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# Fibrates, glitazones, and peroxisome proliferator-activated receptors

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## Abstract

Several decades ago, fibrates were approved for the treatment of dyslipidemia, whereas thiazolidinediones were screened in animal models to improve glucose homeostasis and subsequently developed for the treatment of type 2 diabetes. Relatively recently, these drugs were found to act via peroxisome proliferator-activated receptors, nuclear receptors which control lipid metabolism and glucose homeostasis. In this historical perspective, we discuss the history of discovery of the peroxisome proliferator-activated receptors, from the clinical development of their agonists to the subsequent discovery of these receptors and their mechanisms of action, to finally evoke possibilities of targeted pharmacology for future development of selective peroxisome proliferator-activated receptors modulators.

**MESH Keywords** Animals ; Antilipemic Agents ; history ; therapeutic use ; Blood Glucose ; drug effects ; Clofibric Acid ; history ; therapeutic use ; Diabetes Mellitus, Type 2 ; drug therapy ; history ; Drug Discovery ; history ; Dyslipidemias ; drug therapy ; history ; History, 20th Century ; History, 21st Century ; Humans ; Hypoglycemic Agents ; history ; therapeutic use ; Lipid Metabolism ; drug effects ; Peroxisome Proliferator-Activated Receptors ; agonists ; history ; Thiazolidinediones ; history ; therapeutic use ; Treatment Outcome

## Fibrates and glitazones: classical pharmacology and clinical use

### Discovery of Fibrates

The first fibrates were synthesized in the mid-1950s. Their clinical development came from the observation, in 1953, of researchers in France that, among 80 synthesized structures derived from dehydrocholic acid, phenylethyl acetic acid and certain other disubstituted acetic acids exhibited hypocholesterolemic properties in rats and humans<sup>1, 2</sup>. Several years later, Imperial Chemical Industries (ICI) laboratories in England screened several branched-chain fatty acids initially developed as plant hormone analogues and found that several oxyisobutyric acid derivatives decreased total lipid and cholesterol concentrations in rat plasma and liver. The most effective compound with minimal toxicity, discovered by Thorp and Waring in 1962<sup>3</sup>, was ethyl- $\alpha$ -4-chlorophenoxyisobutyrate (CPIB) which was called clofibrate. This compound was previously described in 1947 by two Italian chemists, Galimberti and DeFranceschi<sup>4</sup>, and evoked in 1956 by French researchers, Julia et al, working on plant growth-regulating substances<sup>5</sup>. Clofibrate decreased lipids in animal models<sup>3</sup>. Its mode of action was not known but, initially, the hypolipidemic effect of CPIB was attributed to seasonal variations in adrenal and thyroid function, and the administration of androsterone in rats and monkeys potentiated the hypocholesterolemic effect of CPIB<sup>6</sup>. Subsequently, several clinical studies were performed on clofibrate as monotherapy or in combination with androsterone. These trials showed that clofibrate decreases lipid levels in hypercholesterolemic patients, mainly as the result of a reduction in the very low density lipoprotein (VLDL), and less in the low density lipoprotein (LDL) fraction, that it was well tolerated in long-term treatment and that the coadministration of androsterone was not necessary for its hypolipidemic effect<sup>7, 8</sup>. However, the mode of action of clofibrate was still unclear and 30 years of intensive research was needed to discover it. Researchers were worried by the fact that long-term treatment with clofibrate induces hepatomegaly in rats as the result of unexplained proliferation of hepatic cytoplasmic inclusion bodies called peroxisomes<sup>9</sup>. Nevertheless, clofibrate was approved in the United States in 1967 for the treatment of hyperlipidemias. Further studies suggested that the peroxisome proliferation and the hypolipidemic properties of clofibrate were independent<sup>10, 11</sup>, but the mechanism was not known. Several years later, two other hypolipidemic compounds, Wy-14,653 and tibric acid, were found to be more potent than clofibrate in increasing peroxisome proliferation in livers of rats and mice, despite being structurally different from clofibrate and the other oxyisobutyric acid derivatives<sup>12</sup>.

