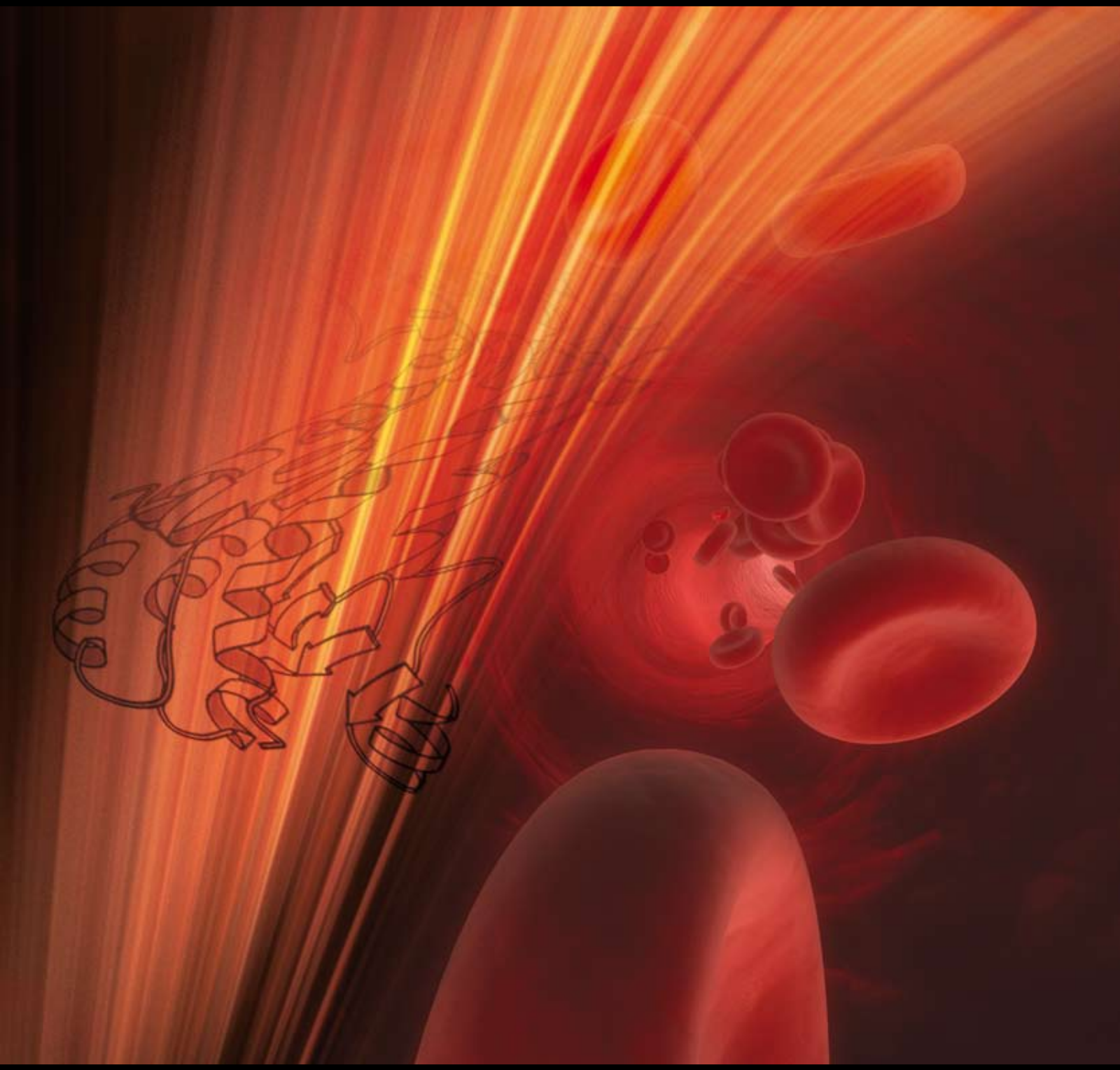
PPAR Research

PPARs in Viral Disease

Guest Editor: Lawrence Serfaty and Jacqueline Capeau



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Editorial
PPARs in Viral Disease

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This special issue of PPAR Research contains an exciting array of valuable reviews examining the relationship between PPARs and various viral infections, varying from HIV to HCV and HBV. This assembly of reviews includes a group of three complimentary reviews addressing different facets of the cross-talk between HIV infection and antiretroviral treatment as well as the potential beneficial role of PPARγ in HIV-associated lipodystrophy. First, Giralt et al. detail the cross-talk between HIV infection and PPARγ leading to the reported negative impact of this interplay on adipose tissue physiology. They also present literature on the reciprocal, yet contradictory roles of PPARα and PPARγ on HIV-1 replication and transcription. Second, Caron et al. describe the effect of ART on adipocyte PPARγ expression in vitro, in animal models, and in HIV-infected patients. These authors also report on the intriguing connection between PPARγ, activated macrophages found in the adipose tissue and ART. In the third review of this series, J. Sutinen closely examines the results of 14 different clinical trials which evaluated if the PPARγ thiazolidinedione agonists could be useful in the treatment of HAART-associated metabolic complications in HIV-infected patients. Upon careful evaluation of the reported results, Sutinen concludes that these agonists produce a very modest, if any, effect on lipoatrophic subcutaneous adipose tissue, despite their improvement of insulin sensitivity in treated patients. The review by Doran and coworkers focuses on the studies dealing with the potential role of PPARγ in HIV-1-associated bone disease. These authors put forth a provocative hypothesis which stipulates a potential role for PPARγ in the reduced bone mass associated with HIV-1 infection and treatment. Specifically, they suggest a possible dysregulation of the activity of PPARγ in undifferentiated stromal cells or in partially differentiated preosteoblast and preadipocyte cells. The liver which is impacted not only by HIV but also by HBV and HCV is the focus of several excellent reviews in this special issue. In a review by Lemoine and coauthors, the role that PPARs play in HIV infection, in terms of associated metabolic disorders, disease progression, coinfections with HBV or HCV, and response to antiviral treatment, is featured. In addition, the summary by Lemoine et al. of the experimental and clinical data regarding PPARS in HIV-associated liver disease provides a rationale for the use of PPARγ agonists as therapeutic agents in these patients. Further, Negro reviews experimental and clinical data suggesting that HCV may interfere with hepatic insulin signaling, possibly involving the downregulation of the PPARγ. Capitalizing on the known involvement of PPARs in lipid metabolism, inflammatory process, and fibrogenesis, Dharancy et al. have summarized experimental and human studies showing a diminished expression and function of PPARα and PPARγ during HCV infection. These authors also review the potential benefits of nonhepatotoxic PPARα/γ agonists as therapeutic agents to treat chronic hepatitis C. In another valuable review by Dubuquoy and coauthors, the potential of PPARs to modulate HBV transcription and replication is summarized. The authors report that, in HBV transgenic mouse model, activation of PPARα increased the transcription and replication of HBV, thus concluding that modulating the PPARα/RXR heterodimer may be an interesting therapeutic option to control HBV infection. We are assured that the reviews presented in this special issue, on the interplay

between PPARs and viral disease, will be highly useful for those with interest in the field.

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Review Article

HIV-1 Infection and the PPARγ-Dependent Control of Adipose Tissue Physiology

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PPARγ is a ligand-dependent master transcription factor controlling adipocyte differentiation as well as multiple biological processes taking place in other cells present in adipose tissue depots such as macrophages. Recent research indicates that HIV-1 infection-related events may alter adipose tissue biology through several mechanisms involving PPARγ, ranging from direct effects of HIV-1-encoded proteins on adipocytes to the promotion of a proinflammatory environment that interferes with PPARγ actions. This effect of HIV-1 on adipose tissue cells can occur even in the absence of direct infection of adipocytes, as soluble HIV-1-encoded proteins such as Vpr may enter cells and inhibit PPARγ action. Moreover, repression of PPARγ actions may relieve inhibitory pathways of HIV-1 gene transcription, thus enhancing HIV-1 effects in infected cells. HIV-1 infection-mediated interference of PPARγ-dependent pathways in adipocytes and other cells inside adipose depots such as macrophages is likely to create an altered local environment that, after antiretroviral treatment, leads to lipodystrophy in HIV-1-infected and HAART-treated patients.

