Regulation of Triglyceride Metabolism by PPARs: Fibrates and Thiazolidinediones have Distinct Effects

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The molecular mechanism by which hypolipidemic fibrates and antidiabetic thiazolidinediones exert their hypotriglyceridemic action are discussed. Increased activity of lipoprotein lipase (LPL), a key lipolytic enzyme, and decreased levels of apolipoprotein C-III (apo C-III) seem to explain the hypotriglyceridemic effects of compounds. Both fibrates and thiazolidinediones exert their action by activating transcription factors of the peroxisome proliferator activated receptor (PPAR) family, thereby modulating the expression of the LPL and apo C-II genes. First, treatment of rats with PPARα activators, such as fibrates induced LPL mRNA and activity selectively in the liver. In contrast, the thiazolidinediones, which are high affinity ligands for PPARγ, have no effect on liver, but induce LPL mRNA and activity levels in adipose tissue. In hepatocytes, fibrates, unlike the thiazolidinediones, induce LPL mRNA levels, whereas in preadipocyte cell lines the PPARγ ligand induces LPL mRNA levels much quicker and to a higher extent than fibrates. Second, apo C-III mRNA and protein production strongly decrease in livers of fibrate- but not thiazolidinedione-treated animals. Fibrates also reduced apo C-III production in primary cultures of rat and human hepatocytes. The modulation of the expression of the LPL and apo C-III genes by either PPARα or γ activators, correlates with the tissue-specific distribution of the respective PPARs: PPARγ expression is restricted to adipose tissues, whereas PPARα is expressed predominantly in liver. In both the LPL and apo C-III genes, sequence elements responsible for the modulation of their expression by activated PPARs have been identified which supports that the transcriptional regulation of these genes by fibrates and thiazolidinediones contributes significantly to their hypotriglyceridemic effects in vivo. Whereas thiazolidinediones predominantly affect adipocyte LPL production through activation of PPARγ, fibrates exert their effects mainly in the liver via a PPARα mediated reduction in apo C-III production. This tissue-specific transcriptional regulation of genes involved in lipid metabolism by PPAR activators and/or ligands might have important therapeutic implications. J Atheroscler Thromb, 1996; 3: 81–89.

Key words: Atherosclerosis, Gene expression, Lipoproteins, Nuclear receptors, Peroxisomes, Transcription factors

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Abbreviations
Apo: Apolipoprotein
CAT: Chloramphenicol Acetyl Transferase
EMSA: Electrophoretic Mobility Shift Assay
LPL: Lipoprotein Lipase

PPAR: Peroxisome Proliferator Activated Receptor (m: mouse, ha: hamster, x: xenopus)
PPRE: Peroxisome Proliferator Response Element
RXR: Retinoid X Receptor

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Peroxisome Proliferator Activated Receptors

PPARs are members of the superfamily of nuclear hormone receptors that function as ligand-dependent transcription factors (reviewed in (1)). Upon ligand activation they regulate the expression of genes containing specific response elements, called PPREs (2, 3). Three receptor subtypes of peroxisome proliferator activated receptors (PPAR), termed α, δ (or β), and γ, have been identified (4-14). These receptors heterodimerize with the retinoid X receptor (RXR) and alter the transcription of target genes after binding to PPREs, which consist of a hexameric nucleotide direct repeat of the recognition motif (TGACCT) spaced by 1 nucleotide (DR-1). After activation, PPARs control the expression of genes implicated in intracellular and extra-cellular lipid metabolism, such as the genes encoding enzymes involved in the peroxisomal β-oxidation pathway (2, 3, 15-18), cytochrome P450 4A6 (19, 20), 3-hydroxy-3-methylglutaryl-coA synthase (21), medium chain acyl-coA dehydrogenase (22), adipocyte fatty acid binding protein aP2 (11), apolipoproteins A-I and A-II (23, 24), lipoprotein lipase (25), and the acyl-coA synthetase gene (26).

