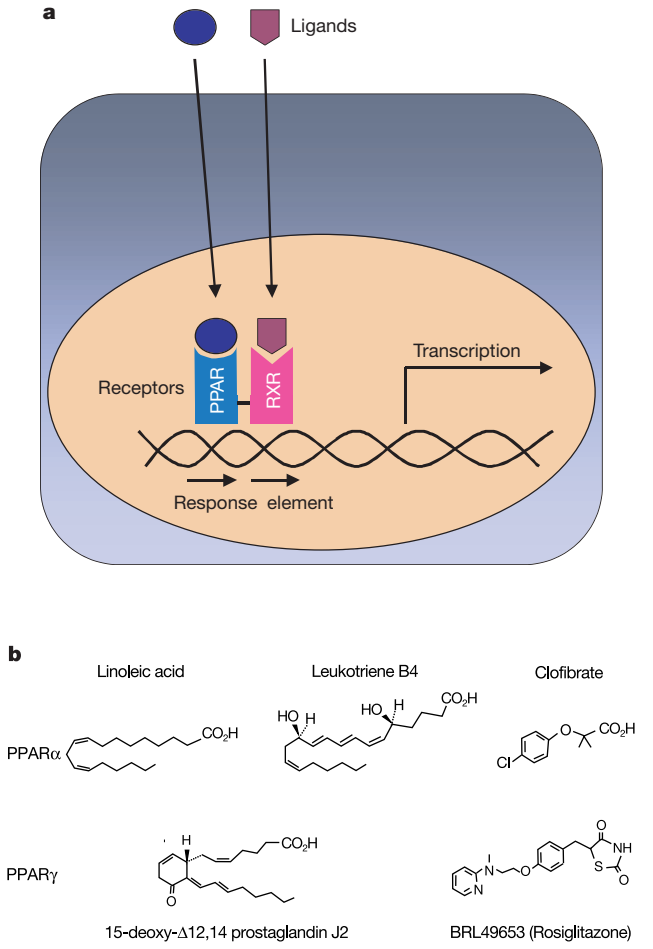

Figure 1 PPARs at the basic level. **a**, Basic mechanism of action of nuclear hormone receptors. Nuclear hormone receptors bind to a specific sequence in the promoter of target genes (called hormone response elements), and activate transcription upon binding of ligand. Several nuclear hormone receptors, including the retinoic acid receptor, the vitamin D receptor and PPAR, can bind to DNA only as a heterodimer with the retinoid X receptor, RXR, as shown. **b**, Structure of some PPARα and PPARγ ligands.

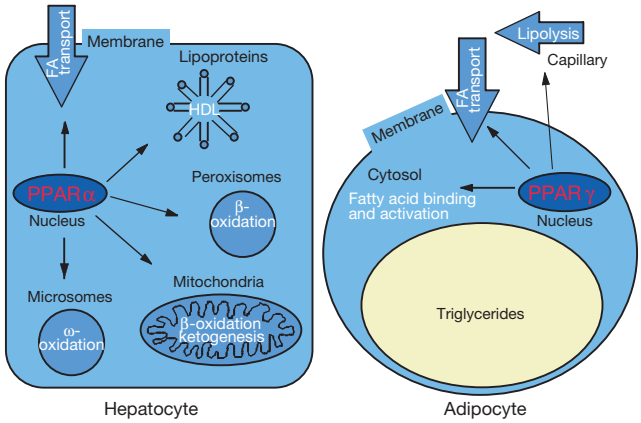

Figure 2 Action of PPARα and PPARγ at the cellular level. PPARα stimulates oxidation of fatty acids in various organelles, such as mitochondria, peroxisomes and microsomes. It also stimulates uptake of fatty acids and synthesis of lipoproteins. PPARγ stimulates lipolysis of circulating triglycerides and the subsequent uptake of fatty acids into the adipose cell. It also stimulates binding and activation of fatty acids in the cytosol, events that are required for synthesis of triglycerides. FA, fatty acid; HDL, high density lipoprotein.