1. Introduction

A complex set of metabolic alterations, preferentially involving adipose tissue, has emerged in recent years in a substantial number of HIV-1-infected patients under highly-active antiretroviral treatment (HAART). This is the so-called HIV-1/HAART-associated lipodystrophy syndrome. Disturbances in adipose tissue of these patients range from lipoatrophy of subcutaneous adipose tissue to visceral adipose accumulation or lipomatosis [1]. Moreover, during recent years, basic research on adipose tissue biology has succeeded in identifying major molecular players in the differentiation and function of adipose tissue. Among them, the peroxisome proliferator-activated receptor γ (PPARγ)subtype of PPARs has emerged as a master transcriptional regulator of adipose cells [2]. Although we are still far from a full understanding of the molecular basis of HIV-1 lipodystrophy, research has been actively undertaken in this area and has examined the role of alterations in PPARγ-dependent pathways in eliciting the syndrome [3, 4]. Regardless of the potential effects of antiretroviral drugs on PPARγ, several recent findings suggest that HIV-1 infection-related events may cause disturbances in the PPARγ-dependent pathways of control of adipose tissue physiopathology, and they are summarized in the present review.

2. Adipose Tissue, HIV-1 Infection, and Lipodystrophy

The concepts concerning the causal basis of HIV-1 associated lipodystrophy have evolved substantially in the last few years. The initial identification of HIV-1 lipodystrophy was coincident with the introduction of drugs from the protease inhibitor family for use in antiretroviral treatment, and these drugs were at first considered to be causative of the syndrome [5]. After it was recognized that patients without protease inhibitor treatment could also develop lipodystrophy, the syndrome was associated with HAART overall. Although several drugs of HAART regimes are known at present to be more prone to induce the appearance of lipodystrophy or of some of their particular features (abdominal fat hypertrophy

versus peripheral lipoatrophy) than others [6], no single class of drugs can account for elicitation of the overall syndrome. On the other hand, there have been suggestions that antiretroviral treatment causes lipodystrophy only when acting upon HIV-1-infected patients, and that events related to HIV-1 infection are intrinsically associated with the development of the syndrome. Evidently, there are no data on long-term antiretroviral treatment of non-HIV-1-infected patients that could establish the specific role of HAART independent of the HIV-1 infection, and a single two-week study of nucleotide-analog reverse transcriptase inhibitor treatment of non-infected volunteers indicated the appearance of only a few features of the lipodystrophy syndrome [7].

Some data have indicated that mild alterations of adipose tissue biology are already present in nontreated HIV-1-infected patients [8]. The studies of body composition in the era before HAART reported a disproportionate loss of body fat mass in men with advanced HIV-1 disease or AIDS [9]. This was attributed to the progression towards AIDS-related wasting and associated diseases. However, some data suggested that weight loss and depletion of body fat may precede the progression to AIDS, even in adults with normal CD4+ lymphocyte counts [10]. Further studies confirmed an indirect effect of HIV-1 viremia leading to effects similar to AIDS wasting. Studies by Visnegarwala et al. of HIV-1-infected men with CD4+ lymphocyte counts >200 cells/mm^3 suggested that malnutrition due to decreased caloric intake and increased energy demands associated with an active opportunistic infection was not contributing factors in explaining reduced body weight [11]. It was concluded that reduction in fat mass is related to HIV-1 infection itself, independent of the additive effects of opportunistic illnesses. Similar conclusions were obtained from the analysis of dyslipemia. Before the introduction of HAART, the early appearance of hypertriglyceridemia and enhanced lipolysis was observed in HIV-1-infected patients before the onset of overt illness [12, 13]. Recent studies have confirmed that HIV-1 infection-induced changes in lipolysis are unrelated to the further effects of HAART leading to full-blown lipodystrophy [14]. Finally, several rodent models relevant to HIV-1 infection events have shown metabolic disturbances in the absence of any exposure to antiretroviral drugs. For instance, transgenic mice expressing the HIV-1 accessory viral protein R (Vpr) in liver and adipose tissue exhibit altered systemic fat metabolism [15]. Disturbances of PPARγ, as a master regulator of adipose tissue differentiation and function, may play a major role in the alterations of adipose mass and lipid metabolism elicited by HIV-1 infection, and this issue is summarized in the present review.

3. PPARγ and Its Pivotal Role in Adipose Tissue Function

PPARγ is highly expressed in adipose tissue, where it plays a key role in adipose tissue development and function. There are two major splice variants of PPARγ, γ1 and γ2, which differ in their N-terminal region (PPARγ2 contains an additional 30 amino acids) and in their tissue-specific expression; PPARγ2 is found almost exclusively in white and brown adipose tissues whereas PPARγ1 is also relatively abundant in macrophages and endothelial cells [16–18]. The activity of both isoforms is regulated by posttranscriptional modifications and by ligand-dependent transactivation and recruitment of coactivators. For instance, phosphorylation inhibits the transcriptional activity of PPARγ [19] and promotes sumoylation, which further reduces its transcriptional activity [20]. PPARγ forms heterodimers with retinoid *X* receptors (RXRs) to bind to specific DNA sequences in its target genes. In the absence of ligands, corepressors such as nuclear receptor corepressor (N-CoR) or silencing mediator of retinoid and thyroid (SMRT) receptors bind to these heterodimers and recruit histone deacetylases to repress transcription (reviewed in [21]). Binding of ligands to PPARγ triggers conformational changes that allow the recruitment of transcriptional coactivators, including members of the steroid receptor coactivator (SRC) family [22] and PPARγ-coactivator 1α (PGC-1α) [23] that ultimately recruit histone acetyltransferase coactivators such as p300/CBP or PCAF [21].

However, the natural ligands for PPARγ remain unknown. Recent studies have provided functional evidence for an unidentified natural ligand that is produced transiently during adipogenesis [24]. There is also evidence that small lipophilic compounds, such as polyunsaturated fatty acids and fatty acid derivatives (eiocosanoids) bind and activate this receptor [25], thus supporting the concept that PPARγ is a nutrient sensor that finely regulates metabolic homoeostasis in response to different nutritional states.

Regarding synthetic ligands, it is clear that members of the thiazolidinedione (TZD) family of antidiabetic drugs are high-affinity agonists for PPARγ [26]. TZDs have been reported to enhance insulin sensitivity in animals and humans [27]. Furthermore, cellular, genetic, and pharmacological studies have provided strong evidence both that TZDs function via PPARγ, and that adipose tissue is the main site where the insulin-sensitizing effects of PPARγ are produced (reviewed in [28]).

It was reported that TZDs induce adipocyte differentiation even before they were known to be ligands of PPARγ [29]. By now, the key role of PPARγ as a master regulator of adipogenesis has been clearly established, and gain-of-function experiments have demonstrated that PPARγ is sufficient to induce adipocyte differentiation in the presence of an appropriate ligand [30]. However, loss-of-function experiments to prove that PPARγ is required for this process have been more difficult, since PPARγ homozygous inactivation results in embryonic death due to placental alteration, in a developmental stage before there is any adipose tissue development [31]. Later, however, studies utilizing chimeric mice [32] and adipose-specific PPARγ knockout mice [33] confirmed the essential role of PPARγ in adipose tissue differentiation.

