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GW501516 (Cardarine): Pharmacological and Clinical Effects

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ABSTRACT

GW501516/Cardarine is a nuclear hormone that activates the PPARδ genes involved in cell differentiation and exerts a primary regulation in maintaining health conditions and preventing diseases. PPARδ activation stimulates the mitochondrial function in various tissues improving lipid metabolism and energy homeostasis.

PPARδ receptors are expressed mainly in active metabolic tissues such as skeletal muscles, the heart, the liver, and neurons. PPARδ activation increases fatty acid oxidation, saving glycogen stores, so they find indications for the treatment of metabolic diseases, especially dyslipidemia and type-2 diabetes mellitus (T2DM), and hypertriglyceridemia. The pancreatic β-cell function is improved. In the heart, PPARδ improves cardiomyocytes function and reduces fibrosis. In the liver, it reduces the fat deposit, suppressing inflammation. Cardarine seems to be effective in preventing β-amyloid deposition and Alzheimer's disease. In the skeletal muscles, cardarine induces a strong activation of mitochondrial biogenesis, increasing oxidative muscle capacity and endurance exercise capacity, and for this reason, it was included in the list of doping agents by WADA. However, some controversies on cancer risk stimulation exist. This review aims to evaluate the clinical benefits and side effects of GW501516/cardarine.

Keywords

GW501516, Cardarine, PPARδ agonist, Cardarine and cardioprotection, Cardarine and endurance, Antiinflammatory effect of Cardarine, Cardarine and obesity.

Introduction

Cardarine, or GW501516 or Endurobol, is a synthetic agonist of the peroxisome proliferator-activated receptor delta (PPARδ). The substance originated by combinatorial chemistry and structure-based drug design resulting from collaborative research between GlaxoSmithKline and Ligand Pharmaceuticals in the 1990s [1].

Although none of the selective PPARδ agonists have been approved for human therapy, some synthetic compounds are still under clinical evaluation for efficacy and safety. Some of these, ASP0367 and ASP1128, have been produced by Mitobridge, Astellas Pharma, Cambridge, USA; MBX8025 or Seladelpar by CymaBay Therapeutics, NJ, USA; and REN-001 by Renere Pharmaceutical, San Diego, USA. The GlaxoSmithKline and Ligand Pharmaceuticals intended to develop a PPARδ agonist to treat hyperlipidemia. Preclinical studies revealed that Cardarine could actively accelerate the metabolism, reducing adiposity, increasing fatty acids beta-oxidation in muscle [2], and improving the physical performance in mice [3]. Unfortunately, despite the promising results, later studies revealed that the drug allowed cancer to develop rapidly in various organs. Those results caused an interruption of further research on the drug. However, the effect of cardarine to enhance training adaptation or even increase endurance without exercise in mice [4] quickly gained popularity with athletes and bodybuilders as the "wonder pill," and if FDA did not approve Cardarine, and the World Anti-Doping Agency (WADA) banned it, many athletes got it illegally on the black market. The drug, called Cardarine, Endurobol, GW501516, GW516, and others, is present in online shopping as a research compound, indicating "not for human use."

GW501516/cardarine has been classified mistakenly also as a Selective Androgen Receptor Modulator (SARM). However, the compound is not an androgen receptor activator. It is instead a selective agonist of the PPARδ receptor [5], displaying high affinity and potency for PPARδ with >1,000-fold selectivity over PPARα and PPARγ [6]. However, despite the initial promising results, later studies revealed that the drug leads cancer to develop rapidly in several organs, but unquestionably, drugs able to act on PPARs are potential of great clinical interest.

The Peroxisome Proliferator-Activated Receptors PPARs

PPARs belong to the nuclear receptor superfamily of nuclear hormone control of genes involved in cell differentiation. They play a crucial role in controlling lipid, glucose, and energy homeostasis, whose physiological functions are the control of metabolism and energy homeostasis [7]. Three PPAR receptors have been identified, PPARα, PPARδ (or PPARδ), and PPARδ. Ligand X receptors bind to peroxisome proliferator response elements (PPREs) to regulate the transcription of target genes [8]. The three PPAR isoforms, despite sharing a high degree of structural homology, have different tissue distributions, distinct functional roles, and ligand-binding properties [9]. The PPARα, PPARδ, and PPARγ are expressed mainly in active metabolic tissues such as skeletal muscles, the heart, the liver, and macrophages [10]. The characteristics of these receptors have been reviewed elsewhere [11].

The nuclear receptor PPARs are primary regulators in maintaining health conditions and preventing diseases [12,13], cell proliferation, differentiation, and survival [14], immune response [15], and T-cell function [16]. PPARδ agonists were shown to play a crucial role in whole-body lipid homeostasis and insulin sensitivity and are active in skeletal muscle, liver, and adipose tissue [17].

PPARδ elicits a transcriptional cascade in skeletal muscle, increasing fatty acid oxidation. In this way, they permit the sparing of glucose, which enables muscle to function, improving running endurance without decreasing systemic glucose levels or increasing muscle lactic acid [18].

Owing to their crucial metabolic regulatory roles, many PPAR agonists have been used to treat metabolic diseases, especially dyslipidemia and type-2 diabetes mellitus (T2DM), and hypertriglyceridemia [19]. Likewise, thiazolidinediones (TZDs), potent PPARγ activators, are insulin sensitizers in T2DM patients [20]. The clinical efficacy of fibrates and TZDs has pushed the search for various PPARα or γ agonists and stimulated the investigation of novel PPAR modulators, including selective PPARδ activators, dual-PPAR, and pan-PPAR agonists [9]. The lipid-lowering activity of PPARα agonists and the insulin-sensitizing effect of PPARγ agonists are well-established and have been widely used to ameliorate dyslipidemia and T2DM [21,22]. Aside from dyslipidemia and T2DM, PPARs have profound implications also on other aspects of metabolic syndrome, like diabetic complications [23], non-alcoholic fatty liver disease, as well as non-metabolic disorders, including neurodegenerative diseases [24], depression, cancer, autoimmune diseases [25], inflammatory diseases, such as ulcerative colitis [26], and psoriasis [27].

PPARδ has been less explored than α and γ receptors due to concerns about its side effects. Still, it was shown to play an essential role in lipid homeostasis and insulin sensitivity in skeletal muscle, liver, and adipose tissue [17], but also in adipogenesis, wound cure, and keratinocyte differentiation [28]. This review aims to consolidate experimental evidence of PPARδ agonist GW501516/cardarine to highlight its effectiveness and developmental challenges for treating various diseases based on preclinical and clinical data.

PPARδ and lipid metabolism

The importance of PPARδ in lipid catabolism has been described in various essential tissues, such as skeletal muscle, which induces fatty acids beta-oxidation [2], in adipose tissue, which prevents obesity [29], protecting the heart from cardiomyopathy [30], and liver [31] pointing at PPARδ as a potential drug target in the treatment of metabolic diseases. Hypolipidemic drugs, unsaturated fatty acids, and their derivatives can directly activate PPAR receptors, particularly PPARα and PPARδ [17,32,33].

It is well known that the PPARδ gene is expressed in rodent pancreatic beta-cells [34], where, in conjunction with PPARα, it has been shown to mediate the effects of unsaturated fatty acids on gene expression in some cell types [33]. The PPARδ mRNA is also present in the human endocrine pancreas, although slightly less than PPARα [35], and affects pancreatic beta cell mass and insulin secretion in mice [36]. However, its role is still under-explored in humans. It is known, however, that the PPARδ ligand cardarine reverses metabolic abnormalities associated with obesity, such as dyslipidemia and insulin resistance, in humans [37], monkeys, and mice [1,38].