a direct connection between PPARγ and glucose homeostasis has not been easy to establish because skeletal muscle, which accounts for the TZD-mediated increase in glucose disposal, expresses only trace amounts of PPARγ. To explain this paradox, a mechanism has been proposed by which TZDs divert fatty acids away from skeletal muscle by increasing their uptake in adipose tissue, and so reduce the deleterious effects of fatty acids on muscle insulin action. In this model, the hypoglycaemic effect of TZDs is secondary to their hypolipidaemic effect. However, mice that lack adipose tissue can still benefit from the action of TZDs, indicating that adipose tissue is dispensable for mediating the hypoglycaemic effects of TZDs. It is also possible that the effect of TZDs on glucose homeostasis might be via an alternative mechanism not involving PPARγ, as is the case for the inhibitory effect of troglitazone on cholesterol synthesis[16]. New ligands specific for PPARγ will be useful in refining these observations and working out the mechanisms of glucose homeostasis.

Fibrates are potent hypolipidaemic drugs. In the past few years they have been used increasingly to treat cardiovascular disease. Fibrates, which include gemfibrozil, bezafibrate and fenofibrate, bind PPARα with high affinity and it is believed that most of their effects on disease progression are mediated by PPARα. Fibrates lower plasma triglyceride levels markedly and increase high density lipoprotein (HDL) levels. The former effect occurs by stimulating hepatic fatty acid oxidation and reducing apoCIII expression, whereas the latter effect is due to induction of apolipoprotein-AI and apolipoprotein-AII expression, both mediated by PPARα. Fibrates may also have a hypoglycaemic and thus anti-diabetic effect, as a consequence of their hypolipidaemic action. Accordingly, it is interesting to compare PPARα agonists and PPARγ agonists. Though they act through different receptor isotypes, both groups of compound have potent hypolipidaemic properties which may be at the basis of their hypoglycaemic effect. Future studies will have to establish whether fibrates or other PPARα agonists may be applicable in the treatment of type II diabetes.

The heterodimer between PPAR and RXR can also be activated by ligand binding to RXR. Synthetic ligands specific for RXR have been tested as an alternative treatment for diabetes. Compounds such as LG1069 and LG100268 have a potent hypoglycaemic effect in animal studies. The effects of these RXR agonists on glucose homeostasis have not been fully established, but they are probably mediated by the PPAR:RXR heterodimer.