PPARγ also plays an important role in regulation of lipid metabolism in mature adipocytes. Activation of PPARγ increases both fatty acid uptake and its storage into adipocytes by promoting the transcription of genes

such as those encoding lipoprotein lipase, fatty acid binding protein-4 (aP2/FABP4), phosphoenolpyruvate carboxykinase (PEPCK) [34–37], and also glucose transporter GLUT-4, in order to increase fatty acid synthesis [38]. These effects of PPARγ may underlie its insulin-sensitizing effects. Thus, together with the proadipogenic role of PPARγ (glucose homeostasis requires adequate amounts of adipose tissue), the improvement of lipid storage in this tissue will prevent ectopic lipid accumulation in nonadipose tissues such as liver, skeletal muscle, and β-cells. Furthermore, PPARγ has been reported to induce transcription of the PGC-1α gene in adipose tissue [39]. The coactivator PGC-1α promotes mitochondrial biogenesis, thus leading to an increase in fatty acid oxidation in adipose tissue, which may protect against adipocyte hypertrophy [40]. Finally, adipose tissue has endocrine functions, and PPARγ regulates expression of genes encoding adipokines such as adiponectin, leptin, resistin, or cytokines such as TNFα. Activation of PPARγ promotes the expression of a pro-insulin-sensitizing adipokine profile (i.e., induction of adiponectin and reduction of TNFα gene expression) thus involving the cross-talk between adipose tissue and other insulin-sensitive organs (liver, skeletal muscle) in the insulin-sensitizing effects of PPARγ [41].

Evidence from human mutations in PPARγ has further underlined the importance of PPARγ in the development of adipose tissue, in the maintenance of glucose and lipid homeostasis and more generally in the control of energy balance (reviewed in [42]). Patients harboring mutations in the ligand-binding domain of PPARγ have a stereotyped phenotype characterized by partial lipodystrophy, severe insulin resistance, dyslipidemia, hepatic steatosis, and hypertension, thus identifying PPARγ as playing a molecular role in the pathogenesis of the metabolic syndrome [43, 44].

PPARγ is also expressed in macrophages and endothelial cells, that is, cells that are present in adipose tissue [17, 18, 45]. In endothelial cells, activation of PPARγ has antiproliferative, antiangiogenic, and anti-inflammatory effects [45]. PPARγ is induced during macrophage differentiation, and its activation increases the expression of macrophage-specific markers, such as CD14 and CD11b [17, 46]. However, loss-of-function approaches have demonstrated that PPARγ is not essential for monocyte/macrophage differentiation either in vivo or in vitro [47, 48] but selective deletion of PPARγ in macrophages results in increased insulin resistance [49]. Recently, macrophage-mediated inflammation in adipose tissue has been proposed to play a central role in the pathogenesis of insulin resistance [50]. Two types of macrophages, proinflammatory M1 and anti-inflammatory M2, are present in adipose tissue and their relative abundance may change dynamically through recruitment of polarized monocytes from the blood (macrophage infiltration) or through the effects of local cytokines on macrophages in adipose tissue. Activation of PPARγ by TZDs has now been reported to increase the proportion of anti-inflammatory M2 macrophages in adipose tissue [51]. Furthermore, TZDs also act through PPARγ to inhibit the expression of inflammatory mediators in macrophages, and as reported above, to negatively regulate expression of cytokines such as IL-6, TNF-α, and monocyte chemoattractant protein—1 (MCP-1/CCL-2) in adipocytes [52]. In summary, activation of PPARγ improves adipose tissue function by having a beneficial effect on the adipocyte—macrophage relationship, which may result in prevention of insulin resistance.

4. HIV-1 Infection and PPARγ

Recent studies revealed that expression of marker genes of adipogenesis, such as those encoding GLUT-4, adiponectin, or lipoprotein lipase is already altered in subcutaneous adipose tissue from HIV-1-infected patients in the absence of treatment [53]. These genes are known targets of PPARγ, and the expression of PPARγ itself is also reduced in HIV-1-infected patients without treatment, relative to healthy controls [53]. In fact, in this same sense, it has been observed that PPARγ expression is lower in HIV-1-infected and HAART-treated patients with lipodystrophy relative to healthy controls [54], but similar when compared to levels in antiretroviral-naive patients [55]. These findings point to the possibility that HIV-1 infection and associated events interfere with the action of PPARγ as a master transcriptional controller of adipogenesis and, in a broader sense, of adipose tissue biology, thus contributing to the appearance of lipodystrophy. Recent experimental evidence is supportive of this possibility (see below). However, a relevant role of HAART in worsening potential HIV-1-mediated alterations in PPARγ expression cannot be excluded. In this sense, it has been recently reported that a 6-month interruption of antiretroviral treatment results in a significant amelioration of PPARγ levels in patients formerly under HAART containing protease inhibitors [56]. On the other hand, one of the features of HIV-1-associated lipodystrophy is insulin resistance. It cannot be excluded that PPARγ impairment in HIV-1-infected patients may contribute to reduced insulin sensitivity, as PPARγ is a known target of drugs with insulin-sensitizing properties (see above).

It must be taken into account that the action of HIV-1 infection in adipose tissue and PPARγ activity should not be considered only in relation to adipocytes. As stated above, adipose tissue depots contain, in addition to adipocytes, other cells such as preadipocytes, macrophages, or endothelial cells. Recently, even lymphocytes have been reported to be present inside adipose depots [57, 58]. As these other cell types (e.g., macrophages or endothelial cells) also express PPARγ, they could themselves be sensitive to HIV-1-mediated disturbances in PPARγ expression and activity. Moreover, cells inside adipose tissue can release regulatory factors (such as adipokines or cytokines) or metabolites (free fatty acids) capable of influencing surrounding cells, for instance preadipocytes and adipocytes, and the overall pathways of gene regulation dependent on PPARγ.

5. How May HIV-1 Infection Affect PPARγ Pathways in Adipose Tissue?

The possibility that HIV-1 infection could influence PPARγ activity leads to the first consideration; whether the cells

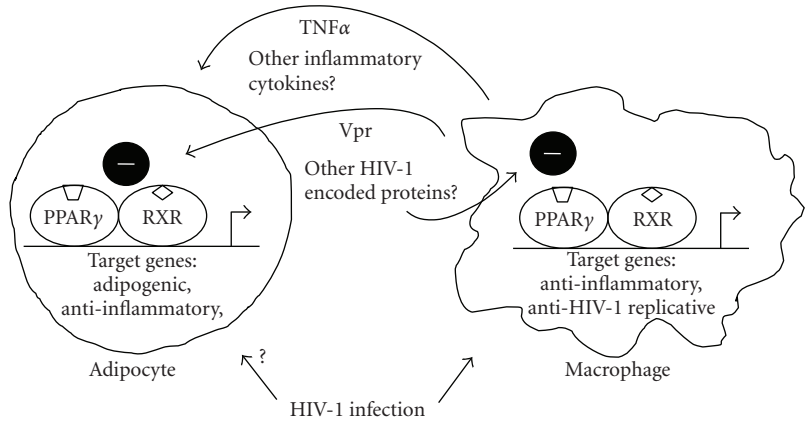

Figure 1: Schematic representation of the potential effects of HIV-1 infection on PPARγ-mediated pathways in adipocytes and macrophages. HIV-1 infection of macrophages may lead to the synthesis of HIV-1 encoded proteins, that is, Vpr, with negative effects on the expression of PPARγ target genes. This may lead to reduced expression of anti-inflammatory genes as well as promotion of HIV-1 replication. Release of HIV-1-encoded proteins as well as enhanced production of inflammatory cytokines, that is, TNFα and other, by macrophages as a consequence of HIV-1 infection may lead to impaired PPARγ action in adipocytes and preadipocytes, thus impairing adipogenesis and fat accretion. Direct effects of HIV-1 infection in line with what happens in macrophages cannot be excluded. Similar events to those depicted for macrophages could be considered to occur in endothelial cells or even lymphocytes present in adipose tissue depots as a consequence of HIV-1 infection.