Disturbed by concerns about the clofibrate-induced hepatomegaly in murine species, Thorp and colleagues tried to modify the clofibrate structure to identify more potent hypolipidemic fibrate drugs with minimum toxicity. To do so, they used a test measuring the displacement of albumin-bound L-thyroxine because they thought that clofibrate exhibited indirect hypocholesterolemic activities because of its capacity to bind to albumin and displace albumin-bound proteins such as L-thyroxine. Two analogs were selected: methyl-clofenapate and clobuzarit. Despite higher-potency and lipid-lowering activity when compared with clofibrate, methyl-clofenapate was withdrawn from clinical studies not only because of the induction of strong hepatomegaly and peroxisome proliferation in rats, but also because of the occurrence of hepatic carcinomas in rats that, at that time, were thought to be prognostic for humans. Clobuzarit was less hypolipidemic than clofibrate; however, it induced a significant decrease in plasma fibrinogen levels and was oriented toward the treatment of rheumatoid arthritis due to its anti-inflammatory properties.

Meanwhile, several pharmaceutical companies performed intensive research to improve the pharmacological and pharmacokinetic activities of clofibrate. Many modifications were tested: the phenoxy-2-methyl-2-propionic acid chain of clofibrate was preserved and the Cl atom was substituted by different hydrophobic groups. However, none of the obtained phenylketone molecules were interesting hypolipidemic drugs, except for the benzoyl derivative with a Cl atom in position 4, which was called procetofen. Thus, procetofen was synthesized in 1974 and was introduced in clinical practice in France the same year. Procetofen, which significantly decreased plasma lipid concentrations in hyperlipidemic patients<sup>13</sup>, was later called fenofibrate to comply with World Health Organization nomenclature guidelines. Fenofibrate was demonstrated to exhibit improved pharmacokinetic and pharmacological properties when compared with clofibrate.

Other fibrates were also introduced in the late 1970s and early 1980s, such as gemfibrozil in the United States and bezafibrate and ciprofibrate in Europe. However, at that time, the clinical use of fibrates was limited by the demonstration that clofibrate and other hypolipidemic drugs (eg, nafenopin, Wy-14.643, and tibric acid)<sup>14</sup> induced hepatic carcinogenesis in rats and mice, suggesting a potential deleterious effect of these drugs in humans. Fortunately, in 1983, it was shown that humans and nonhuman primates seemed to be resistant to this peroxisome proliferative effect<sup>15</sup>; further epidemiological studies in humans confirmed the absence of increased risk of cancer in patients treated with fibrates, allowing these drugs to be safely used in the clinic<sup>16, 17</sup>. Consequently, a renewed interest in fibrates appeared in the 1990s when the mode of action of fibrates became understood and the results of several clinical trials such as the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) study, which demonstrated the efficacy of gemfibrozil on cardiovascular events, were demonstrated<sup>18</sup>. The recent demonstration of beneficial effects on microvascular complications with fenofibrate in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study<sup>19</sup>, which corroborates earlier findings with clofibrate<sup>20</sup>, have further consolidated the interest for these drugs. Although the FIELD study showed mitigated results with respect to macrovascular complications of diabetes mellitus and although statins are now the first-line hypolipidemic drugs, fibrates are still a widely prescribed class of hypolipidemic drugs; with gemfibrozil and fenofibrate primarily used in the United States and bezafibrate and ciprofibrate available in Europe.

### Discovery of the glitazones

Thiazolidinediones (TZD), also termed glitazones, are widely prescribed in the treatment of type 2 diabetes as monotherapy or in combination with sulfonylureas, metformin, dipeptidyl peptidase-4 inhibitors and insulin. However, their discovery, in the early 1980s, was quite surprising when Japanese researchers tried to synthesize more potent fibrate hypolipidemic drugs. Indeed, in 1975, Takeda laboratories in Japan synthesized 71 analogues of clofibrate, i.e. alkanolic acids containing a biphenyl ether moiety, and tested them for hypolipidemic properties. Interestingly, some of these compounds showed both hypolipidemic and hypoglycemic effects in diabetic mice<sup>21, 22</sup>. Thus, extensive structure-activities relationship studies led to the discovery of ADD-3878, also called ciglitazone<sup>23, 24</sup>, which was shown to normalize hyperglycemia, hyperinsulinemia and hypertriglyceridemia in animal models of type 2 diabetes without provoking hypoglycemia<sup>25</sup>. Consecutively, another glitazone, troglitazone (CS-045), discovered by Sankyo Company in 1988<sup>26</sup>, decreased insulin resistance by both increasing insulin-stimulated glucose utilization and reducing hepatic glucose production. Troglitazone was the first TZD approved for clinical use in the United States in 1997; unfortunately, it was subsequently withdrawn worldwide because of liver toxicity<sup>27</sup>. Meanwhile, an intensive insulin resistance targeted drug research program had been initiated by SmithKline in the United Kingdom to develop insulin sensitizers more potent than ciglitazone. BRL-49653, synthesized in 1988 and later known as rosiglitazone, was shown to normalize blood glucose levels and to improve tissue sensitivity in rodent models and in patients with type 2 diabetes, to be orally active, and to be more selective and more potent (1 mg/kg) than ciglitazone (150 mg/kg)<sup>28, 29</sup>. In parallel, Takeda laboratories in Japan developed another TZD compound, pioglitazone (AD-4833)<sup>30</sup>, which also ameliorates glucose and lipid profiles in patients with type 2 diabetes. Rosiglitazone and pioglitazone were approved in the United States in 1999 for the treatment of type 2 diabetes; today, they represent the only two glitazones used in the treatment of type 2 diabetes.