The transcriptional activity of the PPAR subtypes is enhanced by a multitude of chemical compounds including fatty acids, thiazolidinedione antidiabetic agents, prostaglandins, peroxisome proliferators, and fibrate hypolipidemic drugs. Whereas all these compounds are known to activate PPARs, only for PPARγ ligands have been identified. PPARγ directly binds antidiabetic thiazolidinediones (27, 28) and prostaglandin derivatives (27, 29) but not the other activators. Fatty acids activate more selective the PPARδ type, whereas fibrates are more selective PPARα activators (10). Furthermore, the fibrate WY-14.643 was shown to have almost no activity on PPARα (30). In addition to ligand selectivity for the PPAR subtypes, the expression patterns of the various PPAR subtypes are also distinct. PPARα is predominantly expressed in liver, heart, kidney, intestinal mucosa and brown adipose tissue, all tissues with high catabolic rates of fatty acid and peroxisomal metabolism (4, 31, 32). PPARδ is abundantly and ubiquitously expressed, whereas PPARγ presents a much more restricted expression (32). In fact, the mPPARγ2 isoform is predominantly expressed in adipose tissue (11, 33, 34). The pivotal role of mPPARγ2 in adipocyte differentiation can be deduced from the following observations: 1) The induction of an adipocyte phenotype in fibroblast and muscle cells over-
Triglyceride and PPARs

expressing mPPARγ2 (34, 35). 2) Several key adipocyte-specific genes are induced by PPAR activators and contain functional PPREs in their regulatory sequences (11, 25, 26, 36). 3) Finally, arachidonic acid-derived PPARγ ligands (37) are potent inducers of adipocyte differentiation.

Since most of the target genes of PPAR are involved in the control of lipid and energy metabolism and since PPAR itself is activated by lipids, such as fatty acids and prostaglandins, it is evident that the family of PPARs plays a pivotal role in the translation of nutritional signals into changes in gene expression. Taking its pivotal role in controlling lipid metabolism into account it is expected that PPAR has also a regulatory role in the pathogenesis of hyperlipidemia and atherosclerosis. Similarly, it is expected that PPAR has a major function in controlling cell proliferation and differentiation, and development as suggested by the capacity of peroxisome proliferators to reverse developmentally defined patterns of gene expression (such as for LPL (38)), and induce differentiation (for example adipocyte differentiation is enhanced by PPARγ (34, 35)).

Apo C-III and LPL Two Important Players in Triglyceride Metabolism

Apo C-III is a major component of plasma chylomicrons and very low density lipoproteins (VLDL) and is a minor component of high density lipoproteins (HDL). Apo C-III is a 79 amino acid glycoprotein produced predominantly in the liver and to a lesser extent in the intestine. Although it is a very abundant apolipoprotein in human plasma its exact function in vivo is not yet fully understood. Apo C-III has been shown to inhibit hydrolysis of triglycerides by LPL in vitro (39, 40). It also inhibits apo E-mediated clearance of lipoproteins by liver cells (41, 42), although it does not appear to affect binding of lipoproteins to the LDL receptor-related protein (43). These observations are compatible with apo C-III playing a role in the catabolism of triglyceride-rich lipoproteins. Further evidence for a role of apo C-III in triglyceride metabolism comes from a study in subjects deficient in apo C-III (due to a deletion of the apo A1/C3/A4 region). These patients exhibit an increased catabolic rate of VLDL and an unusually efficient conversion of VLDL to intermediate density lipoproteins and LDL (40). In contrast, elevated apo C-III synthetic rates have been observed in hypertriglyceridemic patients (44). Furthermore, genetic studies have identified several apo C-III gene polymorphisms which may be associated with increased plasma apo C-III levels and hypertriglyceridemia (45, 46). The strongest evidence for a role of apo C-III in lipid homeostasis comes, however, from studies in transgenic animals. In animals overexpressing apo C-III marked hypertriglyceridemia was found (47, 48) whereas in animals mutant for both apo C-III alleles hypotriglyceridemia was observed (49). The animals with a targeted disruption of the apolipoprotein C-III gene were furthermore protected from postprandial hypertriglyceridemia, a predisposing factor for the development of atherosclerotic vascular disease (49). Recently, it has become clear that apo C-III might not only play a role in the metabolism of triglyceride-rich lipoproteins but that it may also be implicated in the metabolism of the atherogenic LDL particles. Like LpB particles, complex apo B containing particles such as LpE : B or LpC-III : B are also atherogenic. In fact in males with angiographically documented CAD, LpC-III : B showed a significant correlation with the severity of CAD. This result corresponds with those of a population study in which three cities (Belfast, Strasbourg, Toulouse) were compared (50). LpC-III : B levels were significantly increased in the population with the highest coronary mortality rate (Belfast) (50). Also within each of the...
studies an association between LpC-III : B concentrations and CAD was found (50).