present in adipose tissue depots can be infected by HIV-1. The capacity of HIV-1 to infect adipocytes is controversial. Whereas some initial reports indicated that adipocytes could be infected [59, 60], later data appeared to exclude this possibility [61]. However, more recently, it was reported that substantial infection of adipocytes could take place when TNFα was present in the medium [62]. High levels of TNFα are found in adipose tissue from HIV-1-infected patients even before treatment and are part of a proinflammatory environment already present in adipose tissue as a consequence of long-term HIV-1 infection [53, 63]. It should be noted that studies on the capacity of HIV-1 to infect adipose cells have focused on mature adipocytes and less is known concerning preadipocytes. In adipose depots, resident preadipocytes are found and they can differentiate into mature adipocytes. Lipoatrophic situations combine a loss of adipose cells (primarily via apoptosis) and an incapacity of preadipocytes to differentiate and replenish the depot; thus any direct or indirect effect of HIV-1 infection that interferes with PPARγ could lead to this impaired differentiation. Recent identification of PPARγ gene mutations causative of lipodystrophies of genetic origin supports the notion that abnormal PPARγ function can lead to lipodystrophy [42, 64].

Evidently, if a cell of the adipocyte lineage is infected by HIV-1, it will be exposed to gene products of HIV-1 and to their potential effects on PPARγ actions. A reported example of this is Nef, a 27 kDa HIV-1-encoded protein that localizes in the cytoplasm as well as nucleus of infected cells [65]. It has been shown that nuclear Nef results in a reduction in the expression of PPARγ and of PPARγ gene targets in human T cells and macrophages as well as interfering with fat accumulation in cell lines [66]. The effects of Nef were specific in impairing PPARγ-dependent, but not PPARα-dependent, transcriptional activity.

However, the most relevant evidence of interference of PPARγ pathways by HIV-1-encoded products concerns the HIV-1 accessory protein Vpr. Kopp and collaborators have recently shown that Vpr suppresses the differentiation of adipocytes in cell culture by interfering with PPARγ-dependent transactivation of target genes [67]. Vpr acts as a corepressor of PPARγ by interacting with the ligand-binding domain of the receptor in an agonist-dependent manner. Remarkably, this effect could be observed when Vpr was added to the adipose cell culture media thus indicating that, as already shown in other cell types [68], exogenous Vpr can enter the cell and interfere with metabolic pathways. These findings are highly relevant to an understanding of the etiopathogenesis of lipodystrophy in HIV-1-infected patients. Thus, it is expected that adipose cells can be affected by Vpr either due to direct infection by HIV-1, or indirectly, because Vpr is known to be present as a soluble protein in the circulation of HIV-1 infected patients [69]. Moreover, adipocytes and preadipocytes may be exposed to local concentrations of Vpr higher than those in the overall circulation, given the proximity of cells such as macrophages (or even resident lymphocytes) that can be infected and release Vpr.

Finally, recent research has revealed several features of the biology of PPARγ, in relation to adipogenesis, in which HIV-1 infection may be hypothesized to interfere. Thus, several players in cell cycle control, such as cyclin D3 and CDK4, have been reported to promote adipogenesis through interaction with PPARγ [70, 71], whereas E2F4 represses PPARγ during adipogenesis [72]. HIV-1 infection may interfere with the cell cycle machinery, and specifically the HIV-1-encoded proteins Vpr and Tat have been recognized as being capable of interacting with CDK4, cyclin D3, and E2F4 [73–75]. However, the capacity of these interactions

to influence PPARγ-dependent pathways in adipocytes or in other cells present in adipose depots, and their consequences for metabolism, remains to be explored. Similarly, a number of reports have indicated that HIV-1-encoded Tat or Vpr can interact with known coactivators of PPARγ required for its transcriptional activity, such as p300/CBP or PCAF [76, 77]. It cannot be excluded that HIV-1 infection-mediated events affect PPARγ activity in adipose tissue through these interactions, although experimental evidence for this is lacking at present.

6. The Reciprocal Issue: the Effect of PPARγ on HIV-1 Biology, and the Implications of This for Adipose Tissue Pathophysiology

What we have described up to now provides evidence that HIV-1 infection may alter PPARγ activities. However, several reports also indicate the occurrence of reciprocal events, that is, the action of PPARs and particularly PPARγ on HIV-1 biology, especially on the replication and transcription of the HIV-1 genome. The capacity of nuclear receptors to interact with the long-terminal repeat of HIV-1 was recognized several years ago [78]. It was observed that heterodimers of RXR and PPARα were capable of binding a region between -356 to -320 in the long terminal repeat. Accordingly, PPARα agonists such as clofibric acid were shown to activate HIV-1 transcription [79]. This effect, which may be relevant in tissues such as liver which express high levels of PPARα, is not expected to be involved in alterations of white adipocytes and preadipocytes which are almost devoid of PPARα [80].

In contrast, it was reported recently that the PPARγ agonist ciglitazone inhibits HIV-1 replication in a dose-dependent manner in acutely-infected human monocyte-derived macrophages and in latently-infected and viral entry-independent U1 cells, suggesting an effect at the level of HIV-1 gene expression [81]. Cotransfection of PPARγ wild-type vectors and treatment with PPARγ agonists inhibited HIV-1 promoter activity in U937 cells, and activation of PPARγ also decreased HIV-1 mRNA stability following actinomycin D treatment. Thus, natural and synthetic PPARγ agonists may play a role in controlling HIV-1 infection in macrophages [81, 82]. Similar observations were obtained by Skolnik et al. who observed that activation of PPARγ, and also of PPARα, by specific agonists also decreased HIV-1 replication in peripheral blood mononuclear cells acutely infected with HIV-1, in a chronically-infected monoblastoid cell line and in alveolar macrophages from HIV-1-infected subjects and uninfected controls [83]. The precise mechanisms of action of PPARγ on HIV-1 are not fully known and, in addition to the potential direct interaction with specific regions of the long-terminal repeat mentioned above, indirect effects via nuclear factor κB have also been proposed on the basis of the effects of PPARγ and its ligand rosiglitazone impairing nuclear factor κB-mediated enhancement of HIV-1 replication in macrophages [84].

All these findings indicate the occurrence of a potential cross-talk between PPARγ and HIV-1 that could reinforce the activity of HIV-1 proteins in cells harboring PPARγ. Thus, a reduction in PPARγ levels and/or activity as a consequence of HIV-1 infection may lead to depression of such a pathway of potential inhibition of HIV-1 transcription and could create an environment prone to enhancement of HIV-1 gene expression, establishing a "vicious cycle" further augmenting adipose pathogenesis.

7. Conclusions

In summary, research to date indicates that HIV-1 infection-related events may alter adipose tissue and contribute to development of the full-blown lipodystrophy syndrome after antiretroviral treatment. The role of HIV-1 infection of cells in adipose tissue, of soluble proteins released by infected cells as well as of the indirect effects elicited by the mild proinflammatory environment associated with viral infection, is issues expected to be the subject of intense research in the near future. For all these aspects, PPARγ appears as a main candidate for the mediation of pathogenic events. Moreover, a full understanding will be required of the relationships among the complex set of cell types that, in addition to adipocytes, are present in adipose tissue depots. Macrophages, endothelial cells, preadipocytes, and perhaps even lymphocytes are present in adipose depots and establish a complex regulatory cross-talk that is altered by HIV-1 infection, and which may ultimately lead to disturbances in adipocytes and in the whole adipose mass (see Figure 1). All of these cell types express PPARγ, and the pivotal role of this receptor in adipogenesis, insulin sensitivity, lipid metabolism, and inflammatory pathways point to this receptor as a key target of future research on adipose tissue disturbances in the HIV-1/HAART-associated lipodystrophy syndrome.