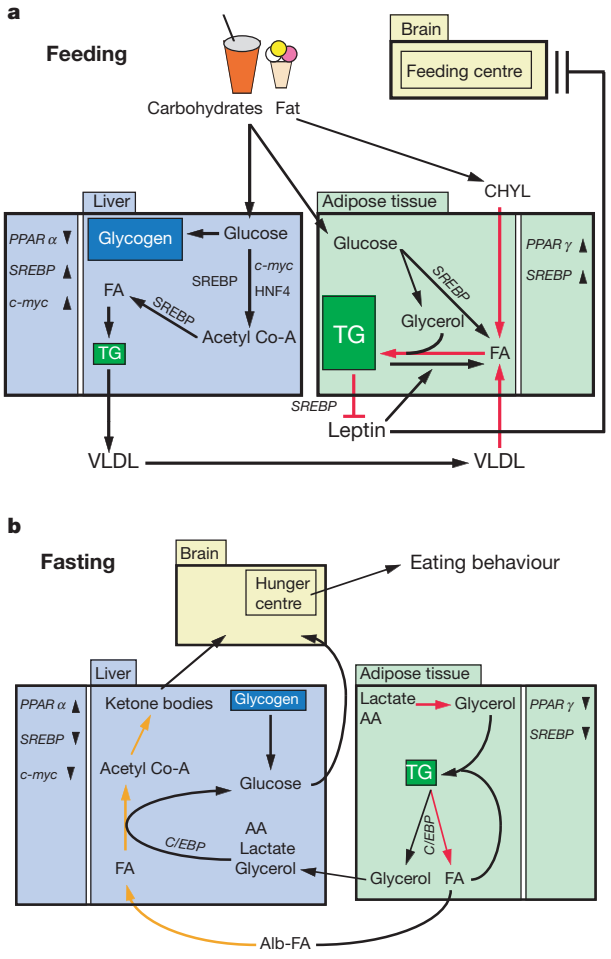

Figure 3 Overview of the roles of PPARα and PPARγ during feeding and fasting. **a**, PPARγ is mainly active in the fed state when it stimulates accumulation of fat in the adipose tissue. **b**, PPARα is mainly active in the fasting state when it stimulates oxidation of fatty acids in the liver. The particular metabolic steps that are regulated by PPARα and PPARγ are shown in orange and red, respectively. The roles of the transcription factors C/EBP, c-myc, HNF4 and SREBP, which are transcriptional regulators of metabolism, have been illustrated. The expression of the indicated proteins is increased (▲) or decreased (▼). AA, amino acids; CHYL, chylomicrons; FA, fatty acids; TG, triglycerides.

Atherosclerosis

Atherosclerosis is a complex disease to which many factors contribute. It is characterized by a gradual build-up of lipids in the arterial wall (atherosclerotic plaque), often leading to sudden obstruction of blood flow after rupture of this plaque. Endothelial dysfunction resulting in chronic inflammation of the vascular wall, proliferation of smooth muscle cells and formation of foam cells is important in the development of atherosclerosis. As well as regulating plasma lipoprotein concentrations, PPARα and PPARγ may affect foam cell formation, modulate the inflammatory response and influence plaque stability. PPARα may also decrease the plasma concentration of pro-atherosclerotic proteins such as fibrinogen and C-reactive protein[17].

In 1996 it was proposed that PPARα may be involved in inflammation, as it is the nuclear receptor for the eicosanoid LTB_4. Mice lacking PPARα display a prolonged response to an inflammatory stimulus, indicating that PPARα has an anti-inflammatory action[18]. Fibrates can modulate inflammation by inhibiting cytokine (TNFα, interleukins) production in a PPARα-dependent manner[19]. In contrast, fibrates markedly increase plasma tumour necrosis factor-α (TNFα) levels in mice, an effect also mediated by PPARα[20]. However, in rodents increased TNFα may be secondary to the hypolipidaemic and peroxisome proliferative effects of fibrates. It is clear that PPARα plays a role in the inflammatory process but much still needs to be learned about the details of its function.

PPARγ has also been implicated in inflammation. In monocytes/macrophages, PPARγ has been proposed to reduce cytokine (TNFα, interleukin-1β, interleukin-6) production by inhibiting the activity of pro-inflammatory transcription factors such as AP-1, STAT and NF-κ. This anti-inflammatory effect of PPARγ could be beneficial in the treatment of atherosclerosis. In addition, PPARγ may reduce expression of metalloproteinases such as MMP-9, which are implicated in plaque destabilization[21].

In contrast, PPARγ may promote atherosclerosis by stimulating the uptake of oxidized LDL, a critical event in foam cell formation. A feed-forward mechanism has been proposed whereby oxidized fatty acids that enter the cell through oxidized LDL can activate PPARγ, further stimulating uptake of oxidized LDL.

However, the effect of TZDs and 15-deoxy-$D^{12,14}$-prostaglandin J_2 on atherosclerosis and inflammation do not always correspond[22]. This suggests that at least one type of ligand, probably 15-deoxy-$D^{12,14}$-prostoaglandin J_2, acts independently of PPARγ, although PPARγ-independent effects of TZDs have been reported as well[16]. Use of antisense and/or gene-targeting technology should clarify the role of PPARγ in inflammation and atherosclerosis. Without this information and without clear data from clinical trials, the link between PPARγ and atherosclerosis remains speculative.

Cancer

PPARα mediates the hepatocarcinogenic effect of certain peroxisome proliferators in rodents. However, no carcinogenic effect of peroxisome proliferators has been found in humans, possibly because expression of PPARα is much lower in human liver than in rodent liver or due to other species-specific differences.