Results by Ravnskjaer et al. [39] demonstrated that PPARδ activation increased phospholipase 2 (PLA2), which, in turn, activates the hydrolysis of arachidonic and linoleic acid from phospholipids in a rat insulinoma cell line (INS-1E). Glucose also increased the level of reactive oxygen species, which promoted the peroxidation of these polyunsaturated fatty acids (PUFAs) to generate the compound 4-hydroxy-2E-nonenal (4-HNE). The latter mimicked the amplifying effect of stimulated insulin secretion after pre-exposure to high glucose and cardarine in the insulin cell line and isolated rat islet the fatty acids. The results were explicitly blocked by the selective PPARδ antagonist, GSK0660, or by silencing PPARδ expression [39]. Furthermore, cardarine can ameliorate hyperglycemia, insulin resistance, and lipotoxicity in beta cells [40]. In different rat models of T2DM, Li et al. [41] demonstrated that the PPARδ agonist protected beta cells *in vivo*, upregulating GRP40 and activating the Akt/Bcl-2/caspase-2 pathway. They also showed that the protective effect was similar to that of pioglitazone, but at the difference, cardarine reduced body weight.

Daoudi et al. [42] discovered a different contributing mechanism

that could significantly improve glucose homeostasis by PPARδ stimulation. They demonstrated that cardarine administration in wild-type and ob/ob mice improved glucose tolerance, and they gave evidence that the mechanism could be indirectly mediated by the stimulation of small intestine glucagon-like peptide-1 (GLP-1) production. GLP-1 is an incretin physiologically secreted after nutrient ingestion that acts on endocrine pancreas beta cells to enhance insulin secretion. These results contribute to expanding the interest of PPARδ as a target for treating DT2M.

More recently, Zhuo et al. [43], in a rat model of gestational diabetes mellitus, found that cardarine could improve the blood glucose level, reduce the apoptosis rate of islet cells and inhibit the expression of lipid metabolism-related factors in the blood. Therefore, the PPARδ agonists may be a promising therapeutic target for treating insulin resistance in patients with T2DM and, possibly, with gestational diabetes in pregnant women.

The antidiabetic effect of PPARδ is partly due to the activation of 5' adenosine monophosphate-activated protein kinase (AMPK) that increases insulin activity, improving glucose control [44]. AMPK, a sensing glucose signal, is activated by reduced energy intake and glucose starvation [45]. In addition, diet restriction, physical exercise, and the drug metformin can start the AMPK [46].

PPARδ is a central transcriptional regulator of fat burning in adipose tissue through activating the long-chain fatty acids β-oxidation [29]. Therefore, PPARδ can potentially be effective in treating obesity, reducing adipogenesis and its associated disorders [47]. In sum, these results provide evidence for a repressive role for PPARδ in β-cell mass and insulin exocytosis, adding a new light on PPARδ metabolic action.

The overexpression of PPARδ can increase glycogen storage and increase mitochondrial activity in association with 5' adenosine monophosphate-activated protein kinase (AMPK) expression and myocyte enhancer factor-2 (Mef2) [48]. The essential activators of AMPK are diet restriction, physical exercise, and metformin administration [46]. It can be argued, therefore, that the most evident effect of cardarine/GW501516 is observed synergically with exercise training to increase endurance capacity [4].

PPARδ is essential to increasing the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) that stimulates mitochondrial biogenesis in skeletal muscle during exercise [49]. PPARδ ablated mice showed changes in muscle fiber type associated with reduced oxidative capacity and running duration [50].

Cardarine increased fatty acids oxidation preventing the down-regulation of AMPK caused by a high-fat diet in the liver and amplifying the PGC-1alpha-lipin 1-PPAR-α pathway [51]. AMPK exerts a prominent role in fatty acid oxidation [52], and the PPARδ-PGC-1 pathway contributes to the hypo-triglyceridemic effect of the drug. Genetic and pharmacological studies showed that AMPK is required for maintaining glucose homeostasis [53]. The activation of AMPK by pharmacological agents presents a unique challenge, given the complexity of the biological control of ATP synthesis and consumption and its deregulation in cancer [54]. PPARs regulate the triglycerides acids (TCA) cycle flux [55], promoting endurance exercise [18] and energy production [56].

GW501516/Cardarine in Oxidative Stress and Inflammation

In vitro pharmacologic experiments on cardiac rat cells showed that PPARδ activation inhibited the oxidative stress induced by hydrogen peroxide [57]. The PPARδ agonist, GW0742, inhibited the hydrogen peroxide-induced apoptosis and increased cardiac cell viability, reducing the reactive oxygen/nitrogen species and matrix metalloproteinases, which can be associated with cardiomyocyte apoptosis. Cardarine exerts a potent anti-inflammatory effect abolishing the effect of TNF-α induce expression of adiponectin and insulin receptors in adipocytes [58], preventing the IL-6-induced STAT3 activation, and reducing the cytokine-induced insulin resistance in hepatic cells [59].

Cardarine prevents TNF-α-induced Nuclear factor B (NF-κB) activation in human HaCaT cells (an immortalized human keratinocyte line) [60]. NF-κB is a ubiquitously expressed transcription factor controlling the expression of numerous genes involved in inflammation. *In vitro*, in the AC16 cell line derived from human cardiomyocytes, PPARδ activation can reduce the inflammatory response to the exposition of the saturated fatty acid palmitate [61]. In mice fed a high-fat diet, it blocks lipid-induced inflammatory pathways [61].

Cardarine reduces the inflammatory state in white adipose tissue (WAT) and hepatic stellate cells (HSCs), reducing lipogenesis and insulin resistance. Damages in the liver are alleviated by increasing the beta-oxidation process and decreasing the expression of the genes involved in lipogenesis and gluconeogenesis [62].

Effects of PPAR mixed agonists in type-2 diabetic patients

The antidiabetic effect of PPARδ is partly due to the activation of AMPK [44], which increases insulin activity, improving glucose control. AMPK, a sensing glucose signal, is activated by reduced energy intake and glucose starvation [45]. Cardarine exerts a therapeutic effect on T2DM, improving insulin activity, glucose, and fat metabolism and activating beta-cells activity. A randomized, double-blind study of 16 weeks duration with aleglitazar, a mixed PPARα/γ agonist, in single daily doses of less than 300 mcg, showed a beneficial effect on glucose regulation without side effects or cardiac dysfunction and edema [63], lowering postprandial plasma triglyceride and HbA1c [64], improved glycemic control [65].

Neuroprotective effect of GW501516/Cardarine

PPARα agonists found a clinical application in neurodegenerative disorders, which includes Parkinson's disease (PD) [66], Alzheimer's disease (AD) [67], Huntington's disease (HD), Amyotrophic Lateral Sclerosis (ALS) [68], and multiple sclerosis (MS) [69]. The neuroprotective effect of PPAR agonists *in vitro* and *in vivo* models of PD regulates the expression of a set of genes

involved in cell survival [66].

PPARα activation may improve mitochondrial function and oxidative stress and reduce neuroinflammation in the brain, suggesting the potential application of PPAR agonists in treating neurodegenerative disorders [70]. Many studies reported the beneficial effects of PPARα agonists on cognitive behavior in preclinical AD models [71].

In animal models, the PPARγ agonist treatment reduced proinflammatory cytokines, increased microglial phagocytosis, and matured oligodendrocyte progenitor cells (OPC). RNA-sequencing analyses of microglia revealed that PPARγ activation down-regulated several proinflammatory genes, which have a role in PD and ALS, contributing to neurological recovery [72].

In the ALS animal model, the PPARϒ agonist pioglitazone reduced iNOS, NF-kappa-B, and 3-nitrotyrosine immunoreactivity in the spinal cord, improved motor performance, delayed weight loss, and extended survival [68]. The neuroprotective effect of PPAR-ϒ was also demonstrated in models of ALS in Drosophila, mitigating locomotor dysfunction [73]. Cardarine suppresses the generation of neurotoxic amyloid β following the decrease in beta-secretase 1 (BACE1) expression [74]. BACE1 is the enzyme that reduces the amyloid β deposition in neurons; the inhibitors of BACE1 are involved in the rate-limiting step of the amyloid precursor protein (APP), leading to the generation of the neurotoxic amyloid β protein [75]. Together, these results indicate that PPARδ attenuates BACE1 expression via SOCS1-mediated inhibition of signal transducer and activator of transcription signaling, thereby suppressing BACE1-associated generation of neurotoxic amyloid β deposition [74].