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Review Article

Antiretroviral-Related Adipocyte Dysfunction and Lipodystrophy in HIV-Infected Patients: Alteration of the PPARγ-Dependent Pathways

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Lipodystrophy and metabolic alterations are major complications of antiretroviral therapy in HIV-infected patients. In vitro studies using cultured murine and human adipocytes revealed that some protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs) were implicated to a different extent in adipose cell dysfunction and that a chronic incubation with some PIs decreased mRNA and protein expression of PPARγ. Defective lamin A maturation linked to PI inhibitory activity could impede the nuclear translocation of SREBP1c, therefore, reducing PPARγ expression. Adipose cell function was partially restored by the PPARγ agonists, thiazolidinediones. Adverse effects of PIs and NRTIs have also been reported in macrophages, a cell type that coexists with, and modulates, adipocyte function in fat tissue. In HIV-infected patients under ART, a decreased expression of PPARγ and of PPARγ-related genes was observed in adipose tissue, these anomalies being more severe in patients with ART-induced lipoatrophy. Altered PPARγ expression was reversed in patients stopping PIs. Treatment of patients with agonists of PPARγ could improve, at least partially, the subcutaneous lipoatrophy. These data indicate that decreased PPARγ expression and PPARγ-related function, resulting from ART-induced adipose tissue toxicity, play a central role in HIV-related lipoatrophy and metabolic consequences.

1. Introduction

HIV-associated lipodystrophy (LD) is a disorder characterized by a selective damage of the adipose tissue resulting in part from antiretroviral drugs [1, 2]. The LD syndrome includes progressive subcutaneous fat loss and/or central fat accumulation along with dyslipidemia, glucose alterations, and insulin resistance, altogether generating cardiovascular dysfunctions [3, 4]. Recent studies have hypothesized that HIV itself could play a role in the LD phenotype (see Giralt et al. [5]). However, the risk of developing fat tissue redistribution has been related in priority to the antiretroviral treatment (ART) and mainly to the use of two classes of drugs, protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs) [6–8]. Lipoatrophy in the face and extremities has been linked repeatedly to the use of stavudine (and to a lesser extend zidovudine) among NRTIs [7, 9, 10] and increases with long-term exposure [11]. PIs have been mainly associated with central fat accumulation along with insulin resistance. However, nelfinavir or indinavir can independently decrease limb fat level in patients cotreated with NRTIs [7, 12]. Peripheral fat loss and central fat accumulation can occur simultaneously, though lipoatrophy may emerge as the more dominant feature on prolonged treatments [12, 13]. Recently, a role for the nonnucleoside analog efavirenz in lipoatrophy has been reported but needs to be confirmed [14].

The pathogenesis of adipose cell dysfunction includes the mitochondrial toxicity of NRTIs [15–19] and the adverse effects of PIs and NRTIs on the adipocyte differentiation status [17, 20–26], insulin sensitivity [27, 28], survival [17, 18, 23, 29], ability to secrete a variety of adipokines [30–33],

and longevity [19, 34]. The oxidative stress induced by both PIs and NRTIs at the fat cell level [19, 28, 33–35] probably plays a major role in the setup of lipodystrophy.

Severe adipose tissue alterations have been reported in HIV-infected patients with ART-related lipodystrophy. Lipoatrophic adipose tissue biopsies present major histological alterations with decreased and heterogeneous size of adipocytes, increased fibrosis, altered mitochondria, and macrophage infiltration [1, 2, 36–38], consistent with a profound remodeling of subcutaneous fat tissue. The presence of isolated fat droplets, macrophages, and apoptotic cells in the enlarged vascular stroma argues for a progressive destruction of subcutaneous adipocytes [1, 2, 29, 37, 39, 40].

PPARγ is expressed in priority in adipocytes. It is also expressed in different other cell types including macrophages and regulates genes associated with growth, differentiation, insulin sensitivity, inflammation, and immunity [41–46] (see [5]). PPARγ plays an essential role in the development and normal function of white adipocytes, where it mediates part of the regulatory effect of dietary fatty acids on gene expression [43, 47], regulates the differentiation program [48] and insulin sensitivity [45]. PPARγ also controls the production and secretion of adipokines such as leptin and adiponectin, which are important mediators of insulin action in peripheral tissues [42]. In brown adipocytes, PPARγ also controls the adipogenic program and the switch from white to brown adipocytes [49]. In macrophages, PPARγ controls alternative activation and improves insulin resistance [50]. It plays an important role in macrophage inflammation and cholesterol homeostasis and inhibits the production of proinflammatory cytokines through inhibition of the NFκB and AP-1 pathways [48, 51–54].

Loss-of-function or dominant-negative mutations in the PPARG gene in humans (see [5]), and genetically-induced PPARγ deficiency in mice [55, 56] are responsible for lipodystrophic syndromes with insulin resistance, showing the primarily involvement of PPARγ defects in adipose tissue development and metabolic roles. Alternatively, other causes of adipocyte differentiation defects lead to a secondary decreased PPARγ expression and/or function, that further contribute to adipose tissue dysfunction, as shown in vivo in murine models [57] or in vitro [58–60].

In that setting, the implication of PPARγ in the ART effect has been demonstrated both in vitro, in cultured adipocytes and macrophages, and ex vivo, in adipose tissue samples from patients, and has been confirmed by the beneficial effects, at least partial, of the PPARγ agonists, thiazolidinediones. PPARγ defects, although probably secondary to the multiple deleterious consequences of ART on adipose tissue, play a central role in ART-related lipodystrophy and metabolic alterations.

2. Effects of ART on PPARγ Expression and Signaling in Cultured Adipocytes

PPARγ contributes to the setup of the differentiation program and to insulin sensitivity. PIs and NRTIs, the two major classes of antiretrovirals associated with lipodystrophy in HIV-infected patients, may interfere at several steps of PPARγ signaling in adipose cells, such as differentiation, insulin action, oxidative stress, inflammation, and mitochondrial function.

A number of studies have clearly shown that the first generation PIs, indinavir, nelfinavir, and ritonavir, used at concentrations comparable to their Cmax in patients' serum or at suprapharmacological concentrations, impaired adipocyte differentiation [20, 21, 23, 25, 26, 32, 61–67]. They were also shown to induce insulin resistance [21, 23, 27, 33, 62, 67–70] in murine and human cultured adipocytes. This was associated with a reduced protein and mRNA expression of PPARγ in both murine [20, 21, 25, 26, 64] and human adipocytes [24, 66, 71, 72]. Interestingly, decreased PPARγ expression was also observed in mature adipocytes chronically incubated with PIs, consistent with PI-induced adipose cell dedifferentiation.

Most PIs (nelfinavir, indinavir, saquinavir, ritonavir, and amprenavir) were shown to acutely inhibit insulin activation of glucose uptake in cultured adipocytes, via a direct inhibition of the glucose transporter Glut4 [73]. Indinavir and nelfinavir also altered the activation of proximal steps in insulin signaling as revealed by decreased phosphorylation of extracellular-regulated kinase (ERK) 1/2 and Akt/protein kinase B. Accordingly, distal events in insulin signaling pathways, glucose transport, and lipogenesis were also affected [21, 30, 74]. Regarding PPARγ, cell imaging studies revealed that indinavir and nelfinavir but not amprenavir severely decreased nuclear expression of PPARγ [21], indicating for the first time that the transcriptional activity of PPARγ may be defective in PI-treated cells. The beneficial effect of rosiglitazone [21, 23, 32] confirmed the implication of PPARγ in PI action, and indicated that PIs act upstream of PPARγ in its signaling cascade to alter adipocyte differentiation and insulin sensitivity. Recent data of our laboratory further support the implication of PPARγ in PI action by showing that two angiotensin II-receptor blockers (telmisartan and irbesartan), that display partial PPARγ agonist activity [75], prevented the PI effects on lipid accumulation and insulin response in murine and human adipocytes (Boccara F. et al., unpublished results).

