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REVIEW

New Therapeutic Target for Metabolic Syndrome: PPARδ

SADAO TAKAHASHI, TOSHIYA TANAKA* AND JURO SAKAI*,**

Third Department of Internal Medicine, Faculty of Medical Sciences, University of Fukui, Fukui 910-1193, Japan
**Laboratory for Systems Biology and Medicine, Research Center for Advanced Science and Technology, University of Tokyo, Komaba 153-8904, Japan*
***Exploratory Research for Advanced Technology of Japan Science and Technology Corporation, Aomi 153-0064, Japan*

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PEROXISOME proliferator-activated receptors (PPARs) are ligand-activated transcriptional factors belonging to superfamily of nuclear hormone receptors related to retinoid, steroid, and thyroid hormone receptors. Three subtypes including PPARα, γ, and β/δ or δ (from now on called PPARδ for simplicity) have been described. PPARs need to heterodimerize with the 9-*cis*-retinoic acid receptor (RXR) to be transcriptional active. PPAR-RXR heterodimers bind to DNA specific sequences called PPAR responsive elements (PPREs) and regulate gene expression programs of fatty acid uptake and oxidation, lipid metabolism and inflammation [1–3]. The three isoforms are the products of distinct genes: the human PPARα gene is mapped on chromosome 22 in the general region 22q12-q13.1, the PPARγ gene is located on chromosome 3 at position 3p25, whereas PPARδ gene is assigned to chromosome 6, at position 6p21.1-p21.2. The roles of PPARα and PPARγ in particular have been exclusively studied because their agonists have been used in the clinical market as hypolipidemic fibrates and insulin sensitizers, thiazolidinediones (TZDs) respectively. PPARα is mainly expressed in heart, skeletal muscle, kidney, liver, and intestine. Fasted PPARα –/– (knockout) mice show enhanced accumulation of lipid in the liver and heart due to sufferings from severe hypoglycemia and hypothermia. It suggests that PPARα plays a crucial role in fatty acid uptake and oxidation in the liver and heart [4, 5]. Furthermore, PPARα is also involved in the intake of dietary long-chain FA in the gut, hepatic fatty acid synthesis, and control of inflammation. It has been established that fibrates is one of the PPARα ligands, but quest for the endogenous ligand is still ongoing. Mono- and polyunsaturated fatty acid (FA), eicosanoids, long-chain fatty acyl-CoAs, and saturated FAs all bind and activate PPAR α. On the other hand, PPARγ is highly rich in adipocytes and macrophages, and is involved in adipocyte differentiation, lipid storage, and glucose homeostasis. Thiazolidinediones (TZDs), which are selective ligands of PPARγ consistently lower fasting and postprandial glucose levels as well as free fatty acid levels. Like PPARα endogenous ligands, various unsaturated FAs, oxidized lipid species, eicosanoids, and prostaglandins have been shown to activate PPARγ. To the contrary, PPARδ is expressed ubiquitously with a less defined function because selective agonists have not been obtained. However, the development of selective PPARδ activators (GW501516, GW1514, GW0472, Compound F and GW610742) has allowed investigations on the functions of PPARδ to progress (Table 1).

Metabolic syndrome is composed of abdominal obesity, hyperinsulinemia due to insulin resistance, atherogenic dyslipidemia and hypertension [6]. The development of new treatments for metabolic syndrome is an urgent project for decreasing the prevalence of coronary heart disease and diabetes mellitus in the world. PPARs agonists have shed light on ef-

Correspondence to: Juro SAKAI, M.D., Ph.D., Laboratory for Systems Biology and Medicine, Research Center for Advanced Science and Technology, University of Tokyo, Komaba 153-8904, Japan

Table 1. Characterization of PPARα, γ, and δ

	Chromosomal location	Tissue distribution	Native activating factors	Main function	Synthetic agents
PPARα	22q12-q13.1	Liver Kidney Skeletal muscle Cardiac muscle	LTB_4 8S-HETE LPL + VLDL	Hepatic fatty acid oxidation	Fibrates Wy1339 Wy14643 GW647 GW957 GW7647
PPARγ	3p25	Adipocytes Macrophages	15d-PGJ_2 9-HODE 13-HODE	Lipid storage in adipocytes Adipocyte differentiation	TZDs GW1929 GW2090 GW7845 GW9820 AD5075
PPARδ	6p21.1-p21.2	Ubiquitous expressed	PGI_2 $cPGI_2$ Long-chain FA LPL + VLDL	Fatty acid β-oxidation and energy dissipation in skeletal muscle and adipose tissue Decreased glucose output in liver	GW501516 GW1514 GW0742 Compound F L165041 GW610742