PPARδ attenuates neuroinflammation triggered by lipopolysaccharide and neurotoxicity associated with glutamate release by enhancing SOCS1-mediated inhibition of JAK2/STAT1 signaling [76]. Furthermore, PPARδ agonists exert antiapoptotic properties *in vitro*, highlighting their potential neuroprotective activity *in vivo* experimental models of cerebral ischemia and Parkinson's disease [77].

These findings suggest that PPARδ agonists could represent a therapy in other neurodegenerative disorders and stroke [77,78] and may be helpful in understanding the role of these diseases' pathogenesis.

Effects of GW501516/Cardarine on the Heart and cardiovascular functions

GW501516/Cardarine exerts various and interesting effects on cardiac function because it is a primary physiological regulator of cardiac energy metabolism in cardiomyocytes reducing insulin resistance [44] and contrasting the pathological alterations present in heart failure and diabetic cardiomyopathy [79]. Although GW501516/Cardarine can reduce cardiovascular diseases, some controversies exist [80]. PPARδ activation reduces the expression of the NF-kB gene in mice and human cardiac cells and the AMPK mechanism, a lipid-induced inflammatory pathway in human cardiac cells. The transcriptional control of NF-kB entails the progression of heart failure and cardiac hypertrophy [61]. Furthermore, PPARδ attenuates endoplasmic reticulum stress and increases autophagic markers in human cardiac cells induced by palmitate [81].

Although all three PPAR isoforms are present in the myocardium, Teunissen and coworkers demonstrated, by real-time quantitative polymerase-chain-reaction, that PPARδ is by large the most abundant isoform present in the rat cardiac fibroblast and myofibroblast. Another group confirmed the presence of PPARδ, and to a less extent, of PPARα in rat cardiac fibroblasts. They demonstrated that GW501516/Cardarine depressed angiotensin II-stimulated collagen type I expression and collagen synthesis in cardiac fibroblasts in a concentration dependent manner. Furthermore, they confirmed the specificity of the inhibitory effect of GW501516/Cardarine on collagen synthesis, demonstrating that the knockdown of PPARδ can entirely reverse the effect observed via RNA interference [82]. Although experimental studies suggested that PPARδ is a promising therapeutic target for preventing myocardial fibrosis, additional *in vivo* studies need to confirm these results.

PPARα activation inhibits collagen expression in the heart and cardiac fibroblast proliferation, reducing collagen synthesis by 36% in CF [83], suggesting that PPARα represents an attractive molecular target for attenuating cardiac fibrosis and skeletal muscle dysfunction in heart failure [84].

In an animal model of ischemic left ventricular dysfunction, a pathological condition characterized by skeletal muscle weakness and physical exercise intolerance, the administration of GW501516/Cardarine improved physical exercise capacity, endurance function, and skeletal muscle oxidative metabolism [85]. These data are very promising in pathological conditions characterized by the poor quality of life of the patients and the absence of effective treatments, except for physical exercise that can partially improve peripheral metabolism in patients with chronic heart failure [86].

Aleglitazar, a PPARα/γ agonist, in a multicenter, randomized, double-blind, placebo-controlled trial, improved cardiovascular outcomes in patients with diabetes and high risk of coronary disease. The study was conducted on 7,228 patients treated daily with 150 μg of the agonist or placebo [87]. However, a recent meta-analysis showed that pioglitazone, aleglitazar, a PPARα/γ agonist, significantly decreased the estimated glomerular filtration rate, significantly increased body weight, gastrointestinal bleeding, bone fractures, heart failure, cardiovascular death, and malignancy in comparison of the control group [88]. Considering more harm than benefit, Aleglitazar testing has been halted. PPAR-ϒ ameliorates autoimmune myocarditis in experimental animal models [89]. In human pluripotent stem cells derived by cardiomyocytes, PPARδ activation induces metabolic and contractile maturation [90].

Effects on Liver

Girroir et al. [91] found that the liver is a tissue that expresses the highest levels of PPARδ in mice. PPARα is also present and serves as the master regulator of hepatic lipid metabolism during fasting, suppressing inflammation. The acute response causes many genes' down-regulation in various immunity-related pathways [92].

GW501516/Cardarine was shown to be an efficient PPARδ agonist on endogenous genes of the mouse liver hepatocytes, acting dose-dependently [93]. GW501516/Cardarine can also activate PPARα, β and ϒ exerting a significant metabolic effect in the liver, activating glycolysis/lipogenesis, and increasing fat oxidation, reducing body fat and fasting plasma triglycerides [37]. Most of the results are related to the activation of lipid metabolism, reducing body weight, and hepatic steatosis in mice [94]. In the human liver cells, PPARδ can reduce insulin resistance by inhibiting IL-6 induced signal transducer and the activator of transcription 3 (STAT3) [59]. GW501516/Cardarine administration protected against the inflammatory state in white adipose tissue and liver damage in high fructose-fed mice [62] and protected hepatocytes inhibiting fibrosis in animal models of liver fibrosis [95].

GW501516/Cardarine is particularly effective in non-alcoholic fatty liver disease regulating the production of very low-density lipoprotein receptor (VLDLR) and serum triglyceride levels [96] and liver fibrosis [97]. A double-blind, randomized, placebo-controlled trial showed that GW501516/Cardarine decreased liver enzyme levels and the majority of lipids, biomarkers of inflammation, and fibrosis [98,99].

GW501516/Cardarine and physical performance

Physical exercise induces changes in several transcriptional gene regulators in skeletal muscle, significantly contributing to metabolic changes [100]. Notably, exercise activates glycogen catabolism [101], increases insulin sensitivity [102], fatty acids utilization [103], and exerts an anti-inflammatory effect [104]. Furthermore, physical exercise regulates the AMPK gene activity and AMPKα2 gene methylation in human blood and eminently in the skeletal muscle [105]. AMPK is another endurance gene regulator of energy expenditure [106], and its activation maintains cellular energy stores, switching on catabolic pathways that produce ATP, primarily by enhancing oxidative metabolism and mitochondrial biogenesis [107]. In human macrophage analysis, co-activation of AMPK and PPARδ increased the expression of FAO genes [108]. Cardarine prevents the down-regulation of AMPK induced by high fat diet amplifying the PGC.1 pathway [51].