The effect of ritonavir on insulin signaling has been particularly studied since this commonly prescribed PI is associated with dyslipidemia and metabolic disorders in HIV-infected patients [67, 76, 77]. Ritonavir induced insulin resistance in cultured adipocytes [24, 32, 64]. Another study reported that ritonavir reduced differentiation and insulin sensitivity in human preadipocytes and adipocytes but surprisingly without decreasing PPARγ2 gene expression [68]. However, the protein expression and the activation of PPARγ have not been evaluated in this study.

The mechanism whereby PIs alter adipose cell differentiation and insulin sensitivity is obviously complex and multifactorial. Impaired SREBP-1 nuclear penetration [21, 22] may inhibit the activation of PPARγ or related adipogenic transcription factors thus leading to defective adipogenesis and insulin resistance. When going further into the mechanism of PI action, we and others demonstrated that some PIs prevented the maturation of lamin A/C [22, 34, 78],

a nuclear membrane protein essential for normal nuclear membrane folding and for nuclear penetration of SREBP-1 [59, 79, 80]. Defective SREBP-1c signaling may explain the decreased differentiation and insulin resistance of PI-treated cells and the ability of PPARγ agonists to overcome the PI effects on fat cell differentiation and insulin response [21].

NRTI therapy is also associated with fat tissue disease in HIV-infected patients. In murine adipose cell lines and primary cultured human adipocytes, stavudine and zidovudine, but not other NRTIs (tenofovir, abacavir, didanosine, and lamivudine), alter lipid storage [23, 31, 33, 81]. They also decrease the expression and secretion of adiponectin in cultured human and murine adipocytes [23, 32, 33, 82] and induce oxidative stress, suggesting that they could secondarily participate to the insulin resistance setup [33]. The negative effect of NRTIs on PPARγ expression and signaling has been reported only in a few studies. Stavudine or zidovudine have a modest, or no effect, on adipose cell differentiation assessed by the gene expression profile of differentiating adipocytes [25] and by protein and mRNA expression of adipogenic transcription factors, among them PPARγ [20, 25, 31, 32, 82]. Altered adipocyte lipid phenotype and insulin sensitivity resulting from NRTI treatment are suspected to result from their mitochondrial toxicity [15–18]. We recently reported that stavudine or zidovudine, but not other NRTIs, triggers mitochondrial oxidative stress and premature senescence in cultured fibroblasts and adipocytes [19]. Stavudine also altered in human preadipocytes [72] the expression of the PPARγ coreceptor 1-alpha (PGC1-α) a transcriptional coactivator upregulated by thiazolidinediones which controls mitochondrial function and biogenesis, and metabolic pathways and integrates insulin signaling and mitochondrial function [83, 84]. Stavudine increased its expression together with mitochondria number [72]. Thus, conversely to PIs, in vitro, thymidine analogs have no or mild detrimental effect on PPARγ function.

The non-NRTI class of antiretrovirals has not yet, as a class, been associated with long-term toxicity [7] even if efavirenz was shown in one study to be associated with lipoatrophy [14]. Very few studies report experimental in vitro findings on the effects of the non-NRTIs efavirenz or nevirapine on white adipose cell functions. Efavirenz but not nevirapine induced a delayed and moderate reduction in lipid accumulation in both murine and human cultured adipocytes, and decreased SREBP-1c and PPARγ expression [85].

3. Effect of ART on PPARγ Expression and Function in Animal Models

Ritonavir was shown to increase lipogenesis [86] and to induce insulin resistance in animal models [87]. In mouse fat tissue, it partially inhibits the function of PPARγ as shown by the decreased induction of PPARγ target genes by rosiglitazone [88]. Lopinavir-ritonavir but not atazanavir decreased by 25% the weight of peripheral inguinal fat in mice treated for 8 weeks, while the profound epididymal adipose tissue depot was not affected. The expressions of SREBP-1c and of its target gene fatty acid synthase were increased in the peripheral inguinal fat while that of PPARγ tended to be decreased in the two depots and that of its target gene adiponectin was not modified [89]. Even if not entirely conclusive, these data are in favor of an altered expression and/or function of PPARγ induced by some PIs in murine models.

4. Effect of ART on PPARγ Expression and Function in Patients' Adipose Tissue

Studies performed on human adipose tissue samples studied ex vivo concerned, at first, healthy controls treated with ART. Mallon et al. [90] reported that a 2-week treatment with stavudine/lamivudine or zidovudine/lamivudine resulted in an increased expression of PGC1α and PPARα and a decreased expression of PPARγ without any modification in the expression of SREBP1. Altered expression of PGC1α was correlated with upregulation of nuclear genes involved in transcription regulation of mtRNA and oxidation of fatty acids suggesting a central role for PGC1 in nuclear response to mitochondrial dysfunction.

Several studies evaluated the expression of PGC1α and PPARγ in adipose tissue from long-term ART treated HIV-infected patients with lipodystrophy. A decreased expression of the two factors was reported in abdominal fat from lipodystrophic patients as compared to controls [36, 37] and to non-lipodystrophic patients [91]. A decreased expression of PPARδ was also found in this latter study. Accordingly, a decreased expression of the transcription factor SREBP-1 was also reported [36, 91, 92]. PPARγ adipose tissue expression was found decreased in HIV-infected patients as compared to noninfected controls by Giralt et al. [5] but the major decrease was observed in naïve versus ART-treated patients, without differences between lipodystrophic and nonlipodystrophic patients, arguing for a major role for the virus itself. The expression of PGC1α was increased. The group of D. Nolan and S. Mallal observed that the PPARγ2 mRNA level was similar in fat from treatment-naïve patients and in patients under PI or zidovudine but lower in patients under stavudine. However, noninfected controls were not evaluated in that study [93]. Interestingly, adipose tissue dysfunction appears more severe in peripheral than in abdominal subcutaneous adipose tissue, as shown by the decreased expression of PPARγ, C/EBPα, and adiponectin in adipose tissue from thigh versus abdomen [94]. Therefore, a strong alteration in PPARγ expression was found in most studies using HIV-infected patients' subcutaneous fat samples.

To examine the reversibility of adipose tissue alterations in HIV-infected patients, adipose tissue biopsies were studied before and after a 6-month interruption of ART in the Lipostop study. Adipose tissue inflammation improved markedly, with fewer infiltrating macrophages and fewer TNFα- and IL6-expressing cells. mRNA expression of PPARγ and of markers of mitochondrial function and biogenesis (cytochrome oxidase subunit 2 and PGC1α) improved after PI withdrawal. In patients who stopped taking stavudine

or zidovudine, adipose tissue inflammation, mitochondrial status, and SREBP-1 expression were improved [95]. Since PGC1α is playing a leading role in mitochondria function [84], this indicates that altered PGC1α and PPARγ expression induced by some ART may be involved in mitochondria dysfunction observed in patients' fat [90, 95].