Lipoprotein lipase (LPL), a secreted enzyme with a relative molecular mass of 57,000 which functions as a homodimer, is another important factor in triglyceride metabolism. LPL synthesis occurs in several cell types and tissues including adipocytes, monocytes, neonatal (but not adult) liver, skeletal and cardiac muscle (reviewed in (51)). After secretion LPL binds to glycosaminoglycans on the luminal surface of the capillary endothelium, where it hydrolyzes core triglycerides in triglyceride-rich lipoproteins, such as chylomicrons and VLDL (reviewed in (51)). The released free fatty acids are either oxidized to generate ATP in muscle, stored in adipose tissue, or secreted in milk by the mammary gland. LPL therefore occupies a pivotal position in both lipoprotein and energy metabolism. Recently LPL has been shown to have several additional functions including retention of LDL and VLDL particles on the cell surface and in the subendothelial matrix. Furthermore LPL acts as a ligand for the LDL receptor-related protein (LRP) and as such is not only involved in the metabolism but also the removal of TG-rich lipoproteins from the circulation. A large body of evidence links diminished LPL activity to hypertriglyceridemia. Indeed, subjects with familial LPL deficiency, a relatively rare autosomal recessive disorder, suffer from severe fasting hypertriglyceridemia due to the accumulation of chylomicrons (52). However, more subtle abnormalities in LPL activity can also lead to elevations in triglyceride concentrations (53, 54). Most interestingly diminished LPL activity was found in a large subset of patients with familial combined hyperlipidemia, a frequent hereditary cause of CAD (53, 54). A mechanism explanation for the role of LPL in familial combined hyperlipidemia, a disease characterized by increased secretion of apo B-containing particles, was presented by Williams et al. (55). These investigators showed that the net secretion rate of apo B-containing particles is reduced by enhanced re-uptake of nascent lipoproteins, a mechanism facilitated after modification of the lipoproteins by LPL (55).

**Fig. 3.** Simplified scheme indicating the mechanisms by which fibrates and thiazolidinediones reduce the triglyceride concentration. This triglyceride-lowering involves changing the transcription rates of the LPL (induction in adipose tissue by thiazolidinediones) and apo C-III genes (reduction in adipose tissue by fibrates).

**Fibrates**

- Liver
  - PPARα
  - Transcription of apo C-III gene
  - Decreased apo C-III concentration
  - Altered lipoprotein composition
  - TG-rich particles

**Thiazolidinediones**

- Adipose tissue
  - PPARγ
  - Transcription of LPL gene
  - Increased LPL concentration
  - Increased lipolytic activity

**Alterd Transcription of the APO C-III and LPL Genes caused by Activated PPARs**

Fibrates and thiazolidinediones are known to lower serum triglyceride levels (56, 57), but the exact mechanism by which they affect triglyceride levels is currently unknown. Clinical studies suggested that part of their beneficial effects on lipoprotein metabolism is due to the induction of LPL activity (reviewed in (57)) and to their apo C-III lowering effects. Given the pivotal role of PPAR in lipid metabolism, it was investigated whether the hypotriglyceridemic actions of fibrates and thiazolidinediones were mediated by a PPAR-induced change in transcription of these genes.