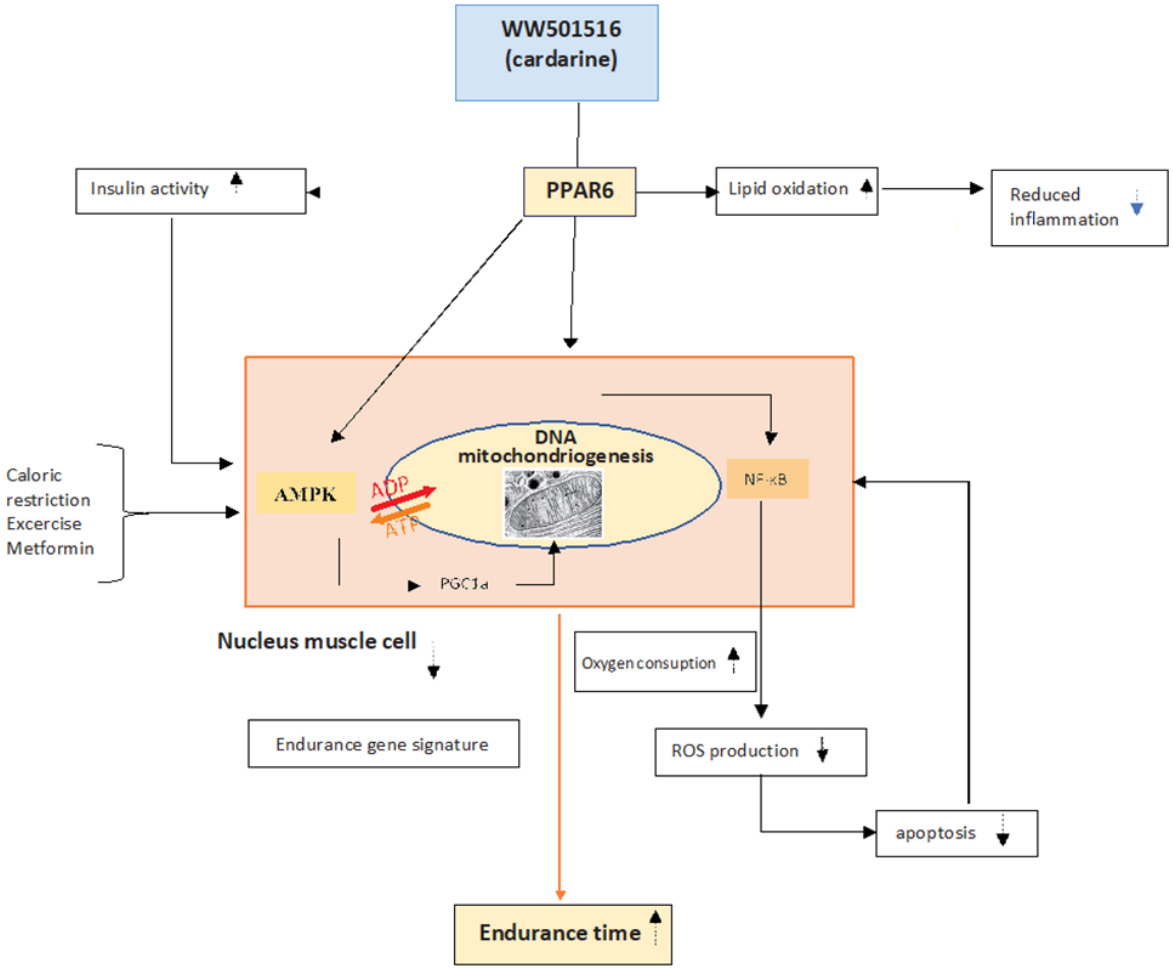

Figure 1: Effect of Cardarine (GW501516) on lipid metabolism and mitochondria.

Many studies identified PPARδ activation's relevant role in the transcriptional regulation of skeletal muscle metabolism [109]. These receptors control muscle development and oxidative capability [110], regulating muscle fibers metabolism and increasing endurance. In muscle fibers, PPARδ receptors increase the PGC1-α expression involved in switching obesity in T2DM [50].

The overexpression of PPARδ in transgenic mice increased oxidative muscle capacity and running endurance by about 100% [111]. Sprecher et al. [112] demonstrated that the PPARδ agonist Cardarine was active in humans and mice, increasing the duration of running time by 60-75%. Narkar et al. [4] found that a 4-week combined administration of Cardarine and exercise training increased the running time by 68% and the distance run by 70%. This phenotype induced by Cardarine was linked to a transcriptional booster identified in the exercise-activated AMPK. The PPARδ agonist works synergistically with exercise increasing endurance performance and mitochondria biogenesis; furthermore, the AMP-mimetic, 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR), increases endurance in a PPARδ manner [4]. AICAR is an activator of the AMPK signaling pathway, which regulates metabolism, hypoxia, exercise, nucleotide synthesis, (and cancer) [113]. These findings demonstrated that a pharmacologic intervention targeting AMPK-PPARδ signaling could reprogram muscle endurance. In trained mice, it was shown that the pharmacological activation of AMPK and PPARδ-potentiates the effects of exercise [114]. Studies conducted in transgenic animals with overexpression or knockout signals showed that AMPK-PPARδ is a critical regulator of endurance performance during exercise [111,115]. The role of AMPK is determinant in genetic adaptation because it is a metabolic sensor detecting the low ATP level during exercise [116] and is directly activated by exercise [117].

Chen et al. [3] found that mechanisms behind enhanced running capacity differ for Cardarine and exercise training. Training increases energy availability by promoting the catabolism of proteins and gluconeogenesis. In contrast, Cardarine reduces glucose utilization by skeletal muscle through a switch in mitochondrial substrate preference from carbohydrate to fatty acids consumption [118], increasing running endurance capacity [18].

The study determined the increased levels of intermediate metabolites and critical enzymes in fatty acid oxidation pathways following training and/or treatment. It was found that exercise training increased serum inositol, glucogenic amino acids, and branch-chain amino acids. During Cardarine administration, an increased serum galactose and β-hydroxybutyrate was observed, and a serum unsaturated fatty acid level, independently of training. These levels increased even more when combined with exercise training. The strong activation of mitochondrial biogenesis induced by Cardarine induces a switch of muscle fibers from type 2 (glycolytic metabolism) to type 1 (oxidative metabolism), and muscle fiber variation was correlated with PPARδ and PGC1 mRNA [119]. The skeletal muscles have plasticity and can convert between different fiber types in response to exercise training and motoneuron activity [120,121]. Skeletal muscle fibers are distinct into type I (slow/oxidative) and type II (fast/glycolytic) fibers. They express significant differences in fast contraction, rapid metabolism, and susceptibility to fatigue [122].

The conversion of muscle fibers from type IIb to type IIa and type I showed increased mitochondrial biogenesis in response to the calcium signaling pathway and the transcriptional cofactor (PGC-1α) [123]. Furthermore, in transgenic mice with VP16-PPARδ fusion cDNA, another marker for type1 fibers, such as myoglobin and cytochrome c and b, were significantly increased, indicating a higher degree of fibers transformation [111]. The mitochondrial DNA increased 2-3-fold, evidencing marked mitochondrial biogenesis. The effect on endurance is significant because in PPARδ null mice, the running time is only 38%, and the distance walked of 34% compared to wild-type counterparts.

Narkar et al. [4] showed that AICAR administration, even in sedentary mice, induced metabolic gene changes for four weeks and enhanced running endurance by 44%. The study demonstrated that activating the AMPK-PPARδ pathway can enhance training adaptation and increase endurance without exercise.

GW501516/Cardarine and cancer

The function of PPARδ in different types of cancer is highly controversial. Many studies indicate that PPARδ can inhibit or promote tumorigenesis in different tissues [124] depending on the varying contribution of PPARδ to endothelial cell proliferation, inflammation, tumor cell proliferation, make differentiation, or apoptosis [125]. These processes are critically involved in cancer growth, and different approaches could give rise to opposing results. Cardarine inhibited tumorigenicity in undifferentiated nasopharyngeal carcinoma promoting apoptosis of cancer cells [126]. In invasive bladder cancer cells, the PPARδ agonist induced an apoptotic effect on invasive bladder cancer cells [127], and the progression of invasive cancer cells was inhibited by Cardarine exposure [128]. Wagner et al. [129] showed that PPARδ agonist favors tumor angiogenesis while decreasing tumor cell proliferation in Lewis lung carcinoma, independently from their action on different cancer cell types. Therefore, the use of PPARδ agonists in these patients can be dangerous. In patients with colorectal cancer, cardarine accelerated the progression and invasion of the tissue [130] and mediated the effect of diet in promoting colorectal cancer [131] and metastatic gastric cancer [132].

Activating PPARδ can stimulate breast and prostate cancer cell growth [133]. It was shown that the activation of the T47D cells caused an increased expression of the proliferation marker Cdk2 and vascular endothelial growth factor alpha (VEGF-α), suggesting that PPARδ may initiate cellular proliferation and angiogenesis. It is to note, however, that the study was conducted on immortalized prostate and breast cancer cell lines. Other studies evidenced that PPARδ did not increase the risk of human cancer cell growth

[134,135]. However, all these results have been obtained *in vitro* on human cells.