Decreased PPARγ expression was also strongly correlated with increased expression of inflammatory cytokines such as IL-6 and TNF-α and decreased expression and circulatory levels of adiponectin which is involved in liver and muscle insulin sensitivity [1, 36, 37, 91, 96]. These data confirm that altered PPARγ function in adipose tissue plays a role in overall insulin resistance associated with lipodystrophy, as reported in genetically-determined PPARγ dysfunctions [45]. In accordance, the study from Sutinen et al. [97] reported the effects on adipose tissue of a 24-week treatment with the PPARγ agonist rosiglitazone compared with placebo in HIV-infected patients with lipodystrophy. The expression of adiponectin, PPARγ, and PGC1α significantly increased while that of IL-6 decreased. Expression of other genes involved in lipogenesis, fatty acid metabolism, or glucose transport, such as PPARδ, and SREBP-1, remained unchanged. Rosiglitazone also significantly induced an increase in serum adiponectin concentration, which was inversely correlated with the changes in fasting serum insulin concentration and liver fat content. Such data have led to conduct clinical trials using thiazolidinediones to try to reverse peripheral fat loss. Even if the results obtained with rosiglitazone were disappointing (see [97]), possibly due to the ongoing presence of stavudine in the ART regimen, recent data obtained with pioglitazone are more promising and reveal, in patients not treated with stavudine, an improvement of peripheral fat [98] further supporting a role for PPARγ dysfunction in lipoatrophy.

5. PPARγ Expression and Fat Hypertrophy in HIV-Infected Patients

The lipodystrophic phenotype observed in HIV-infected patients associates, to different extent, peripheral lipoatrophy and fat hypertrophy in different fat depots. In particular, a buffalo hump has been observed in a number of patients. The group of F. Villaroya showed that buffalo humps from HIV-infected patients displayed a brown adipose tissue phenotype with both specific uncoupling protein 1 (UCP1) expression and mitochondrial dysfunctions [99]. However, there were no significant changes in the expression of other UCP genes or of that of markers of adipogenesis including PPARγ, PGC1α, and adiponectin relative to controls. A more extensive analysis indicated that buffalo hump tissue does not express a complete brown adipocyte phenotype but rather a distorted brown-versus-white phenotype associated with enhanced proliferation [2]. In addition, buffalo humps failed to show increased expression of TNFα or the macrophage marker CD68 indicating the absence of a local inflammatory status. Since adipose tissue inflammation and the presence of proinflammatory cytokines has been presumed to play a role in subcutaneous fat lipoatrophy in HIV-infected patients, this absence of inflammation could explain, at least in part, the absence of fat loss observed in that depot.

The effect of antiretrovirals on brown adipocytes has been evaluated in two studies. In primary culture of differentiated murine brown adipocytes, neither the cell differentiation nor the level of PPARγ was modified by the treatment with a series of NRTI including stavudine and zidovudine. By contrast, regarding the NNRTI, nevirapine increased and efavirenz decreased brown adipocyte differentiation and PPARγ expression. PGC1α expression was not modified by the drugs except for its increase in response to stavudine and nevirapine [100]. In the T37i brown adipocyte cell-line, indinavir, stavudine, and zidovudine alone or in association impaired PPARγ2 and UCP1 expression together with a strong inhibition of cell differentiation and mitochondrial functions, although the 3T3-F442A white adipocyte cell line, studied under similar conditions, was less severely affected [26]. Therefore, brown fat can also be a target of antiretrovirals. Since the presence of brown adipose tissue in normal humans has been recently reassessed [101], it would be important to further evaluate its alterations in HIV-infected patients under ART.

Increased visceral fat is also a characteristic feature of HIV-related lipodystrophy. However, samples from patients are difficult to obtain and no study, up to now, has reported specific data obtained with HIV-infected patients' visceral fat. A few studies compared the effect of antiretrovirals on adipocytes issued from subcutaneous and visceral fat from noninfected subjects but the expression of PPARγ or PGC1 was not evaluated.

6. PPARγ and Macrophages

PPARγ plays an important role in macrophage function and phenotype and exerts an overall anti-inflammatory function (see [5]). Recent data have shown that adipose tissue from obese individuals presents macrophage infiltration as well as increased number of "M1" or "classically activated" macrophages. Importantly, the agonists of PPARγ have been shown to alter macrophage phenotype to "M2" or an "alternatively activated" anti-inflammatory phenotype and may induce macrophage specific cell death [102]. PIs could alter PPARγ in macrophages by increasing PPARγ mRNA expression resulting in foam cell formation [103]. In the Lipostop study [95], we observed that stopping ART resulted in an improvement of adipose tissue function associated with a decreased number of M1 but not M2 macrophages together with an increased expression of PPARγ. This can result from modified PPARγ expression both in adipocytes and macrophages.

7. Conclusion

In vitro and in vivo data strongly suggest that altered PPARγ function plays a role in HIV-related lipodystrophy as a result of a multifactorial toxicity of ART on adipose tissue. In vitro studies investigating the effect of individual antiretrovirals have clearly revealed that some PIs inhibit PPARγ functions, probably at the earlier step of SREBP1c

activation. Ex vivo studies of adipose tissue, both in healthy volunteers and in HIV-infected patients, confirmed these data but also point to a possible toxicity of NRTI, principally stavudine and to a lesser extent, zidovudine. Since PPARγ is playing a central role in adipose tissue differentiation and function, decreased PPARγ expression could be expected to be involved in the pathophysiology of lipodystrophy. Importantly, both adipocytes and macrophages present in patients' adipose tissue can be affected at the PPARγ level. Adipose tissue dysfunction could induce insulin resistance and deregulate adipokine secretion with increased release of proinflammatory cytokines and decreased adiponectin, alterations which will impact on the liver and muscles.

Most studies in that setting evaluated the expression and function of PPARγ and only scarce data are available for PPARα and PPARδ.

Using thiazolidinediones to reverse fat lipoatrophy was a logical proposition. However, trials using rosiglitazone were disappointing, in part due to the absence of discontinuation of stavudine. Pioglitazone was more promising and resulted in some recovery of limb fat further arguing for a role for PPARγ in initial fat alteration.

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Review Article

Is PPARγ a Prospective Player in HIV-1-Associated Bone Disease?

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Currently infection with the human immunodeficiency virus-1 (HIV-1) is in most instances a chronic disease that can be controlled by effective antiretroviral therapy (ART). However, chronic use of ART has been associated with a number of toxicities; including significant reductions in bone mineral density (BMD) and disorders of the fat metabolism. The peroxisome proliferator-activated receptor gamma (PPARγ) transcription factor is vital for the development and maintenance of mature and developing adipocytes. Alterations in PPARγ expression have been implicated as a factor in the mechanism of HIV-1-associated lipodystrophy. Both reduced BMD and lipodystrophy have been well described as complications of HIV-1 infection and treatment, and a question remains as to their interdependence. Interestingly, both adipocytes and osteoblasts are derived from a common precursor cell type; the mesenchymal stem cell. The possibility that dysregulation of PPARγ (and the subsequent effect on both osteoblastogenesis and adipogenesis) is a contributory factor in the lipid- and bone-abnormalities observed in HIV-1 infection and treatment has also been investigated. This review deals with the hypothesis that dysregulation of PPARγ may underpin the bone abnormalities associated with HIV-1 infection, and treats the current knowledge and prospective developments, in our understanding of PPARγ involvement in HIV-1-associated bone disease.

1. Introduction

Aside from the serious effects on the cells of the immune system, HIV-1 infection and its treatment have been associated with disorders in other tissues, most notably bone [1, 2] and adipose [3–6] tissues, where reduced bone mineral density (BMD) and abnormalities of the lipid metabolism (lipodystrophy, dyslipidemia, and insulin resistance) have been described. In both disorders (particularly those of the adipose tissue), antiretroviral treatment is believed to play a major role, but the contribution of underlying HIV-1 infection has yet to be elucidated, and therefore cannot be ignored as a potential causative factor.