## Fibrates, glitazones and PPARs: from classic to molecular pharmacology

### PPAR $\alpha$ and fibrate action

In the mid-1980s, Reddy et al showed that fibrates increase the transcription of peroxisomal fatty acid  $\beta$ -oxidation genes in liver rat<sup>31</sup>, whereas our studies in Leuven, Belgium, at the end of the 1980s showed that fibrates regulate the expression of genes involved in lipoprotein metabolism<sup>32</sup>. The triggering event in the understanding of fibrate action occurred in 1990 when two researchers from the Central Toxicology Laboratories of ICI, Isseman and Green, identified a novel member of the steroid hormone receptor superfamily of ligand-activated transcription factors. The receptor was structurally related but clearly different from steroid hormone receptors, could be activated by a wide range of molecules including several fatty acids and the pharmacological class of fibrates, was thought to mediate the peroxisome proliferation response<sup>33</sup> and was, therefore, called Peroxisome Proliferator-Activated Receptor (PPAR), later PPAR $\alpha$  (NR1C1). This receptor was expressed at a high level in the liver, kidney and heart. Further studies showed that PPAR activation increases the transcription of specific PPAR target genes after heterodimerization with the Retinoid X Receptor (RXR), and binding of the complex to specific transcriptional regulatory elements called Peroxisome Proliferator Response Elements (PPREs)<sup>34, 35</sup>. It was hypothesized that

the hypolipidemic effects of fibrates were mediated through PPAR $\alpha$ 36 , which was proven by using knockout (KO) mouse models that allow the study of the in vivo role of this receptor. Thus, using the PPAR $\alpha$  KO mouse37 , it was shown that fibrates decrease plasma lipid levels and induce hepatomegaly and hepatic peroxisome proliferation in a PPAR $\alpha$ -dependent manner. Moreover, species differences in PPAR $\alpha$  function, particularly between murine species and humans, were found at the level of PPAR $\alpha$  expression, ligand activation and biological responses, especially with respect to its role in peroxisome proliferation in rats and mice, but not humans38 .

By using molecular biology and functional genomic technologies, target genes of PPAR $\alpha$  were identified with roles not only in lipid homeostasis but also in other pathways. Thus, in humans, the increase in serum high density lipoprotein-cholesterol (HDL-C) concentrations is related to the gene activation of two major HDL apolipoproteins (apo), apoA-I and apoA-II, by PPAR $\alpha$ 39 . Moreover, the TG-lowering action of fibrates, related to decreased synthesis and increased catabolism of VLDL, was associated with the inhibition of apo C-III expression, a well-known inhibitor of lipoprotein lipase (LPL), and the induction of LPL and apoA-V expression. In addition to its major role in lipid homeostasis, PPAR $\alpha$  has exhibited additional pleiotropic effects on endothelial dysfunction, myocardial ischemic injury and immune-inflammatory responses. At that time, PPAR $\alpha$  was found to be expressed in the major cell types of the atherosclerotic plaque including macrophages, smooth muscle cells, endothelial cells and lymphocytes; its activation resulted in direct antiatherogenic effects in the artery wall40 . PPAR $\alpha$  activation reduces the recruitment and adhesion of mononuclear cells to the endothelium, decreases atherosclerotic plaque inflammation and proliferation of smooth muscle cells, and facilitates cholesterol efflux by increasing the expression of the transporters scavenger receptor-BI and ATP-binding cassette transporter A-1 in macrophages. Thus, these molecular actions of PPAR $\alpha$  increased our understanding of the hypolipemic effects of fibrates and suggested an interesting potential of these drugs in the control of cardiovascular disease and its risk factors.