In mice with mammary carcinomas, the administration of Cardarine reduced the tumor latency by eee months, showing a direct role of PPARδ in the pathogenesis of mammary tumorigenesis [136]. This finding suggests a therapeutic role of PPARδ antagonists to prevent and treat this disease. The complex interaction between PPARδ and cell proliferation makes future studies necessary to unravel the precise function of PPARδ in carcinogenesis. However, regenerative biology and tissue development work synergistically with cancer interaction depending fundamentally on the peripheral nervous system, which controls normal tissue development, homeostasis, plasticity, and regeneration. The nervous system essentially contributes to the initiation and growth of cancer, making it challenging to compare cellular and animal studies to the situation *in vivo* in humans [137].

Conclusions

Many studies have shown in animals and humans very promising effects of cardarine on metabolism increasing fatty acids oxidation, exerting anti-obesity and anti-diabetic actions, and essential effects on the liver, skeletal muscles, and heart function. Figure 2. In particular, Cardarine exerts specific effect on various organ and tissues; particularly on body fat, skeletal muscles, heart, brain. Despite the exciting results, human studies were halted very early due to their propensity to stimulate carcinogenesis in some animal models. Activation of different PPARs can interfere with tumor-suppressing or promoting growth in various tissues. The knowledge of the complex mechanisms involved in the PPARs interactions with its endogenous ligands and other nuclear receptors, coactivators, and repressors' action still need to be completed: Moreover, Cardarine seems to be a more selective PPARδ agonist in humans than in rodents, make unrealistic the translation of experimental results resulted from rodent the humans.

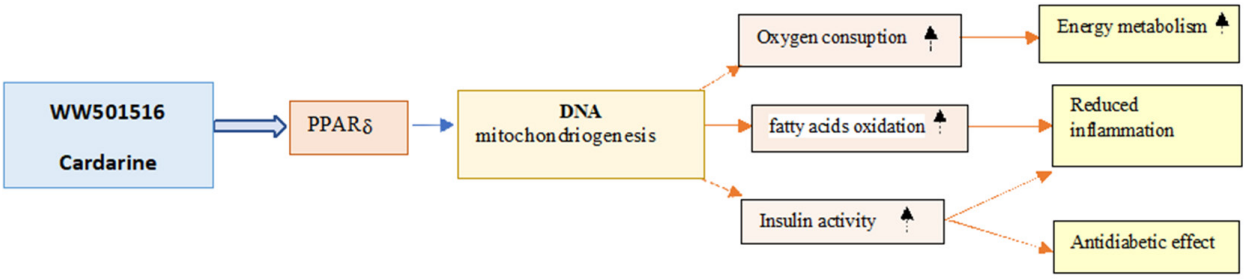

Figure 2: Effect of Cardarine on cell metabolism.

In conclusion, there is a lot of work to do to define the exact role of PPARδ in different conditions, but with the exciting hope of achieving exceptional results.

References

1. Oliver WR Jr, Shenk JL, Snaith MR, et al. A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc Natl Acad Sci U S A. 2001; 98: 5306-5311.
2. Tanaka T, Yamamoto J, Iwasaki S, et al. Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. Proc Natl Acad Sci U S A. 2003; 100: 15924-15929.
3. Chen W, Gao R, Xie X, et al. A metabolomic study of the PPARdelta agonist GW501516 for enhancing running endurance in Kunming mice. Sci Rep. 2015; 5: 9884.
4. Narkar VA, Downes M, Yu RT, et al. AMPK and PPARdelta agonists are exercise mimetics. Cell. 2008; 134: 405-415.
5. Lagu B, Kluge AF, Tozzo E, et al. Selective PPARdelta Modulators Improve Mitochondrial Function: Potential Treatment for Duchenne Muscular Dystrophy (DMD). ACS Med Chem Lett. 2018; 9: 935-940.
6. Kasuga J, Nakagome I, Aoyama A, et al. Design, synthesis, and evaluation of potent, structurally novel peroxisome proliferator-activated receptor (PPAR) delta-selective agonists. Bioorg Med Chem. 2007; 15: 5177-5190.
7. Brown JD, Plutzky J. Peroxisome proliferator-activated receptors as transcriptional nodal points and therapeutic targets. Circulation. 2007; 115: 518-533.
8. Chan LSA, Wells RA. Cross-Talk between PPARs and the Partners of RXR: A Molecular Perspective. PPAR Res. 2009; 2009: 925309.
9. Tan CK, Zhuang Y, Wahli W. Synthetic and natural Peroxisome Proliferator-Activated Receptor (PPAR) agonists as candidates for the therapy of the metabolic syndrome. Expert Opin Ther Targets. 2017; 21: 333-348.
10. Tan NS, Vazquez-Carrera M, Montagner A, et al. Transcriptional control of physiological and pathological processes by the nuclear receptor PPARbeta/delta. Prog Lipid Res. 2016; 64: 98-122.
11. Mirza AZ, Althagafi II, Shamshad H. Role of PPAR receptor in different diseases and their ligands: Physiological importance and clinical implications. Eur J Med Chem. 2019; 166: 502-513.
12. Wagner N, Wagner KD. The Role of PPARs in Disease. Cells. 2020; 9: 2367.
13. Cheng HS, Tan WR, Low ZS, et al. Exploration and Development of PPAR Modulators in Health and Disease: An Update of Clinical Evidence. Int J Mol Sci. 2019; 20: 5055.

14. Lin Y. Editorial: Important Roles of PPAR in Stem Cell Differentiation. Curr Stem Cell Res Ther. 2016; 11: 176.
15. Christofides A, Konstantinidou E, Jani C, et al. The role of peroxisome proliferator-activated receptors (PPAR) in immune responses. Metabolism. 2021; 114: 154338.
16. Choi JM, Bothwell ALM. The nuclear receptor PPARs as important regulators of T-cell functions and autoimmune diseases. Mol Cells. 2012; 33: 217-222.
17. de Lange P, Lombardi A, Silvestri E, et al. Peroxisome Proliferator-Activated Receptor Delta: A Conserved Director of Lipid Homeostasis through Regulation of the Oxidative Capacity of Muscle. PPAR Res. 2008; 2008: 172676.
18. Fan W, Waizenegger W, Lin CS, et al. PPARdelta Promotes Running Endurance by Preserving Glucose. Cell Metab. 2017; 25: 1186-1193.e4.
19. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019; 139: e1082-e1143.
20. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach: Update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia. 2015; 38: 140-149.
21. Bruckert E, Labreuche J, Deplanque D, et al. Fibrates effect on cardiovascular risk is greater in patients with high triglyceride levels or atherogenic dyslipidemia profile: a systematic review and meta-analysis. J Cardiovasc Pharmacol. 2011; 57: 267-272.
22. Palmer SC, Mavridis D, Nicolucci A, et al. Comparison of Clinical Outcomes and Adverse Events Associated With Glucose-Lowering Drugs in Patients With Type 2 Diabetes: A Meta-analysis. JAMA. 2016; 316: 313-324.
23. Hong F, Xu P, Zhai Y. The Opportunities and Challenges of Peroxisome Proliferator-Activated Receptors Ligands in Clinical Drug Discovery and Development. Int J Mol Sci. 2018; 19: 2189.
24. Sato T, Hanyu H, Hirao K, et al. Efficacy of PPAR-gamma agonist pioglitazone in mild Alzheimer disease. Neurobiol Aging. 2011; 32: 1626-1633.
25. Ormseth MJ, Oeser AM, Cunningham A, et al. Peroxisome proliferator-activated receptor gamma agonist effect on rheumatoid arthritis: a randomized controlled trial. Arthritis Res Ther. 2013; 15: R110.
26. Pedersen G, Brynskov J. Topical rosiglitazone treatment improves ulcerative colitis by restoring peroxisome proliferator-activated receptor-gamma activity. Am J Gastroenterol. 2010; 105: 1595-1603.
27. El Eishi N, Hegazy R, Abou Zeid O, et al. Peroxisome proliferator receptor (PPAR) beta/delta in psoriatic patients before and after two conventional therapeutic modalities: methotrexate and PUVA. Eur J Dermatol. 2011; 21: 691-695.
28. Hamblin M, Chang L, Fan Y, et al. PPARs and the cardiovascular system. Antioxid Redox Signal. 2009; 11: 1415-1452.
29. Wang YX, Lee CH, Tiep S, et al. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell. 2003; 113: 159-170.
30. Cheng L, Ding G, Qin Q, et al. Cardiomyocyte-restricted peroxisome proliferator-activated receptor-delta deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy. Nat Med. 2004; 10: 1245-1250.
31. Zhou Z, Ren Q, Jiao S, et al. Discovery of new and highly effective quadruple FFA1 and PPARalpha/gamma/delta agonists as potential anti-fatty liver agents. Eur J Med Chem. 2022; 229: 114061.
32. Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc Natl Acad Sci U S A. 1997; 94: 4312-4317.
33. Chevillotte E, Rieusset J, Roques M, et al. The regulation of uncoupling protein-2 gene expression by omega-6 polyunsaturated fatty acids in human skeletal muscle cells involves multiple pathways, including the nuclear receptor peroxisome proliferator-activated receptor beta. J Biol Chem. 2001; 276: 10853-10860.
34. Chuang JC, Cha JY, Garmey JC, et al. Research resource: nuclear hormone receptor expression in the endocrine pancreas. Mol Endocrinol. 2008; 22: 2353-2363.
35. Groop LC, Bonadonna RC, DelPrato S, et al. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Invest. 1989; 84: 205-213.
36. Iglesias J, Barg S, Vallois D, et al. PPARbeta/delta affects pancreatic beta cell mass and insulin secretion in mice. J Clin Invest. 2012; 122: 4105-4117.
37. Riserus U, Sprecher D, Johnson T, et al. Activation of peroxisome proliferator-activated receptor (PPAR)delta promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. Diabetes. 2008; 57: 332-339.
38. Serrano-Marco L, Rodriguez-Calvo R, El Kochairi I, et al. Activation of peroxisome proliferator-activated receptor-beta/-delta (PPAR-beta/-delta) ameliorates insulin signaling and reduces SOCS3 levels by inhibiting STAT3 in interleukin-6-stimulated adipocytes. Diabetes. 2011; 60: 1990-1999.
39. Ravnskjaer K, Frigerio F, Boergesen M, et al. PPARdelta is a fatty acid sensor that enhances mitochondrial oxidation in insulin-secreting cells and protects against fatty acid-induced dysfunction. J Lipid Res. 2010; 51: 1370-1379.
40. Cohen G, Riahi Y, Shamni O, et al. Role of lipid peroxidation and PPAR-delta in amplifying glucose-stimulated insulin secretion. Diabetes. 2011; 60: 2830-2842.
41. Li J, Xu S, Liu Y, et al. Activated PPARbeta/delta Protects