Treatment of adult rats in vivo with fibrates results in a decrease in plasma triglyceride concentrations. This is accompanied by a tissue-specific, dose- and time-dependent, reversible decrease of liver apo C-III gene transcription. In contrast, fibrates reinduce liver LPL gene expression (adult liver normally does not produce LPL) (38). Since all three fibrates (fenofibrate, clofibrate and gemfibrozil) tested lower hepatic apo C-III and
increase liver LPL mRNA levels, it appears to be a general effect of fibrates. The changes in apo C-III and LPL expression after fibrate administration are a result of fibrate action per se, and not merely a consequence of alterations in plasma lipid and lipoprotein concentrations, since treatment of isolated rat or human hepatocytes with fibrates, results respectively in a down- and up-regulation of apo C-III and LPL gene expression (25, 58). Nuclear run-on experiments indicated that the effects of fibrates on liver apo C-III and LPL gene expression are at the transcriptional level (38, 58). Furthermore, results from transfection experiments indicate that repression of apo C-III by fibrates is mediated by sequences located within 1kb upstream of the transcription initiation site. Interestingly, one of these cis-elements located in this region contains a DR1 sequence, which has been shown to mediate transregulation of apo C-III gene expression by other members of the nuclear receptor superfamily, such as HNF-4, Arp1, EAR-2 and EAR-3 (59, 60) and therefore may be a potential target site for PPARs. In fact recently, it has been suggested that the decreased apo C-III transcription is due to competition of activated PPARs with the stronger transcriptional activator HNF-4 for binding to this response element (61).

A number of clinical studies suggested that the triglyceride-lowering action of fibrates, thiazolidinediones and polyunsaturated fatty acids is associated with an increase in adipose tissue LPL activity (56, 62-65). LPL is a key enzyme in the metabolism of triglyceride-rich lipoproteins and plays the role of a gatekeeper in energy metabolism by controlling the generation of fatty acids. In contrast to other genes involved in the determination of serum triglyceride levels, such as apo C-III which is only expressed in the liver and intestine, LPL is expressed in multiple tissues, including but not restricted to adipose tissue, liver, heart, mammary gland, macrophages and brain. Our results obtained using cultured hepatocyte and preadipocyte cell lines, as well as in vivo data obtained in rat adipose tissue or liver, establish that LPL mRNA and activity levels can be regulated in a tissue-specific fashion by PPARα activators and PPARγ ligands: fibrates induce LPL preferentially in the liver, whereas the thiazolidinediones are only active in adipose tissue (25). Transient transfection assays of the LPL 5′ regulatory sequences indicated that LPL induction is mediated by a specific response element consisting of a DR1, located between -157 and -169 relative from the transcription initiation site (25). We showed that PPAR/ RXR heterodimers bind to this element in the LPL promoter and mediate its activation by thiazolidinediones and fibric acids (25). However, the PPARE of the LPL promoter binds PPAR/RXR heterodimers with weaker affinity than the ACO PPARE. The almost perfect conservation of the LPL PPARE in the human (TGCCCTTCCCCC) (66) and mouse (TGCCCTTTCCCC) (67) gene indicates that this sequence likely has an important and conserved regulatory role.