LTB_4: leukotriene B_4, 8S-HETE: 8-(S)-hydroxyeicosatetraenoic acid, LPL: lipoprotein lipase, VLDL: very low density lipoprotein, 15d-PGJ_2: 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, HODE: hydroxyoctadecadienoic acid, PGI_2: prostacyclin, $cPGI_2$: carbaprostacyclin, FA: fatty acid

fective therapeutic approaches for correcting some aspect of metabolic syndrome. In this review, we will mainly focus on the recently elucidated function of PPARδ on metabolic syndrome.

Characterization of PPARδ

All PPARs bind to the PPRE on the DNA with the sequence AGGTCANAGGTCA (direct repeat with a single nucleotide spacer) as obligate heterodomers with the RXR. Their activation results in a transcriptional regulation of pathway that control energy homeostasis. For structure, PPARs have an NH_2-terminal region with a ligand-inactivation domain (AF-1), followed by a DNA binding domain (two zinc fingers) and, at the COOH-terminus, a ligand binding and dimerization domain and a ligand-dependent activation domain (AF-2). After the discovery of first member of the PPAR family (PPARα), Schmidt *et al.* discovered a novel member of the steroid hormone receptor superfamily cDNA as NUC1 (later on defined as PPAR β/δ) from a human osteosarcoma cell library [7]. In adult rat, PPARδ is expressed ubiquitously and is the most abundant of the three PPARs in most tissues except adipose tissue. PPARδ mRNA levels are dramatically down-regulated in the liver and kidney by up to 80% after an overnight fast. On the other hand, PPARα expression is up-regulated in the liver and small intestine, and PPARγ expression is decreased by 50% in adipose tissues in the fasted state in mature rat tissues [8]. Prostacyclin (PGI_2), carbaprostacyclin ($cPGI_2$), and saturated fatty acids are PPARδ ligands, but those ligands also activate PPARα or γ. That is the reason why knowledge of the function of PPARδ is emerging slowly. GlaxoSmithKline first identified a high-affinity PPARδ ligand, GW501516 that has more than 1,000-fold selectivity for PPARδ over other subtypes. Chawla *et al.* reported that native very low-density lipoprotein (VLDL) particles might be one of the endogenous sources of PPARδ ligands. Especially triglycerides in VLDL that is hydrolyzed by lipoprotein lipase (LPL) are an efficient activator of PPARδ in macrophages. PPARδ deficient macrophages lost the transcriptional response by VLDL particles [9]. Activation of PPARδ by VLDL particles induces carnitine biosynthesis and lipid mobilization, and subsequently to initiate a fat burning program to prevent macrophage from lipid overload [10]. Those findings are impressive because oxidized low-density lipoprotein (ox-LDL) activates PPARγ and induce the expression of the scavenger receptor, CD36 and the oxysterol

receptor, liver X receptor (LXRα). On the other hand, PPARδ activation induces adipose differentiation-related protein (ADRP) expression but does not induce CD36 or LXRα. It seems that PPARγ and PPARδ are related to ox-LDL and TG-rich lipoprotein respectively.

Three independent PPARδ –/– knockout mice lines

Three independent homozygous PPARδ –/– mice lines have been established. Gonzalez *et al.* ahead established PPARδ –/– mice lacking the last 60 amino acids of the ligand-binding domain [11]. PPARδ –/– mice are smaller than normal from the fetal stage to the postnatal period and myelination of the corpus callosum in brain is altered. Gonadal adipose stores are smaller and CD36 mRNA levels are higher. Enhanced hyperplasia of the epidermis has been detected in the skin of PPARδ –/– mice. They suggested that PPARδ is involved in placental and myelination defects, reduced adipose stores, and impaired wound healing. They observed no embryonic lethality on a pure C57BL/6N background but a lower than expected number of newborn homozygous pups on a mixed genetic background. Second Evans's group eliminated DNA binding domain of PPARδ gene in mice [12]. Over 90% of the embryos PPARδ –/– mice are prone to die due to placental defects and the mice that survive show an extremely lean phenotype. The difference between two knockout mice for lethality is likely due to the different targeting strategies. It is likely that the former truncated receptor mice might retain PPAR activity for survival. Wahli's group also indicated that only few PPARδ –/– mice could be obtained but no null mice line could be established for very high penetrance of a lethal phenotype [13]. Their group has continued to establish third PPARδ –/– mice lacking DNA binding domain and reported in detail that deletion of the PPARδ gene causes a severe failure of the placenta to undergo proper morphogenesis, leading to embryonic lethality at E9.5 to E 10.5 [14]. Those data are compatible to second generated PPARδ –/– mice by Evans's group.