Pancreatic beta Cells in Type 2 Diabetic Goto-Kakizaki Rats from Lipoapoptosis via GPR40. Lipids. 2019; 54: 603-616.

42. Daoudi M, Hennuyer N, Borland MG, et al. PPARbeta/delta activation induces enteroendocrine L cell GLP-1 production. Gastroenterology. 2011; 140: 1564-1574.
43. Zhou J, Zhe R, Guo X, et al. The Role of PPARdelta Agosnist GW501516 in Rats with Gestational Diabetes Mellitus. Diabetes Metab Syndr Obes. 2020; 13: 2307-2316.
44. Vazquez-Carrera M. Unraveling the Effects of PPARbeta/delta on Insulin Resistance and Cardiovascular Disease. Trends Endocrinol Metab. 2016; 27: 319-334.
45. Lin SC, Hardie DG. AMPK: Sensing Glucose as well as Cellular Energy Status. Cell Metab. 2018; 27: 299-313.
46. Timmers S, Konings E, Bilet L, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab. 2011; 14: 612-622.
47. Aprile M, Cataldi S, Perfetto C, et al. In-Vitro-Generated Hypertrophic-Like Adipocytes Displaying PPARG Isoforms Unbalance Recapitulate Adipocyte Dysfunctions *In Vivo*. Cells. 2020; 9: 1284.
48. Gan Z, Burkart-Hartman EM, Han DH, et al. The nuclear receptor PPARbeta/delta programs muscle glucose metabolism in cooperation with AMPK and MEF2. Genes Dev. 2011; 25: 2619-2630.
49. Koh JH, Hancock CR, Terada S, et al. PPARbeta Is Essential for Maintaining Normal Levels of PGC-1alpha and Mitochondria and for the Increase in Muscle Mitochondria Induced by Exercise. Cell Metab. 2017; 25: 1176-1185.e5.
50. Schuler M, Ali F, Chambon C, et al. PGC1alpha expression is controlled in skeletal muscles by PPARbeta, whose ablation results in fiber-type switching, obesity, and type 2 diabetes. Cell Metab. 2006; 4: 407-414.
51. Barroso E, Rodriguez-Calvo R, Serrano-Marco L, et al. The PPARbeta/delta activator GW501516 prevents the down-regulation of AMPK caused by a high-fat diet in liver and amplifies the PGC-1alpha-Lipin 1-PPARalpha pathway leading to increased fatty acid oxidation. Endocrinology. 2011; 152: 1848-1859.
52. Zhang BB, Zhou G, Li C. AMPK: an emerging drug target for diabetes and the metabolic syndrome. Cell Metab. 2009; 9: 407-416.
53. Schultze SM, Hemmings BA, Niessen M, et al. PI3K/AKT, MAPK and AMPK signalling: protein kinases in glucose homeostasis. Expert Rev Mol Med. 2012; 14: e1.
54. Ke R, Xu Q, Li C, et al. Mechanisms of AMPK in the maintenance of ATP balance during energy metabolism. Cell Biol Int. 2018; 42: 384-392.
55. Montal ED, Dewi R, Bhalla K, et al. PEPCK Coordinates the Regulation of Central Carbon Metabolism to Promote Cancer Cell Growth. Mol Cell. 2015; 60: 571-583.
56. Millward CA, Desantis D, Hsieh CW, et al. Phosphoenol-pyruvate carboxykinase (Pck1) helps regulate the triglyceride/fatty acid cycle and development of insulin resistance in mice. J Lipid Res. 2010; 51: 1452-1463.
57. Barlaka E, Gorbe A, Gaspar R, et al. Activation of PPARbeta/delta protects cardiac myocytes from oxidative stress-induced apoptosis by suppressing generation of reactive oxygen/nitrogen species and expression of matrix metalloproteinases. Pharmacol Res. 2015; 95-96: 102-110.
58. Kim WJ, Lee W, Jung Y, et al. PPARbeta/delta agonist GW501516 inhibits TNFalpha-induced repression of adiponectin and insulin receptor in 3T3-L1 adipocytes. Biochem Biophys Res Commun. 2019; 510: 621-628.
59. Serrano-Marco L, Barroso E, El Kochairi I, et al. The peroxisome proliferator-activated receptor (PPAR) beta/delta agonist GW501516 inhibits IL-6-induced signal transducer and activator of transcription 3 (STAT3) activation and insulin resistance in human liver cells. Diabetologia. 2012; 55: 743-751.
60. Barroso E, Eyre E, Palomer X, et al. The peroxisome proliferator-activated receptor beta/delta (PPARbeta/delta) agonist GW501516 prevents TNF-alpha-induced NF-kappaB activation in human HaCaT cells by reducing p65 acetylation through AMPK and SIRT1. Biochem Pharmacol. 2011; 81: 534-543.
61. Alvarez-Guardia D, Palomer X, Coll T, et al. PPARbeta/delta activation blocks lipid-induced inflammatory pathways in mouse heart and human cardiac cells. Biochim Biophys Acta. 2011; 1811: 59-67.
62. Magliano DC, Penna-de-Carvalho A, Vazquez-Carrera M, et al. Short-term administration of GW501516 improves inflammatory state in white adipose tissue and liver damage in high-fructose-fed mice through modulation of the renin-angiotensin system. Endocrine. 2015; 50: 355-367.
63. Henry RR, Lincoff AM, Mudaliar S, et al. Effect of the dual peroxisome proliferator-activated receptor-alpha/gamma agonist aleglitazar on risk of cardiovascular disease in patients with type 2 diabetes (SYNCHRONY): a phase II, randomised, dose-ranging study. Lancet. 2009; 374: 126-135.
64. Rastogi A, Dunbar RL, Thacker HP, et al. Abrogation of postprandial triglyceridemia with dual PPAR alpha/gamma agonist in type 2 diabetes mellitus: a randomized, placebo-controlled study. Acta Diabetol. 2020; 57: 809-818.
65. Krishnappa M, Patil K, Parmar K, et al. Effect of saroglitazar 2 mg and 4 mg on glycemic control, lipid profile and cardiovascular disease risk in patients with type 2 diabetes mellitus: a 56-week, randomized, double blind, phase 3 study (PRESS XII study). Cardiovasc Diabetol. 2020; 19: 93.
66. Chaturvedi RK, Beal MF. PPAR: a therapeutic target in Parkinson's disease. J Neurochem. 2008; 106: 506-518.
67. Wojtowicz S, Strosznajder AK, Jezyna M, et al. The Novel Role of PPAR Alpha in the Brain: Promising Target in Therapy of Alzheimer's Disease and Other Neurodegenerative Disorders. Neurochem Res. 2020; 45: 972-988.