PPARγ has an anti-proliferative effect in pre-adipocytes and possibly in several malignant cell types. PPARγ ligands can induce terminal differentiation of human liposarcoma cells *in vitro* and in patients suffering from advanced liposarcoma[23]. PPARγ ligands also promote terminal differentiation of malignant breast epithelial cells *in vitro*, induce apoptosis and fibrosis of injected breast tumour cells (MCF-7) in mice, and reduce tumour incidence in rats treated with nitrosomethylurea[24]. An anti-tumour effect of PPARγ ligands was also observed in mice injected with prostate tumour cells (PC-3). Unfortunately, the picture is less clear for colon cancer. PPARγ ligands have been reported both to promote and to protect against colon cancer in mice. Interestingly, PPARβ has also been linked to colon cancer. It is a negative target of the APC gene, which is mutated in familial adenomatous polyposis, an inherited disease characterized by numerous colorectal adenomas[25]. In addition, the nonsteroidal anti-inflammatory drug (NSAID) sulindac, which suppresses colorectal tumorigenesis, can antagonize PPARβ. Thus PPARβ may be a critical intermediate in the tumorigenic pathway of the APC gene and be the molecular target for the effect of NSAIDs on colorectal cancer.

There are however two problems associated with the type of studies described above. First, it is difficult to extrapolate *in vitro* data and data from animal models to the human situation. Second, they make the possibly false assumption that all of the effects of PPAR ligands are mediated through activation of PPAR.

Conclusion

Since their discovery in the early 1990s it has become clear that PPARs are crucial in the genetic regulation of complex pathways of mammalian metabolism, including fatty acid oxidation and lipogenesis. Whereas PPARα promotes fatty acid oxidation under conditions of lipid catabolism such as fasting, PPARγ acts at the level of the adipose tissue and promotes lipogenesis under anabolic conditions. Much research is directed towards the identification of high-affinity, high-specificity agonists and antagonists for the treatment of hyperglycaemia, hyperlipidaemia and other metabolic diseases. Further advances in modern technology should assist in answering some of the more pertinent questions relating to PPARs, particularly with respect to the purported role of PPARs in atherosclerosis and cancer. □