Several *in vitro* studies proposed the impact of PPARδ in adipocyte differentiation [14–16]. Furthermore PPARδ –/– mice showed smaller and lean phenotype in all types of fat tissues *in vivo* [11, 12]. But adipose-specific PPARδ –/– mice do not affect adipose tissue mass. Those data indicate that the reduced adiposity in PPARδ –/– mice reflects a response of the tissue to an exogenous stimulus, rather than an intrinsic function of PPARδ in fat cell [12]. Expression of activated PPARδ in adipose tissue (ligand independent active form of PPARδ) leads to leanness but features of irregular adipogenesis are not shown, and no effect of PPARδ on adipocyte differentiation in 3T3-L1 cells is re-confirmed [18]. Those *in vivo* data suggest that the direct effect of PPARδ on adipose differentiation in adipose tissue *in vitro* is in the negative, and peripheral PPARδ functions in systemic lipid metabolism more than the direct effect on adipocyte differentiation that might be working in PPARδ –/– mice.

Lipoprotein metabolism and PPARδ

Elevated triglycerides and low levels of HDL (high-density lipoprotein)-cholesterol are one of the hallmarks of metabolic syndrome. It is well established that plasma levels of HDL-cholesterol are inversely related to the development of atherosclerosis. Furthermore the ATP-binding cassette A1 (ABCA1) protein has been identified as a regulator of reverse cholesterol transport [19]. Leibowitz *et al.* indicated that a weak nonselective PPARδ agonist L-165041, at dose that had no effect on either glucose or triglycerides, raised HDL-cholesterol levels in obese and diabetic db/db mice [20]. The high affinity PPARδ agonist, GW501516 for four weeks also dramatically improved serum lipid profiles, causing a 79% increase in HDL cholesterol level in insulin resistant obese rhesus monkeys, while lowering the levels of small-dense LDL (low density lipoprotein), fasting triglycerides, and fasting insulin. No changes in fasting glucose levels were detected. Those data indicated that PPARδ agonist might correct the hyperinsulinemia in insulin resistant monkeys *in vivo*. GW501516 also showed strong induction of ABCA1 mRNA expression and apoprotein(apo) A-I specific cholesterol efflux in macrophages *in vitro* [21]. Van der Veen *et al.* confirmed the effect of PPARδ agonist on HDL-cholesterol via ABCA1 [22]. Treatment with PPARδ agonist, GW610742 resulted in a 50% increase of plasma HDL-cholesterol in wild-type mice, whereas plasma cholesterol levels remained extremely low in ABCA1 –/– mice. They also found that GW610742 resulted in

a 43% reduction of fractional cholesterol absorption with a significantly reduced expression of the cholesterol absorption protein, Niemann-Pick C1-like 1 in the intestine. Akiyama *et al.* investigated a lipoprotein metabolism in first generated PPARδ –/– mice lacking the ligand-binding domain of the murine PPARδ gene [11, 23]. PPARδ –/– mice showed elevated plasma TG and free fatty acid (FFA), but similar total cholesterol (TC), free cholesterol (FC), and phospholipids (PL) on normal chow diet. During a high fat diet (HFD) challenge for 10 weeks, TG levels remained significantly elevated in the PPARδ –/– mice. PPARδ –/– mice have an increased rate of hepatic VLDL production as well as lowered lipoprotein lipase (LPL) activity with increased hepatic angiopoietin-like proteins (Angptl) 3 and 4 in PPARδ –/– mice compared to wild-mice. Angptl 3 and 4 proteins have been shown to inhibit LPL activity. Those data are compatible with hypotriglyceridemia that was observed in targeted activation of PPARδ [18]. However, in second generated PPARδ –/– mice (deletion of DNA binding domain of PPARδ gene) reported by Barak *et al.* [12], there are no changes in TC, TG, HDL-C and FFA with a normal chow diet; there is no data for lipoprotein profiles during a HFD challenge. In third generated PPARδ –/– mice, they did not examine a systemic lipoprotein metabolism [14]. The reason why there are different lipoprotein patterns in normal chow diet among PPARδ –/– mice lines is unclear. One plausible reason is their method for the deletion of the PPARδ gene like an embryonic lethality as mentioned above.