68. Kiaei M, Kipiani K, Chen J, et al. Peroxisome proliferator-activated receptor-gamma agonist extends survival in transgenic mouse model of amyotrophic lateral sclerosis. Exp Neurol. 2005; 191: 331-336.

69. Bright JJ, Walline CC, Kanakasabai S, et al. Targeting PPAR as a therapy to treat multiple sclerosis. Expert Opin Ther Targets. 2008; 12: 1565-1575.

70. Agarwal S, Yadav A, Chaturvedi RK. Peroxisome proliferator-activated receptors (PPARs) as therapeutic target in neurodegenerative disorders. Biochem Biophys Res Commun. 2017; 483: 1166-1177.

71. Saez-Orellana F, Octave JN, Pierrot N. Alzheimer's Disease, a Lipid Story: Involvement of Peroxisome Proliferator-Activated Receptor alpha. Cells. 2020; 9: 1215.

72. Krishna S, Cheng B, Sharma DR, et al. PPAR-gamma activation enhances myelination and neurological recovery in premature rabbits with intraventricular hemorrhage. Proc Natl Acad Sci U S A. 2021; 118: e2103084118.

73. Joardar A, Menzl J, Podolsky TC, et al. PPAR gamma activation is neuroprotective in a Drosophila model of ALS based on TDP-43. Hum Mol Genet. 2015; 24: 1741-1754.

74. Lee WJ, Ham SA, Lee GH, et al. Activation of peroxisome proliferator-activated receptor delta suppresses BACE1 expression by up-regulating SOCS1 in a JAK2/STAT1-dependent manner. J Neurochem. 2019; 151: 370-385.

75. Moussa-Pacha NM, Abdin SM, Omar HA, et al. BACE1 inhibitors: Current status and future directions in treating Alzheimer's disease. Med Res Rev. 2020; 40: 339-384.

76. Lee WJ, Ham SA, Yoo H, et al. Activation of PPARdelta attenuates neurotoxicity by inhibiting lipopolysaccharide-triggered glutamate release in BV-2 microglial cells. J Cell Biochem. 2018; 119: 5609-5619.

77. Iwashita A, Muramatsu Y, Yamazaki T, et al. Neuroprotective efficacy of the peroxisome proliferator-activated receptor delta-selective agonists *in vitro* and *in vivo*. J Pharmacol Exp Ther. 2007; 320: 1087-1096.

78. Luo Y, Yin W, Signore AP, et al. Neuroprotection against focal ischemic brain injury by the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone. J Neurochem. 2006; 97: 435-448.

79. Montaigne D, Butruille L, Staels B. PPAR control of metabolism and cardiovascular functions. Nat Rev Cardiol. 2021; 18: 809-823.

80. Han L, Shen WJ, Bittner S, et al. PPARs: regulators of metabolism and as therapeutic targets in cardiovascular disease. Part II: PPAR-beta/delta and PPAR-gamma. Future Cardiol. 2017; 13: 279-296.

81. Palomer X, Capdevila-Busquets E, Botteri G, et al. PPARbeta/delta attenuates palmitate-induced endoplasmic reticulum stress and induces autophagic markers in human cardiac cells. Int J Cardiol. 2014; 174: 110-118.

82. Zhang H, Pi R, Li R, et al. PPARbeta/delta activation inhibits angiotensin II-induced collagen type I expression in rat cardiac fibroblasts. Arch Biochem Biophys. 2007; 460: 25-32.

83. Teunissen BE, Smeets PJ, Willemsen PH, et al. Activation of PPARdelta inhibits cardiac fibroblast proliferation and the transdifferentiation into myofibroblasts. Cardiovasc Res. 2007; 75: 519-529.

84. Myers RB, Yoshioka J. Regulating PPARdelta signaling as a potential therapeutic strategy for skeletal muscle disorders in heart failure. Am J Physiol Heart Circ Physiol. 2015; 308: H967-H969.

85. Zizola C, Kennel PJ, Akashi H, et al. Activation of PPARdelta signaling improves skeletal muscle oxidative metabolism and endurance function in an animal model of ischemic left ventricular dysfunction. Am J Physiol Heart Circ Physiol. 2015; 308: H1078-H1085.

86. Okita K, Kinugawa S, Tsutsui H. Exercise intolerance in chronic heart failure--skeletal muscle dysfunction and potential therapies. Circ J. 2013; 77: 293-300.

87. Lincoff AM, Tardif JC, Neal B, et al. Evaluation of the dual peroxisome proliferator-activated receptor alpha/gamma agonist aleglitazar to reduce cardiovascular events in patients with acute coronary syndrome and type 2 diabetes mellitus: rationale and design of the AleCardio trial. Am Heart J. 2013; 166: 429-434.

88. Han CL, Qu CZ. Cardiovascular Risk and Safety Evaluation of a Dual Peroxisome Proliferator-Activated Receptor-Alpha/Gamma Agonist, Aleglitazar, in Patients With Type 2 Diabetes: A Meta-analysis. J Cardiovasc Pharmacol. 2020; 75: 351-357.

89. Yuan Z, Liu Y, Liu Y, et al. Peroxisome proliferation-activated receptor-gamma ligands ameliorate experimental autoimmune myocarditis. Cardiovasc Res. 2003; 59: 685-694.

90. Wickramasinghe NM, Sachs D, Shewale B, et al. PPARdelta activation induces metabolic and contractile maturation of human pluripotent stem cell-derived cardiomyocytes. Cell Stem Cell. 2022; 29: 559-576.e7.

91. Girroir EE, Hollingshead HE, He P, et al. Quantitative expression patterns of peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) protein in mice. Biochem Biophys Res Commun. 2008; 371: 456-461.

92. Kersten S, Stienstra R. The role and regulation of the peroxisome proliferator activated receptor alpha in human liver. Biochimie. 2017; 136: 75-84.

93. Terada M, Araki M, Ashibe B, et al. GW501516 acts as an efficient PPARalpha activator in the mouse liver. Drug Discov Ther. 2011; 5: 176-180.

94. Garbacz WG, Huang JT, Higgins LG, et al. PPARalpha Is Required for PPARdelta Action in Regulation of Body Weight and Hepatic Steatosis in Mice. PPAR Res. 2015; 2015: 927057.

95. Iwaisako K, Haimerl M, Paik YH, et al. Protection from liver fibrosis by a peroxisome proliferator-activated receptor delta agonist. Proc Natl Acad Sci U S A. 2012; 109: E1369-E1376.

96. Zarei M, Barroso E, Palomer X, et al. Pharmacological PPARbeta/delta activation upregulates VLDLR in hepatocytes. Clin Investig Arterioscler. 2019; 31: 111-118.

97. Pawlak M, Lefebvre P, Staels B. Molecular mechanism of PPARalpha action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. J Hepatol. 2015; 62: 720-733.