**Clinical Effects of Treatment with Fibrates**

Due to the novelty of the thiazolidinediones only a limited amount of information is available about their lipid lowering effects in clinical practice and hence the following section mainly contains data about fibrates, for whom it is well established that they reduce plasma triglyceride concentrations. Previous clinical reports have indicated that this hypotriglyceridemic effect of fibrates is accompanied by both a decrease in plasma concentration and synthesis rate of apo C-III (44) and an increase in LPL activity (62, 63, 65, 68, 69). Therefore we propose the following dual action of fibrates. First, a lowered secretion of apo C-III, along with unchanged apo E secretion, may lead to a decreased apo C-III/apo E ratio of triglyceride-rich particles, which then could be more efficiently cleared from plasma. Indeed, studies on transgenic mice have shown that overexpression of apo C-III leads to an hypertriglyceridemia due to a diminished clearance rate of triglyceride-rich lipoproteins containing increased amounts of apo C-III and reduced apo E on the particles (48, 70). This apo C-III induced hypertriglyceridemia could be corrected by simultaneous overexpression of apo E, indicating that apo C-III may be implicated in modulating the apo E-mediated lipoprotein clearance (48). Furthermore, the decrease in apo C-III production after fibrate treatment also results in a decrease in the concentration of the atherogenic LpC-III : B particles (71). Second, enhanced transcription of the LPL gene (in the liver) might result in higher LPL protein and activity levels (62, 63, 65, 68, 69). Since apo C-III has been suggested to be an inhibitor of LPL activity, the decrease in plasma apo C-III concentrations may hence also contribute to an improved lipolysis of triglyceride-rich lipoproteins in plasma (39). Consistent with this hypothesis, fibrate induced increases in LPL activity have been previously observed both in control (62, 68) and hyperlipidemic human subjects (63, 65, 69). Consequently, treatment with fibrates will lead to alterations in plasma apolipoprotein composition, as well as an enhanced lipolytic activity, both favouring the clearance of triglyceride-rich lipoproteins. Therefore, the decrease in apo C-III and the increase in LPL gene expression by fibrates provides a potential mechanism by which these drugs induce a less atherogenic plasma lipoprotein profile.

**Tissue-selective Effects of Different PPAR Activators**

The tissue-selective effects of the PPAR activators/ligands are highly intriguing and provide insight in their effects on triglyceride metabolism. Fibrate treatment reduced apo C-III expression and induced LPL expression strongest in liver, whereas BRL 49653 had no effect on
liver, but induced adipocyte LPL expression. In both the in vivo and in vitro studies, inducibility by either PPARα or γ activators, correlated with the tissue-specific distribution of the respective PPARs. This selectivity of PPARα and PPARγ activators, together with the tissue-specific expression of the respective receptors, allows a tissue-selective activation of genes. Identification of the natural ligands for all PPAR types, as well as the identification of high affinity synthetic agonists and antagonists, will most likely result in chemicals which will be more selective than the currently available compounds, allowing target gene activation in a specific tissue of interest while limiting undesired side effects in other tissues. These compounds might have interesting therapeutic features especially if one considers the context of the widespread use of fibrates in the treatment of diet-resistant hyperlipidemia and the emerging use of thiazolidinediones in treatment of non-insulin-dependent diabetes. In addition to studies aimed at defining new agonists and antagonists, it is of equal importance to understand the distinct structural features of the ligand binding domain of the PPAR subtypes, which allow similar and related receptors to interact with a high degree of specificity with certain ligands or activators.

Conclusions

In conclusion, the role of apolipoprotein C-III and the enzymes LPL, in governing triglyceride metabolism, was discussed. Both clinical as well as laboratory evidence suggested the importance of both components in determining triglyceride concentrations. Next it was investigated whether the hypotriglyceridemic effects of fibrates and thiazolidinediones was mediated by changes in LPL and apo C-III concentrations. LPL mRNA and activity levels and apo C-III production can be regulated in a tissue-specific fashion by PPARα activators and PPARγ ligands. The modulation of LPL and apo C-III gene expression by these compounds is mediated by specific response elements or PPREs in their promoters. In adipose tissue, the increase in LPL production after treatment with thiazolidinediones will enhance the clearance of plasma triglycerides and provide the (pre)adipocytes with additional fatty acids, which can further stimulate the transactivation capacity of PPAR or which can be stored under form of triglycerides. In the liver, the enhanced production of LPL together with the reduced production of apo C-III, contributes to the hypolipidemic action of these compounds. This tissue-selective induction of gene transcription by activators of different PPARs, demonstrates the feasibility of the development of highly specific PPAR subtype-specific agonists and antagonists, which can be used as drugs.

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Triglyceride and PPARs

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