Atherosclerosis and PPARδ

Even though GW501516 increased expression of the reverse cholesterol transporter ABCA1 and induced apoA1-specific cholesterol efflux in macrophages *in vitro* [21]. Opposite findings for atherosclerosis have been reported. Vosper *et al.* reported that PPARδ agonist, compound F promoted lipid accumulation in human macrophages by increasing the expression of the class A and B scavenger receptors (SR-A and CD36) and adipophilin. The expression of ABCA1 was increased, but apoE and cholesterol 27-hydroxylase (CYP27) were repressed by PPARδ agonist. Overall total cholesterol efflux may not be increased because apoE and CYP27 are important mediators of lipid efflux from macrophages [24]. PPARδ was increased by 3–4 fold in vascular smooth muscle cells (VSMCs) treated with platelet-derived growth factor (PDGF) and overexpression of PPARδ in VSMCs increased post-confluent cell proliferation [25]. Those *in vitro* data suggest that PPARδ agonists may be pro-atherogenic in macrophage and VSMCs. Meanwhile it has been accepted that inflammation couples dyslipidemia to atheroma formation [26]. Welch *et al.* proposed that the effect of high concentration of PPARγ agonist is due to PPARδ activation, and established the overlapping transactivation and transrepression functions of PPARδ and PPARγ in macrophages. It seems that PPARδ activation may induce anti-inflammatory effect on macrophages because high dose of rosiglitazone (PPARγ agonist) inhibited induction of lipopolysaccharide target gene in PPARγ deficient macrophages in part by activating PPARδ [27].

The *in vivo* function of macrophage PPARδ in atherosclerosis has been tested with the transplantation of PPARδ –/– or wild-type bone marrow into LDL receptor –/– mice. After 8 weeks HFD, vascular lesion in PPARδ –/– recipients were at least 50% smaller than those of wild-type recipient control [28]. In contrast to *in vitro* data, there are no differences in the expression of SR-A, CD36 and ABCA1 between wild-type and PPARδ –/– macrophages in the absence or presence of GW501516. Furthermore they found that PPARδ controls the inflammation status by its association (pro-inflammatory) and dissociation (anti-inflammatory) from transcriptional repressors. Deletion of PPARδ in macrophages leads to an anti-inflammatory status [low levels of the monocyte chemoattractant protein (MCP-1), interleukin-1β (IL-1β), and matrix metalloproteinase (MMP-9)] and showed less atherosclerosis in LDL receptor –/– mice. It means that deletion of PPARδ enhances the released of anti-inflammatory transcriptional repressors. PPARδ ligands inhibited inflammatory gene expression in wild-type macrophages, mimicking the suppressed inflammation elicited in PPARδ –/– macrophages. An interaction between PPARδ and the inflammatory suppressor protein B cell lymphoma-6 (BCL-6) was detected and BCL-6 was released from PPARδ in a ligand-dependent manner. Overexpression of PPARδ (increased unliganded PPARδ receptor) increases MCP-1 levels in the primary macrophages by decreasing the release of anti-inflammatory repressors. The effect is com-

Table 2. Effect of PPARδ deletion or agonist on atherosclerosis *in vivo*

PPARδ knockout mice

Treatment	Model	Diet	Duration	Lipoprotein profile	Atherosclerotic lesion	Reference
PPARδ –/– BMT	LDL-R –/– ♂	1.25% Chol, 15.8% Fat	8 weeks	None	51% ↓	28