98. Francque SM, Bedossa P, Ratziu V, et al. A Randomized, Controlled Trial of the Pan-PPAR Agonist Lanifibranor in NASH. N Engl J Med. 2021; 385: 1547-1558.

99. Gawrieh S, Noureddin M, Loo N, et al. Saroglitazar, a PPAR-alpha/gamma Agonist, for Treatment of NAFLD: A Randomized Controlled Double-Blind Phase 2 Trial. Hepatology. 2021; 74: 1809-1824.

100. Bassel-Duby R, Olson EN. Signaling pathways in skeletal muscle remodeling. Annu Rev Biochem. 2006; 75: 19-37.

101. Jensen TE, Richter EA. Regulation of glucose and glycogen metabolism during and after exercise. J Physiol. 2012; 590: 1069-1076.

102. Borghouts LB, Keizer HA. Exercise and insulin sensitivity: a review. Int J Sports Med. 2000; 21: 1-12.

103. Kiens B, Roepstorff C. Utilization of long-chain fatty acids in human skeletal muscle during exercise. Acta Physiol Scand. 2003; 178: 391-396.

104. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol (1985). 2005; 98: 1154-1162.

105. King-Himmelreich TS, Schramm S, Wolters MC, et al. The impact of endurance exercise on global and AMPK gene-specific DNA methylation. Biochem Biophys Res Commun. 2016; 474: 284-290.

106. Canto C, Gerhart-Hines Z, Feige JN, et al. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature. 2009; 458: 1056-1060.

107. Diniz TA, de Lima Junior EA, Teixeira AA, et al. Aerobic training improves NAFLD markers and insulin resistance through AMPK-PPAR-alpha signaling in obese mice. Life Sci. 2021; 266: 118868.

108. Kemmerer M, Finkernagel F, Cavalcante MF, et al. AMP-Activated Protein Kinase Interacts with the Peroxisome Proliferator-Activated Receptor Delta to Induce Genes Affecting Fatty Acid Oxidation in Human Macrophages. PLoS One. 2015; 10: e0130893.

109. Dressel U, Allen TL, Pippal JB, et al. The peroxisome proliferator-activated receptor beta/delta agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells. Mol Endocrinol. 2003; 17: 2477-2493.

110. Luquet S, Lopez-Soriano J, Holst D, et al. Peroxisome proliferator-activated receptor delta controls muscle development and oxidative capability. FASEB J. 2003; 17: 2299-2301.

111. Wang YX, Zhang CL, Yu RT, et al. Regulation of muscle fiber type and running endurance by PPARdelta. PLoS Biol. 2004; 2: e294.

112. Sprecher DL, Massien C, Pearce G, et al. Triglyceride:high-density lipoprotein cholesterol effects in healthy subjects administered a peroxisome proliferator activated receptor delta agonist. Arterioscler Thromb Vasc Biol. 2007; 27: 359-365.

113. Visnjic D, Lalic H, Dembitz V, et al. AICAr, a Widely Used AMPK Activator with Important AMPK-Independent Effects: A Systematic Review. Cells. 2021; 10: 1095.

114. Manio MCC, Inoue K, Fujitani M, et al. Combined pharmacological activation of AMPK and PPARdelta potentiates the effects of exercise in trained mice. Physiol Rep. 2016; 4: e12625.

115. Rockl KS, Hirshman MF, Brandauer J, et al. Skeletal muscle adaptation to exercise training: AMP-activated protein kinase mediates muscle fiber type shift. Diabetes. 2007; 56: 2062-2069.

116. Spaulding HR, Yan Z. AMPK and the Adaptation to Exercise. Annu Rev Physiol. 2022; 84: 209-227.

117. McGee SL, Howlett KF, Starkie RL, et al. Exercise increases nuclear AMPK alpha2 in human skeletal muscle. Diabetes. 2003; 52: 926-928.

118. Feng YZ, Nikolic N, Bakke SS, et al. PPARdelta activation in human myotubes increases mitochondrial fatty acid oxidative capacity and reduces glucose utilization by a switch in substrate preference. Arch Physiol Biochem. 2014; 120: 12-21.

119. Kramer DK, Ahlsen M, Norrbom J, et al. Human skeletal muscle fibre type variations correlate with PPAR alpha, PPAR delta and PGC-1 alpha mRNA. Acta Physiol (Oxf). 2006; 188: 207-216.

120. Booth FW, Thomason DB. Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. Physiol Rev. 1991; 71: 541-585.

121. Jarvis JC, Mokrusch T, Kwende MM, et al. Fast-to-slow transformation in stimulated rat muscle. Muscle Nerve. 1996; 19: 1469-1475.

122. Bylund AC, Bjuro T, Cederblad G, et al. Physical training in man. Skeletal muscle metabolism in relation to muscle morphology and running ability. Eur J Appl Physiol Occup Physiol. 1977; 36: 151-169.

123. Wu H, Kanatous SB, Thurmond FA, et al. Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. Science. 2002; 296: 349-352.

124. Peters JM, Gonzalez FJ, Muller R. Establishing the Role of PPARbeta/delta in Carcinogenesis. Trends Endocrinol Metab. 2015; 26: 595-607.

125. Peters JM, Foreman JE, Gonzalez FJ. Dissecting the role of peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) in colon, breast, and lung carcinogenesis. Cancer Metastasis Rev. 2011; 30: 619-640.

126. Ji Y, Li H, Wang F, et al. PPARbeta/delta Agonist GW501516

Inhibits Tumorigenicity of Undifferentiated Nasopharyngeal Carcinoma in C666-1 Cells by Promoting Apoptosis. Front Pharmacol. 2018; 9: 648.

127. Pechery A, Fauconnet S, Bittard H, et al. Apoptotic effect of the selective PPARbeta/delta agonist GW501516 in invasive bladder cancer cells. Tumour Biol. 2016; 37: 14789-14802.

128. Elie-Caille C, Lascombe I, Pechery A, et al. Molecular and nanoscale evaluation of N-cadherin expression in invasive bladder cancer cells under control conditions or GW501516 exposure. Mol Cell Biochem. 2020; 471: 113-127.

129. Wagner KD, Du S, Martin L, et al. Vascular PPARbeta/delta Promotes Tumor Angiogenesis and Progression. Cells. 2019; 8: 1623.

130. Liu Y, Deguchi Y, Tian R, et al. Pleiotropic Effects of PPARD Accelerate Colorectal Tumorigenesis, Progression, and Invasion. Cancer Res. 2019; 79: 954-969.

131. Wang D, Fu L, Wei J, et al. PPARdelta Mediates the Effect of Dietary Fat in Promoting Colorectal Cancer Metastasis. Cancer Res. 2019; 79: 4480-4490.

132. Pollock CB, Rodriguez O, Martin PL, et al. Induction of metastatic gastric cancer by peroxisome proliferator-activated receptordelta activation. PPAR Res. 2010; 2010: 571783.

133. Stephen RL, Gustafsson MC, Jarvis M, et al. Activation of peroxisome proliferator-activated receptor delta stimulates the proliferation of human breast and prostate cancer cell lines. Cancer Res. 2004; 64: 3162-3170.

134. Hollingshead HE, Killins RL, Borland MG, et al. Peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) ligands do not potentiate growth of human cancer cell lines. Carcinogenesis. 2007; 28: 2641-2649.

135. Girroir EE, Hollingshead HE, Billin AN, et al. Peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) ligands inhibit growth of UACC903 and MCF7 human cancer cell lines. Toxicology. 2008; 243: 236-243.

136. Yuan H, Lu J, Xiao J, et al. PPARdelta induces estrogen receptor-positive mammary neoplasia through an inflammatory and metabolic phenotype linked to mTOR activation. Cancer Res. 2013; 73: 4349-4361.

137. Monje M, Borniger JC, D'Silva NJ, et al. Roadmap for the Emerging Field of Cancer Neuroscience. Cell. 2020; 181: 219-222.