### **PPAR $\gamma$ and glitazone action**

After the discovery of PPAR $\alpha$  in 1990, two other genes belonging to the same family, PPAR $\beta$ / $\delta$  (NR1C2) and PPAR $\gamma$  (NR1C3), were cloned in 199236 . PPAR $\gamma$  is a nuclear receptor that regulates gene transcription after activation by small lipophilic fatty acid derivatives or oxidized lipid components of ox-LDL (9-hydroxy and 13-hydroxy octadecadienoic acids (HODE)). In 1994, PPAR $\gamma$  was shown to be a major adipogenic transcription factor41 , 42 and, in 1995, PPAR $\gamma$  was identified as the target of the TZDs43 . Therefore, PPAR $\gamma$  was suggested to mediate the antidiabetic actions of TZDs. Even though the molecular mechanisms by which TZDs exert hypoglycemic and insulin-sensitizing effects are not totally understood, adipose tissue seems to be the major organ implicated. Indeed, PPAR $\gamma$  promotes adipocyte differentiation, stimulates fatty acid storage in adipocytes via the activation of genes, such as LPL, fatty acid transport protein, CD36, acyl-coA synthetase, and decreases free fatty acid secretion, resulting in enhanced adipocyte insulin signaling. This effect can explain the body weight gain induced by TZD administration, a major drawback in the use of these drugs in the treatment of type 2 diabetes. Consequently, plasma free fatty acids are decreased, thus improving lipotoxicity and insulin sensitivity in liver and skeletal muscle. These insulin-sensitizing effects result in long-term glycemic control in patients with type 2 diabetes who receive treatment with TZDs, as shown in a Diabetes Outcome Progression Trial (ADOPT)44 . PPAR $\gamma$  activation also decreases obesity-induced inflammation and insulin resistance by regulating the expression of cytokines (Tumor Necrosis Factor) and adipokines (adiponectin, resistin) in adipose tissue. Moreover, PPAR $\gamma$  is expressed in atherosclerotic plaques; also, the fact that TZDs inhibit inflammatory cytokine secretion by activated macrophages increased their interest as potential antiatherogenic drugs. The PROspective pioglitazone Clinical Trial In macroVascular Events (PROactive study) has indicated the protective effects of pioglitazone against macrovascular diseases in patients with type 2 diabetes45 .

### **PPAR $\beta$ / $\delta$ : a target for novel drugs**

PPAR $\delta$ , also known as nuclear hormone receptor 1 (NUC1) or PPAR $\beta$ , is activated by saturated and polyunsaturated fatty acids and eicosanoids. Synthetic PPAR $\beta$ / $\delta$  ligands have been developed, such as the phenoxyacetic derivatives GW501516 and GW0742, optimized from a library of hydrophobic carboxylates or L165461 obtained from an in silico approach46 , 47 . Although there are no drugs activating PPAR $\beta$ / $\delta$  that are approved for clinical treatment, new synthetic PPAR $\beta$ / $\delta$  agonists are in development for the treatment of dyslipidemia, obesity and/or insulin resistance such as MBX-8025, CER-002 and KD3010. Activation of PPAR $\beta$ / $\delta$  reduces adiposity and improves glucose metabolism and insulin sensitivity in animal models of obesity and insulin resistance, an effect likely due to the capacity of PPAR  $\beta$ / $\delta$  to enhance fatty acid transport and oxidation48 , 49 . Moreover, PPAR $\beta$ / $\delta$  activation improves dyslipidemia by raising HDL50 , a beneficial effect currently investigated in humans. PPAR $\beta$ / $\delta$  is also implicated in the adaptative metabolic response of skeletal muscle to exercise endurance by increasing oxidative muscle fibers51 , 52 . Furthermore, PPAR $\beta$ / $\delta$  is expressed in vascular cells, including endothelial cells, smooth muscle cells and macrophages; PPAR $\beta$ / $\delta$  activation has direct beneficial effects on vascular homeostasis by protecting endothelial cells from inflammation and apoptosis, decreasing proliferation of SMC, decreasing macrophage inflammation and promoting angiogenesis. These effects confer a promising potential of PPAR $\beta$ / $\delta$  agonists for the treatment of the metabolic syndrome in humans.