PPARδ agonist

Agents	Model	Diet	Period	Lipoprotein profile	Atherosclerotic lesion	Reference
GW0742	LDL-R –/– ♂	1.25% Chol, 21% Fat	14 weeks	TG ↓	0%	29
GW0742	LDL-R –/– ♀	0.25% Chol, 15% Fat	16 weeks	TC →, HDL-C →, VLDL ↓	50% ↓	30

BMT: bone marrow transplantation, Chol: cholesterol, TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein cholesterol, VLDL: very low density lipoprotein

pletely reversed by GW501516 (decreased unliganded PPARδ receptor induces anti-inflammatory repressor release). It seems that the amount of unliganded PPARδ receptor determines the inflammatory status. Both ligand activation and loss of PPARδ receptor release the repressor and produce anti-inflammatory effects (Fig. 1). Those results indicate that PPARδ agonists have anti-atherosclerotic effects *in vivo*, however, two contradictory papers have been reported. Li *et al.* showed that GW0742 (5 mg/kg/day) for 14 weeks failed to decrease atherosclerosis in LDL receptor –/– mice but repressed inflammatory gene expression including interferon-γ (IFN-γ), tumor necrosis factor-alpha (TNF-α), MCP-1, vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion moleculae 1 (ICAM-1) [29]. In contrast, Graham *et al.* reported that GW0742 (6 or 60 mg/kg/day) for 16 weeks inhibited atherosclerosis in female LDL receptor –/– mice and resulted in decreased expression of MCP-1, ICAM-1, and TNF-α in aorta [30]. The efficacy of PPARδ agonists on atherosclerosis may be depends on dose of agonist, treatment duration, dose of cholesterol supplementation, or sex (Table 2).

Effect of PPARδ on fatty acid β-oxidation (FAO) and energy dissipation in skeletal muscle and adipose tissue

In skeletal muscle from PPARα –/– mice, there were only minor changes in fatty acid homeostasis and neither constitutive nor inducible mRNA expression of known PPARα target genes was negatively affected. Unlike in liver and heart, PPARδ in skeletal muscle was several-fold more abundant than either PPARα or PPARγ [31]. These results indicate that PPARγ can compensate for PPARα deficiency in skeletal muscle. Four groups independently confirmed that activation of PPARδ including the treatment of PPARδ agonists enhance fatty acid β-oxidation (FAO) and energy uncoupling in skeletal muscle and adipose tissue. Wang *et al.* described that overexpression of constitutive active form of PPARδ in white and brown adipose tissues displays an up-regulation of genes involved in FAO and energy uncoupling, and are protected against HFD-induced obesity, fatty liver, and hyperlipidemia via increasing energy expenditure [18]. Conversely, PPARδ –/– mice on HFD show reduced energy uncoupling and are prone to obesity. Adipose-specific overexpression of PPARδ or treatment with the PPARδ agonist, GW501516, prevents development of obesity in db/db mice. Activation of PPARδ in adipocytes (3T3-L1 preadipocytes) and myotubes (C2C12 cells) *in vitro* promots fatty acid oxidation and energy uncoupling. They also found that a PPARγ co-activator (PGC-1α), a central component of adaptive thermogenesis, is also a potent coactivator of PPARδ. They suggested that PPARδ serves as a widespread regulator of fat burning in skeletal muscle and adipose tissue. Our laboratory also indicated that the PPARδ agonist, GW501516, controls fatty acid oxidation by regulating genes involved in fatty acid transport, FAO, and mitochondrial respiration in rat L6 myotubes [32]. The mRNAs for pyruvate dehydrogenase kinase 4 (PDK4) that spares glucose oxidation and gluconeogenesis, and PGC-1α are robustly induced by