1. Desvergene, B. & Wahli, W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* **20,** 649–688 (1999).
2. Lin, Q., Ruuska, S. E., Shaw, N. S., Dong, D. & Noy, N. Ligand selectivity of peroxisome proliferator activated receptor alpha. *Biochemistry* **38,** 185–190 (1999).
3. Ellinghaus, P., Wolfrum, C., Assmann, G., Spencer, F. & Seedorf, U. Phytanic acid activates the peroxisome proliferator-activated receptor alpha (PPARalpha) in sterol carrier protein 2-/sterol carrier protein x-deficient mice. *J. Biol. Chem.* **274,** 2766–2772 (1999).
4. Moya-Camarena, S. Y., Vanden Heuvel, J. P., Blanchard, S. G., Leesnitzer, L. A. & Belury, M. A. Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARalpha. *J. Lipid Res.* **40,** 426–1433 (1999).
5. Rosen, E. D. *et al.* PPAR is required for the differentiation of adipose tissue *in vivo* and *in vitro*. *Mol. Cell* **4,** 611–617 (1999).
6. Basu-Modak, S. *et al.* Peroxisome proliferator-activated receptor beta regulates acyl-CoA synthetase 2 in reaggregated rat brain cell cultures. *J. Biol. Chem.* **274,** 35881–35888 (1999).
7. Lim, H. *et al.* Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARδ. *Genes Dev.* **13,** 1561–1574 (1999).
8. Foretz, M. *et al.* ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Mol. Cell. Biol.* **19,** 3760–3768 (1999).
9. Foretz, M., Guichard, C., Ferre, P. & Foufelle, F. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic, expression of glucokinase and lipogenesis-related genes. *Proc. Natl Acad. Sci. USA* **96,** 12737–12742 (1999).
10. Rieusset, J. *et al.* Insulin acutely regulates the expression of the peroxisome proliferator-activated receptor-gamma in human adipocytes. *Diabetes* **48,** 699–705 (1999).
11. Fajas, L. *et al.* Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. *Mol. Cell. Biol.* **19,** 5495–5503 (1999).
12. Wang, M. Y., Lee, Y. & Unger, R. H. Novel form of lipolysis induced by leptin. *J. Biol. Chem.* **274,** 17541–17544 (1999).
13. Kubota, N. *et al.* PPAR mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell* **4,** 597–609 (1999).
14. Kersten, S. *et al.* Peroxisome proliferator activated receptor alpha mediates the adaptive response to fasting. *J. Clin. Invest.* **103,** 1489–1498 (1999).
15. Leone, T. C., Weinheimer, C. J. & Kelly, D. P. A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PARalpha-null mouse as a model of fatty acid oxidation disorders. *Proc. Natl Acad. Sci. USA* **96,** 7473–7478 (1999).
16. Wang, M., Wise, S. C., Leff, T. & Su, T. Z. Troglitazone, an antidiabetic agent, inhibits cholesterol biosynthesis through a mechanism independent of peroxisome proliferator-activated receptor-gamma. *Diabetes* **48,** 254–260 (1999).
17. Kockx, M. F. *et al.* Fibrates suppress fibrinogen gene expression in rodents via activation of the peroxisome proliferator-activated receptor-alpha. *Blood* **93,** 2991–2998 (1999).
18. Devchand, P. R. *et al.* Chemical probes that differentially modulate peroxisome proliferator-activated receptor alpha and BLTR, nuclear and cell surface receptors for leukotriene B(4). *J. Biol. Chem.* **274,** 23341–23348 (1999).
19. Delerive, P. *et al.* Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J. Biol. Chem.* **274,** 32048–32054 (1999).
20. Hill, M. R. *et al.* Effect of peroxisome proliferator-activated receptor alpha activators on tumor necrosis factor expression in mice during endotoxemia. *Infect. Immun.* **67,** 3488–3493 (1999).
21. Ricote, M., Huang, J. T., Welch, J. S. & Glass, C. K. The peroxisome proliferator-activated receptor (PPARgamma) as a regulator of monocyte/macrophage function. *J. Leukoc. Biol.* **66,** 733–739 (1999).
22. Thieringer, R. *et al.* Activation of peroxisome proliferator-activated receptor gamma does not inhibit IL-6 or TNF-α responses of macrophages to lipopolysaccharide *in vitro* and *in vivo*. *J. Immunol.* **164,** 1046–1054 (2000).
23. Demetri, G. D. *et al.* Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-gamma ligand troglitazone in patients with liposarcoma. *Proc. Natl Acad. Sci. USA* **96,** 3951–3956 (1999).
24. Suh, N. *et al.* A new ligand for the peroxisome proliferator-activated receptor-gamma (PPAR-gamma), GW7845, inhibits rat mammary carcinogenesis. *Cancer Res.* **59,** 5671–5673 (1999).
25. He, T. C., Chan, T. A., Vogelstein, B. & Kinzler, K. W. PPARdelta is an APC-regulated target of nonsteroidal anti-inflammatory drugs. *Cell* **99,** 335–345 (1999).
26. Barak, Y. *et al.* PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol. Cell* **4,** 585–595 (1999).
27. Valve, R. *et al.* Two polymorphisms in the peroxisome proliferator-activated receptor-gamma gene are associated with severe overweight among obese women. *J. Clin. Endocrinol. Metab.* **84,** 3708–3712 (1999).
28. Ek, J. *et al.* Homozygosity of the Pro12A1a variant of the peroxisome proliferation-activated receptor-gamma2 (PPAR-gamma2): divergent modulating effects on body mass index in obese and lean Caucasian men. *Diabetologia* **42,** 892–895 (1999).
29. Mancini, F. P. *et al.* Pro12A1a substitution in the peroxisome proliferator-activated receptor-gamma2 is not associated with type 2 diabetes. *Diabetes* **48,** 1466–1468 (1999).
30. Barroso, I. *et al.* Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature* **402,** 880–883 (1999).

Acknowledgements

S.K. was supported by fellowships from the European Molecular Biology Organization and the Roche Research Foundation. Work in the author's laboratory is financed by the Swiss National Science Foundation, the Etat de Vaud, and Human Frontier Science Programme.

Correspondence should be addressed to W.W. (e-mail: walter.wahli@iba.unil.ch).