## **Future PPAR agonists: from basic molecular to target-oriented pharmacology**

### **PPAR coagonists**

The discovery of the role of the PPARs in the regulation of lipid and glucose metabolism led the pharmaceutical industry to develop PPAR coagonists. Because many patients with insulin resistance also exhibit dyslipidemia, it was theoretically judicious to create dual PPAR $\alpha$ / $\gamma$  agonists to treat these patients. Indeed, these dual PPAR $\alpha$ / $\gamma$  agonists, also named glitazars, combine the insulin sensitizing effects of PPAR $\gamma$  activation with the lipid-lowering effects of PPAR $\alpha$  agonists, lowering triglycerides, increasing the HDL level and insulin sensitivity, and reducing cardiovascular risk. In animal models of insulin resistance and dyslipidemia, these compounds were promising. However, in humans, despite an improved clinical efficacy on glucose and lipid metabolism, several dual PPAR $\alpha$ / $\gamma$  agonists were withdrawn in late-stage clinical development, mostly because of safety concerns<sup>53</sup>. For example, ragaglitazar development was discontinued in 2004 as the result of the induction of anaemia and urothelial cancer. The development of muraglitazar and tesaglitazar was discontinued in phase 3 clinical trials in 2006 because of oedema and major adverse cardiovascular events (myocardial infarction, stroke, heart failure) for muraglitazar and elevated serum creatinine levels and a decreased glomerular filtration rate for tesaglitazar. These discouraging adverse effects consequently raised questions about the therapeutic potential of such drugs. However, the recent results with aleglitazar showing improved safety, higher efficacy, and encouraging, albeit still short-term, cardiovascular effects<sup>54</sup> give real hope for the future of glitazars.

Dual PPAR $\gamma$ / $\delta$  and PPAR $\alpha$ / $\delta$  agonists are also under investigation as hypolipidemic, hypoglycaemic and antiatherogenic agents. The dual PPAR $\gamma$ / $\delta$  agonist, (R)-3-{2-ethyl-4-[3-(4-ethyl-2-pyridin-2-yl-phenoxy)-butoxy]-phenyl}propionic acid lowered plasma glucose levels and induced less weight gain than rosiglitazone in Zucker diabetic fatty rats<sup>55</sup>. As for the dual PPAR $\alpha$ / $\delta$  agonists, T913659 has shown beneficial lipid effects in primates<sup>56</sup> and GFT505 has shown promising results in a phase 2 clinical trial in the management of dyslipidemia associated with abdominal obesity. However, further clinical investigation is needed to explore their potential clinical use in diabetes-associated macrovascular and microvascular complications.

Finally, the development of agonists combining the effects of the 3 PPARs is under investigation. Interestingly, bezafibrate, an older-generation fibrate, is a weak pan PPAR $\alpha$ / $\gamma$ / $\delta$  agonist. Bezafibrate increases the HDL-C level, decreases TG levels, improves insulin sensitivity and decreases the long-term rate of progression of coronary artery disease. Consequently, novel PPAR pan agonists (PLX-204, GW-625019, GW-677954...) are currently investigated as clinical agents in the treatment of type 2 diabetes and its cardiovascular complications.

### Selective PPAR modulators (SPPARMs)

Resulting from a better understanding of the molecular actions of nuclear receptors, the concept of selective PPAR modulators (SPPARMs), in analogy to selective estrogen receptor modulators (SERMs), emerged recently as a means to optimize the therapeutic potential. This new molecular-target based strategy aims to synthesize PPAR agonist with a target-oriented therapeutic profile, maintaining the desired therapeutic benefit and minimizing the adverse effects of the first-generation PPAR agonists. At the molecular level, each PPAR ligand induces a specific change in PPAR conformation, resulting in the differential recruitment of cofactors and gene-specific transcriptional regulation. Thus, in addition to a panel of common genes regulated in a similar manner by all agonists, each agonist also induces its proper profile of genes, resulting in specific biological effects. The validity of the concept is clinically supported by observations that fenofibrate and gemfibrozil, which both increase HDL-C levels, have distinct effects on human apoA-I concentrations. Thus, new compounds were created with differential gene-regulating properties. This concept of SPPARMs is also particularly relevant for PPAR $\gamma$  agonists. Indeed, because rosiglitazone and pioglitazone display well-documented adverse effects such as body weight gain, increased risk for bone fractures, and edema, the idea is to develop compounds retaining the beneficial effects of these full PPAR $\gamma$  agonists on glucose metabolism without the adverse effects. Several TZD-like and non-TZD-like PPAR $\gamma$  partial agonists or SPPAR $\gamma$  Ms have been synthesised and some of them have entered clinical studies such as halofenate/metaglidasen, DRF-2593 (balaglitazone) or MCC555 (netoglitazone).