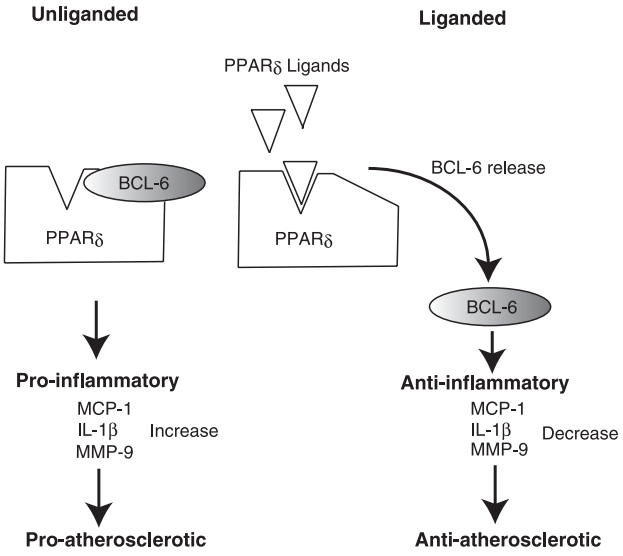

Fig. 1. PPARδ controls an inflammatory switch
Binding of ligand to PPARδ may lead to release of BCL-6, a transcriptional repressor of inflammation. In the absence of ligand, BCL-6 may remain bound to PPARδ, resulting in a pro-inflammatory response. BCL-6; B cell lymphoma-6, MCP-1; monocyte chemoattractant protein-1, IL-1β; interleukin-1β, MMP-9; matrix metalloproteinase-9

GW501516. PPARδ agonists but not PPAR α or γ agonists induces [1-^{14}C] palmitate oxidation in a dose-dependent manner in L6 myotubes. Similarly, PPARδ-mediated increase in FAO is observed in skeletal muscle but not in liver of GW501516-treated mice. Treatment with GW501516 significantly reduces weight gain, but does not affect food consumption in animals fed a HFD. Treatment with a PPARδ agonist also improves insulin resistance induced by HFD, probably as a consequence of increased fat-burning by muscle and overall improvement in systemic lipid metabolism. GW501516 treatment prevents hepatic and intramuscular lipid accumulation, and improves insulin sensitivity in HFD-induced mice or ob/ob mice. It is interesting that the changes in gene expression induced by GW501516 in cultured myotubes and skeletal muscle *in vivo* are very similar to the gene expression profile induced by cold exposure, fasting, and prolonged exercise. We suggest that the physiological role of PPARδ may be a direct switch from glucose metabolism to fatty acid metabolism. It is conceivable that free fatty acids released from adipose tissues on fasting or exercise provide PPARδ ligands to stimulate fatty acid oxidation and thermogenesis in skeletal muscle (Fig. 1). This idea is consistent with the hypothesis that PPARδ acts as a pivotal thermogenic transcription factor [18]. Dressel *et al.* reported a similar effect with GW501516 in C2C12 myotubes, a line of mouse skeletal muscle cells, indicating that PPARδ agonists induces the expression of genes involved in lipid utilization, FAO, cholesterol efflux, and energy uncoupling [33]. It is intriguing that PPARδ agonist increases apolipoprotein-A1 specific efflux of intracellular cholesterol in skeletal muscle even though the effect has been reported in macrophages [21]. Holst *et al.* indicated that 24 hours of fasting up-regulates PPARδ expression in gastrocnemius muscle but not in the heart of mice, and PPARδ agonist GW1514 induces several genes implicated in fatty acid uptake and metabolism in mouse C2C12 myotubes. Furthermore, they generated a Cre/Lox transgenic mouse model allowing overexpression of PPARδ specifically in skeletal muscle *in vivo* [34]. Those mice promote a decrease in body fat content related to a reduced adipocyte size and increased oxidative enzymatic activities. Histological examination shows that the number of oxidative myofibers is increased in muscles. Muscle remodeling in muscle-specific PPARδ overexpression is reminiscent of that provoked by endurance training. They also showed that moderate exercise in wild-type mice increases PPARδ protein in muscle compared to untrained mice in association with the effect of PPARδ in muscle for FAO. Wang *et al.* also showed that the targeted expression of constitutively active form of PPARδ in skeletal muscle or treatment with PPARδ agonist induced oxidative type I fibers in muscle [36]. Genes in those muscles for slow fiber contractile protein, mitochondrial biogenesis, and β-oxidation are up-regulated. These fibers confer resistance to obesity with improved metabolic profiles. PPARδ transgenic mice in muscle remarkably increase and PPARδ –/– mice decrease the exercise endurance capacity. Most recently it has been reported that PPARδ agonists (GW501516 and GW0472) increase glucose uptake independently of insulin in differentiated C2C12 and primary human skeletal myotubes [37]. The effect is coincident with increased expression and phosphorylation of extracellular signal-related kinase (ERK)1/2 mitogen-activated kinase (MAPK), p38 MAPK, and AMP-activated protein kinase (AMPK). PPARδ activation induces both fatty acid β-oxidation and glucose transport in skeletal muscle.

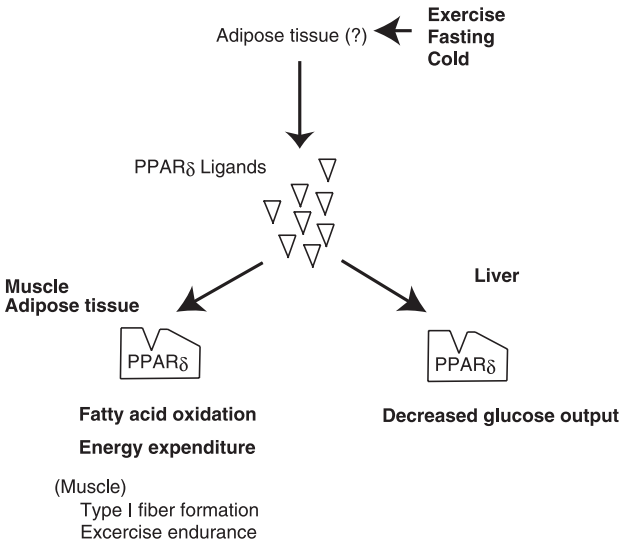

Fig. 2. Mechanism of PPARδ ligands on metabolic syndrome
The effect of PPARδ agonists is similar to the condition by exercise, fasting and cold exposure. Exercise, fasting and cold exposure may produce PPARδ ligands, which effect on muscle, adipose tissue and liver. The combined hepatic and peripheral action of PPARδ ligands could induce a beneficial effect on metabolic syndrome.

Effect of PPARδ on hepatic carbohydrate catabolism

In addition to the beneficial effect of PPARδ on metabolic syndrome in skeletal muscle and adipose tissue by long-term ligand treatment, a direct effect of PPARδ in liver has been proposed [38]. PPARδ –/– mice are glucose intolerant, whereas treatment of diabetic db/db mice with GW501516 for 2 weeks improves insulin sensitivity without weight loss in a case of long-term ligand treatment. Molecular and functional analysis show that PPARδ activation reduces hepatic glucose output by increasing glycolysis and the pentose phosphate shunt and promoting fatty acids synthesis in the liver. As shown in Fig. 2, coupling decreased glucose output in liver with the ability to promote FAO in muscle allow PPARδ to regulate whole-body metabolic homeostasis and insulin sensitivity (Fig. 2).

Cardiomyocytes and PPARδ

The effect of PPARδ on skeletal muscles has expanded the research field to cardiac lipid metabolism. Gilde *et al.* reported that PPARα and PPARδ, but not PPARγ play a prominent role in the regulation of cardiac lipid metabolism [39]. Moreover the PPARδ agonist, GW0742, partly restored the expression of key genes of fatty acid oxidation in mouse adult cardiomyocytes isolated from PPARα –/– mice [40]. Cheng *et al.* examined myocardial fatty acid oxidation in cre-loxP-mediated cardiomyocyte-restricted deletion of PPARδ mice [41]. These mice have cardiac dysfunction, progressive myocardial lipid accumulation, cardiac hypertrophy, and congestive heart failure with reduced survival. PPARδ agonist, GW0742 induced the transcript levels of fatty acid oxidation in cultured cardiomyocytes from PPARδ +/+ mice but the effect is not observed in cardiomyocytes from PPARδ –/– mice. The PPARα agonist, WY14643 restored the decreased transcript levels of fatty acid oxidation in cells from PPARδ –/– mice. Those data indicate that PPARδ is involved in cardiac FAO as does PPARα.

It has been demonstrated that both PPARα and γ activation results in inhibition of inflammatory responses during the pathological progression of myocardial ischemic/reperfusion and hypertrophy [42]. Planavila *et al.* indicated that NF-κB activation down-regulates PPARδ activity, leading to a fall in fatty acid oxidation through a mechanism involving enhanced protein-protein interaction between the p65 subunit of NF-κB and PPARδ. PPARδ agonist, L-165041 inhibits phenylephrene-induced cardiac hypertrophy in cultured neonatal rat cardiomyocytes and lipopolysaccharide (LPS)-induced NF-κB activation in embryonic rat heart-derived H9c2 cells [43, 44]. Ding *et al.* also confirmed that activation of PPARδ using GW0742 or adenovirus-mediated PPARδ overexpression abrogates LPS-induced NF-κB activation [45]. But there is a sharp contrast about the mechanism of NF-κB inactivation by PPARδ agonists. Former group reported L-165041 did not affect the protein level of IκBs, the latter group indicated that PPARδ activation prevented IκB proteolysis. More studies may be needed to elucidate the relation between PPARδ and NF-κB signaling. For apoptosis, GW501516 treatment protected cells from H_2O_2-induced cell death due to an inhibition of H_2O_2-triggaed apoptosis in H9c2 cardiomyoblasts [46].