### Conclusions

Fibrates and TZDs were developed decades ago for the treatment of dyslipidemia and type 2 diabetes. However, since 1990, their mechanism, through the discovery of the PPARs, began to be elucidated. Since that time, PPARs have been shown to be implicated in many processes such as lipid and glucose metabolism, inflammation or cardiovascular functions, thus presenting PPARs as interesting pharmacological targets. In addition, the use of PPAR ligands enabled advancements regarding the physiological roles of PPARs. Although none of the new synthetic PPAR agonists, PPAR coagonists or selective PPAR modulators, have replaced the first-generation fibrates and TZDs, they constitute a panel of promising drug. We are sure that PPARs have not revealed all their secrets and will continue to interest researchers who are trying to understand their physiological roles and their potential as targets for drugs to prevent cardiometabolic disorders.

### References:

1. Mathivat A, Cottet J. Clinical trials on the hypocholesterolemia-producing effect of 2-phenylbutyric acid. Bull Mem Soc Med Hop Paris. 1953; 69: 1030 - 1048
2. Cottet J, Redel J, Krumm-Heller C, Tricaud ME. Hypocholesterolemic property of sodium phenylethylacetate (22 TH) in the rat. Bull Acad Natl Med. 1953; 137: 441 - 442
3. Thorp JM, Waring WS. Modification of metabolism and distribution of lipids by ethyl chlorophenoxyisobutyrate. Nature. 1962; 194: 948 - 949

- 4. Galimberti P, Defranceschi A. Intesi di alcuni derivati  $\alpha$ -isobutirici. *Gazz Chim Ital*. 1947; 77: 431 -
- 5. Julia M, Baillarge M, Thernoff G. Sur quelques nouveaux d $\acute$ riv $\acute$ es aryloxyisobutyriques et apparent $\acute$ s. *Bull Soc Chim Fr*. 1956; 776 - 783
- 6. Thorp JM. Experimental evaluation of an orally active combination of androsterone with ethyl chlorophenoxy-isobutyrate. *Lancet*. 1962; 1: 1323 - 1326
- 7. Oliver MF. Further Observations on the Effects of Atomid and of Ethyl Chlorophenoxyisobutyrate on Serum Lipid Levels. *J Atheroscler Res*. 1963; 3: 427 - 444
- 8. Hellman L, Zumoff B, Kessler G, Kara E, Rubin IL, Rosenfeld RS. Reduction of Serum Cholesterol and Lipids by Ethyl Chlorophenoxyisobutyrate. *J Atheroscler Res*. 1963; 3: 454 - 466
- 9. Hess R, Staubli W, Riess W. Nature of the hepatomegalic effect produced by ethyl-chlorophenoxy-isobutyrate in the rat. *Nature*. 1965; 208: 856 - 858
- 10. Azarnoff DL, Svoboda DJ. Microbodies in experimentally altered cells. VI. Thyroxine displacement from plasma proteins and clofibrate effect. *Arch Int Pharmacodyn Ther*. 1969; 181: 386 - 393
- 11. Svoboda D, Azarnoff D, Reddy J. Microbodies in experimentally altered cells. II. The relationship of microbody proliferation to endocrine glands. *J Cell Biol*. 1969; 40: 734 - 746
- 12. Reddy JK, Krishnakantha TP. Hepatic peroxisome proliferation: induction by two novel compounds structurally unrelated to clofibrate. *Science*. 1975; 190: 787 - 789
- 13. Matzkies F, Schulzky D, Berg G. Procetofen, a new lipid- and urine-acid-reducing substance. *Fortschr Med*. 1978; 96: 1939 - 1941
- 14. Reddy JK, Azarnoff DL, Hignite CE. Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature*. 1980; 283: 397 - 398
- 15. Blumcke S, Schwartzkopf W, Lobeck H, Edmondson NA, Prentice DE, Blane GF. Influence of fenofibrate on cellular and subcellular liver structure in hyperlipidemic patients. *Atherosclerosis*. 1983; 46: 105 - 116
- 16. Bentley P, Calder I, Elcombe C, Grasso P, Stringer D, Wiegand HJ. Hepatic peroxisome proliferation in rodents and its significance for humans. *Food Chem Toxicol*. 1993; 31: 857 - 907
- 17. Cattley RC, DeLuca J, Elcombe C, Fenner-Crisp P, Lake BG, Marsman DS, Pastoor TA, Popp JA, Robinson DE, Schwetz B, Tugwood J, Wahli W. Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Regul Toxicol Pharmacol*. 1998; 27: 47 - 60
- 18. Staels B, Maes M, Zambon A. Fibrates and future PPAR $\alpha$  agonists in the treatment of cardiovascular disease. *Nat Clin Pract Cardiovasc Med*. 2008; 5: 542 - 553
- 19. Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P, Drury P, Kesaniemi YA, Sullivan D, Hunt D, Colman P, d'Emden M, Whiting M, Ehnholm C, Laakso M. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet*. 2005; 366: 1849 - 1861
- 20. Harrold BP, Marmion VJ, Gough KR. A double-blind controlled trial of clofibrate in the treatment of diabetic retinopathy. *Diabetes*. 1969; 18: 285 - 291
- 21. Kawamatsu Y, Saraie T, Imamiya E, Nishikawa K, Hamuro Y. Studies on antihyperlipidemic agents. I. Synthesis and hypolipidemic activities of phenoxyphenyl alkanolic acid derivatives. *Arzneimittelforschung*. 1980; 30: 454 - 459
- 22. Kawamatsu Y, Asakawa H, Saraie T, Imamiya E, Nishikawa K, Hamuro Y. Studies on antihyperlipidemic agents. II. Synthesis and biological activities of 2-chloro-3-arylpropionic acids. *Arzneimittelforschung*. 1980; 30: 585 - 589
- 23. Sohma T, Mizuno K, Tawada H, Sugiyama Y, Fujita T, Kawamatsu Y. Studies on antidiabetic agents. I. Synthesis of 5-[4-(2-methyl-2-phenylpropoxy)-benzyl]thiazolidine-2,4-dione (AL-321) and related compounds. *Chem Pharm Bull (Tokyo)*. 1982; 30: 3563 - 3573
- 24. Sohma T, Mizuno K, Imamiya E, Sugiyama Y, Fujita T, Kawamatsu Y. Studies on antidiabetic agents. II. Synthesis of 5-[4-(1-methylcyclohexylmethoxy)-benzyl]thiazolidine-2,4-dione (ADD-3878) and its derivatives. *Chem Pharm Bull (Tokyo)*. 1982; 30: 3580 - 3600
- 25. Fujita T, Sugiyama Y, Taketomi S, Sohma T, Kawamatsu Y, Iwatsuka H, Suzuki Z. Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, ciglitazone), a new antidiabetic agent. *Diabetes*. 1983; 32: 804 - 810
- 26. Fujiwara T, Yoshioka S, Yoshioka T, Ushiyama I, Horikoshi H. Characterization of new oral antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. *Diabetes*. 1988; 37: 1549 - 1558
- 27. Watkins PB, Whitcomb RW. Hepatic dysfunction associated with troglitazone. *N Engl J Med*. 1998; 338: 916 - 917
- 28. Oakes ND, Kennedy CJ, Jenkins AB, Laybutt DR, Chisholm DJ, Kraegen EW. A new antidiabetic agent, BRL 49653, reduces lipid availability and improves insulin action and glucoregulation in the rat. *Diabetes*. 1994; 43: 1203 - 1210
- 29. Cantello BC, Cawthorne MA, Cottam GP, Duff PT, Haigh D, Hindley RM, Lister CA, Smith SA, Thurlby PL. [[omega-(Heterocyclylamino)alkoxy]benzyl]-2,4-thiazolidinediones as potent antihyperglycemic agents. *J Med Chem*. 1994; 37: 3977 - 3985
- 30. Sohma T, Momose Y, Meguro K, Kawamatsu Y, Sugiyama Y, Ikeda H. Studies on antidiabetic agents. Synthesis and hypoglycemic activity of 5-[4-(pyridylalkoxy)benzyl]-2,4-thiazolidinediones. *Arzneimittelforschung*. 1990; 40: 37 - 42
- 31. Reddy JK, Goel SK, Nemali MR, Carrino JJ, Laffler TG, Reddy MK, Sperbeck SJ, Osumi T, Hashimoto T, Lalwani ND. Transcription regulation of peroxisomal fatty acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase in rat liver by peroxisome proliferators. *Proc Natl Acad Sci U S A*. 1986; 83: 1747 - 1751
- 32. Staels B, van Tol A, Verhoeven G, Auwerx J. Apolipoprotein A-IV messenger ribonucleic acid abundance is regulated in a tissue-specific manner. *Endocrinology*. 1990; 126: 2153 - 2163
- 33. Isseman I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature*. 1990; 347: 645 - 650
- 34. Kliewer SA, Umehono K, Noonan DJ, Heyman RA, Evans RM. Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature*. 1992; 358: 771 - 774
- 35. Tugwood JD, Isseman I, Anderson RG, Bundell KR, McPheat WL, Green S. The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. *Embo J*. 1992; 11: 433 - 439
- 36. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W. Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell*. 1992; 68: 879 - 887
- 37. Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H, Gonzalez FJ. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol*. 1995; 15: 3012 - 3022
- 38. Cheung C, Akiyama TE, Ward JM, Nicol CJ, Feigenbaum L, Vinson C, Gonzalez FJ. Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor alpha. *Cancer Res*. 2004; 64: 3849 - 3854
- 39. Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart JC. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation*. 1998; 98: 2088 - 2093
- 40. Marx N, Duez H, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circ Res*. 2004; 94: 1168 - 1178
- 41. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell*. 1994; 79: 1147 - 1156
- 42. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev*. 1994; 8: 1224 - 1234
- 43. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem*. 1995; 270: 12953 - 12956
- 44. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med*. 2006; 355: 2427 - 2443
- 45. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefebvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Koranyi L, Laakso M, Morkan M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Scherthaner G, Schmitz O, Skrha J, Smith U, Taton J. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet*. 2005; 366: 1279 - 1289