Oncogenesis and PPARδ

Overexpression of the adenomatous polyposis coli (APC) tumor suppressor gene product in a genetically engineered colorectal HT29-derived tumor cell line leads to a decrease in PPARδ mRNA expression. The APC gene and non-steroidal anti-inflammatory drugs (NSAIDs) inhibit tumorigenesis by inhibition of PPARδ [47, 48]. The up-regulation of PPARδ expression activity has also been confirmed in K-Ras transformed intestinal epithelial cells [49]. PPARδ agonist, GW501516 accelerates small-intestine polyp growth in APC^{min} mice and tumorigenesis is inhibited in xenografts of PPARδ –/– colon cancer cells [50, 51]. Those data indicate that PPARδ activation is prone to increase colorectal cancer. To the contrary, Barak *et al.* reported that PPARδ has no influence on intestinal multiplicity [12]. Harman *et al.* and Reed *et al.* showed that PPARδ depletion induces colon carcinogenesis implying a protective effect of PPARδ against colon carcinogenesis in PPARδ-deficient APC^{min} mutant mice (which lack a copy of the adenomatous polyposis coli tumor suppressor gene) [52, 53]. Additionally Reed *et al.* showed that PPARδ is not required for adenoma formation in the context of a defective mismatch repair (MMR) gene leading to a mutator phenotype [54]. Marin *et al.* showed that inhibition of colon polyp multiplicity is found in GW0742 treated PPARβ +/+ mice but the inhibitory effect is not observed in PPARβ –/– mice [55]. Therefore a role for PPARδ in cancer is inconsistent at this time.

Metabolic syndrome and PPARδ polymorphism

The therapeutic potential of PPARδ agonists for metabolic syndrome have been confirmed in animal models. In human, cross-sectional studies have reported an association of single nucleotide polymorphisms (SNPs) of the PPARδ gene with lipid and glucose metabolism. The +294T/C polymorphism in exon 4 of the PPARδ gene was identified and rare C allele had a higher plasma LDL-cholesterol concentration than homozygoutes for the common T allele in middle-aged men [56]. In male subjects with hyperlipidemia from West of Scotland Coronary Prevention Study (WOSCOPS), the C allele carriers had lower HDL cholesterol and homozygoutes had a tendency towards a high risk for coronary artery disease (CAD) [57]. The relation between C allele and lower HDL-cholesterol levels was re-confirmed in female [58]. But negative study in German population was also reported [59]. For diabetes mellitus, one cross-sectional study did not find association between PPARδ polymorphism and type 2 diabetes [60]. Andrulionyté *et al.* found that SNPs in PPARδ modify the conversion from impaired glucose intolerance (IGT) to type 2 diabetes in combination with the SNPs of PGC-1α and PPARγ2 genes [61]. SNPs of PPARδ primarily affected insulin sensitivity by modifying glucose uptake in skeletal muscle but not in adipose tissue [62]. Those findings indicate PPARδ gene might affect lipid and glucose metabolism in human as well as animal models.

Conclusion

New target for metabolic syndrome, PPARδ activation clearly induces energy dissipation in skeletal muscle and adipose tissue. Furthermore PPARδ activation reduces hepatic glucose output by increasing glycolysis and the pentose phosphate shunt and promoting fatty acids synthesis in the liver. It will be exciting to determine whether PPARδ agonists are clinically beneficial for obesity and its associated problems such as type 2 diabetes and hyperlipidemia. However, potential unwanted consequences of PPARδ activation need to be considered. Further work to elucidate the roles of the PPARδ in oncogenesis and organ toxicity will be crucial in determining the safety of chronic PPARδ ligand treatment in human.

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