- 46 . Sznaidman ML , Haffner CD , Maloney PR , Fivush A , Chao E , Goreham D , Sierra ML , LeGrumelec C , Xu HE , Montana VG , Lambert MH , Willson TM , Oliver WR Jr , Sternbach DD . Novel selective small molecule agonists for peroxisome proliferator-activated receptor delta (PPARdelta)--synthesis and biological activity . *Bioorg Med Chem Lett* . 2003 ; 13 : 1517 - 1521
- 47 . Berger J , Leibowitz MD , Doebber TW , Elbrecht A , Zhang B , Zhou G , Biswas C , Cullinan CA , Hayes NS , Li Y , Tanen M , Ventre J , Wu MS , Berger GD , Mosley R , Marquis R , Santini C , Sahoo SP , Tolman RL , Smith RG , Moller DE . Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPARdelta ligands produce distinct biological effects . *J Biol Chem* . 1999 ; 274 : 6718 - 6725
- 48 . Lee CH , Olson P , Hevener A , Mehl I , Chong LW , Olefsky JM , Gonzalez FJ , Ham J , Kang H , Peters JM , Evans RM . PPARdelta regulates glucose metabolism and insulin sensitivity . *Proc Natl Acad Sci U S A* . 2006 ; 103 : 3444 - 3449
- 49 . Qin X , Xie X , Fan Y , Tian J , Guan Y , Wang X , Zhu Y , Wang N . Peroxisome proliferator-activated receptor-delta induces insulin-induced gene-1 and suppresses hepatic lipogenesis in obese diabetic mice . *Hepatology* . 2008 ; 48 : 432 - 441
- 50 . Oliver WR Jr , Shenk JL , Snaith MR , Russell CS , Plunket KD , Bodkin NL , Lewis MC , Winegar DA , Sznaidman ML , Lambert MH , Xu HE , Sternbach DD , Kliewer SA , Hansen BC , Willson TM . A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport . *Proc Natl Acad Sci U S A* . 2001 ; 98 : 5306 - 5311
- 51 . Luquet S , Lopez-Soriano J , Holst D , Fredenrich A , Melki J , Rassoulzadegan M , Grimaldi PA . Peroxisome proliferator-activated receptor delta controls muscle development and oxidative capability . *Faseb J* . 2003 ; 17 : 2299 - 2301
- 52 . Wang YX , Zhang CL , Yu RT , Cho HK , Nelson MC , Bayuga-Ocampo CR , Ham J , Kang H , Evans RM . Regulation of muscle fiber type and running endurance by PPARdelta . *PLoS Biol* . 2004 ; 2 : e294 -
- 53 . Fievet C , Fruchart JC , Staels B . PPARalpha and PPARgamma dual agonists for the treatment of type 2 diabetes and the metabolic syndrome . *Curr Opin Pharmacol* . 2006 ; 6 : 606 - 614
- 54 . Henry RR , Lincoff AM , Mudaliar S , Rabbia M , Chognot C , Herz M . 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
- 55 . Gonzalez IC , Lamar J , Iradier F , Xu Y , Winneroski LL , York J , Yumibe N , Zink R , Montrose-Rafizadeh C , Etgen GJ , Broderick CL , Oldham BA , Mantlo N . Design and synthesis of a novel class of dual PPARgamma/delta agonists . *Bioorg Med Chem Lett* . 2007 ; 17 : 1052 - 1055
- 56 . Wallace JM , Schwarz M , Coward P , Houze J , Sawyer JK , Kelley KL , Chai A , Rudel LL . Effects of peroxisome proliferator-activated receptor alpha/delta agonists on HDL-cholesterol in vervet monkeys . *J Lipid Res* . 2005 ; 46 : 1009 - 1016