PPARγ is a nuclear membrane bound transcription factor which regulates a number of genes involved in adipogenesis from common precursor cells type (mesenchymal stem cells), maturation of preadipocytes, lipid accumulation, and maintenance of adipogenic phenotype [7, 8]. As such, it is not surprising that a number of recent studies have indicated that certain drugs known to be associated with lipodystrophy dysregulate PPARγ [9, 10]. The involvement of PPARγ in HIV-1-associated bone disease is an area that has been little studied to date; however numerous studies suggest that PPARγ plays a role in conditions such as osteoporosis in the absence of HIV-1 or ART, and increased adipocyte content of osteoporotic bone has been reported [10–12]. In addition, osteoblasts—the cells responsible for depositing bone—are derived from mesenchymal stem cells, and evidence suggests that the balance of PPARγ and the pro-osteogenic runt-related transcription factor-2 (RUNX-2) is a key in the determination of mesenchymal stem cell fate [13–15] (see Figure 1). This review will introduce the current knowledge of the role of PPARγ in bone biology in normal and disease states, and discuss its potential as a mechanism for HIV-1-associated bone disease.

2. HIV-1-Associated Bone Disease

Osteoporosis is defined as a reduction in the bone mass and disruption of the microarchitecture of the bone which leads to a greatly increased risk of fractures, while osteopenia

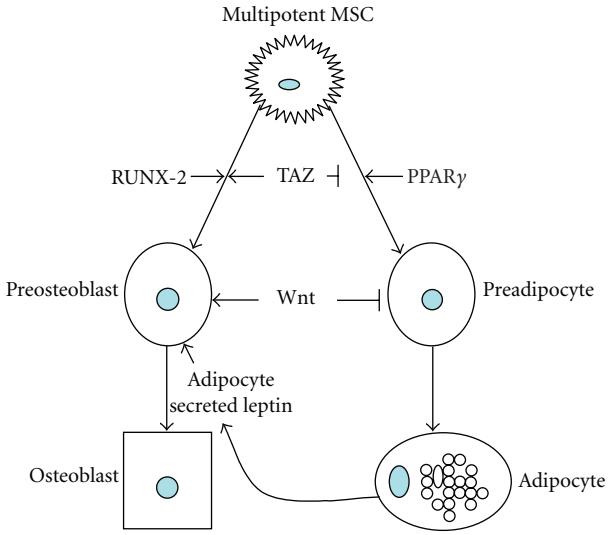

Figure 1: Factors governing normal osteogenesis and adipogenesis from mesenchymal stem cells. Multipotent mesenchymal stem cells can differentiate into a number of cell types, including adipocytes and osteoblasts. ($\perp$ indicates inhibition; $\downarrow$ indicates stimulation). The transcriptional coactivator Taz negatively regulates adipogenesis and promotes osteogenesis through suppression of PPARγ and activation of RUNX-2, while overexpression of PPARγ can reduce bone formation. Also, a number of other factors such as secreted proteins from Wnt family promote the differentiation and maintenance of osteoblasts while reducing the differentiation of the adipocytes. In addition, factors secreted by mature adipocytes, such as leptin and estrogen, can increase bone mass in vivo.

is a lesser reduction in bone density and strength which may remain asymptomatic, but can precede actual osteoporosis. The world health organization (WHO) definitions specify t-scores between -1 and -2.5 as being indicative of osteopenia, while t-scores of less than -2.5 are indicative of osteoporosis [16]. Fractures resulting from osteoporosis affect one in two women and one in five men over the age of 50, and are a significant financial burden to health services, with an estimated combined annual cost of 30 billion Euro in the EU [17].

As will be discussed further, bone remodeling is dependent on the opposing functions of two cell types, osteoblasts, which make new bone (bone formation), and osteoclasts, which destroy old bone (bone resorption). Therefore, the balance between the number and activity of osteoclasts and osteoblasts is crucial in normal bone homeostasis; the perturbation of which can directly lead to increased bone fragility and fracture risk. Two important molecules: macrophage colony-stimulating factor (M-CSF) and receptor for activation of nuclear factor-kappa B ligand (RANKL) produced from osteoblast/stromal cells regulate the differentiation, function, and survival of osteoclasts, while the transcription factors, RUNX-2 and Osterix, have been reported to regulate osteoblast differentiation [18].

2.1. HIV-1 Infection and Bone Disease. Bone metabolism in HIV-infected individuals has been studied since the late 1980s, although the number of early studies is somewhat limited. Before the widespread use of highly active ART, studies indicated that bone mineral metabolism was only minimally affected in HIV-infected patients. Serrano et al. assessed histomorphometry in HIV-positive patients and found that parameters of histomorphometry such as serum osteocalcin were found to be lower in patients who, according to the Centers for Disease Control (CDC) classification, had greater disease severity [19]. Paton et al. reported that 45 HIV-infected patients had marginally lower BMD at the lumbar spine. None of the patients had reduced BMD to levels associated with a diagnosis of osteoporosis [20]. More recently however, it became clear that reduced BMD is also frequent in the absence of therapy [21–24]. In a study by McGowan et al., the prevalence of osteopenia among antiretroviral-naive HIV-positive individuals to be approximately 28%, which is approximately 50% greater than the expected incidence in the general, uninfected population [25]. Studies which have included patients with more advanced HIV disease who have received treatment for longer periods have reported prevalence of 40% to 50% [26, 27], placing reduced BMD among the most common HIV-1-associated metabolic toxicities. Amiel et al. also assessed BMD in 48 HIV-infected treatment-naive patients, 49 HIV-infected patients on protein inhibitors, 51 HIV-infected patients on no-protein inhibitors, and 81 HIV-uninfected control subjects. The results showed a significant decrease of BMD (9%) in all HIV-infected patients compared to the control subjects, occurring concurrently with a lower bone alkaline phosphatase and higher urinary cross-laps/Cr. [28]. The clinical impact of this reduced BMD is beginning to be examined; recent studies in a large American health care system, involving 8526 HIV infected patients and over 2 million control subjects, demonstrated that the prevalence of any fracture type was significantly higher in the HIV-infected population (2.87 versus 1.77 fractures/100 persons, $P = .002$). This study did not specify the treatment status of their subjects, but the data suggests that HIV-1-related fractures are a significant and growing clinical issue [29].

2.2. Antiviral Treatment and Bone Diseases. Antiretroviral treatment (ART) is a complex therapeutic regimen, in which patients typically take 2-3 agents selected from an array of 30 approved antiretroviral agents. ART, in general, comprises of two major therapeutic strategies: a protease inhibitor- (PI-) based regimen and a nucleoside reverse transcriptase inhibitor- (NRTI-) based regimen. The PI-based regimen uses one or two PIs combined with two NRTIs, whereas the NRTI-based regimen uses two NRTIs combined with one non-nucleoside reverse transcriptase inhibitor (NNRTI). With more effective therapies as a result of HAART, the prevalence of HAART-associated bone diseases has increased [30].

A higher incidence of reduced BMD has been clinically associated with both PI and NRTI uses. Tebas et al. determined that in HIV-1 patients receiving PIs about 50% of the patients had osteopenia and other 21% had osteoporosis [31]. This incidence is significantly increased

compared to patients without therapy or normal controls. Studies by Moore et al. confirmed that 71% of HIV-infected patients on PI therapy have reduced BMD [32]. Similarly, Carr et al. reported that 3% of 44 HIV-infected patients receiving NRTIs developed osteoporosis and 22% developed osteopenia [33], while in a study examining HIV-1-infected men Mallon determined a reduction in BMD beginning at 48 weeks postinitiation of treatment [6]. Tsekes et al. determined BMD and whole body fat by dual energy X-ray absorbance (DEXA) of HIV-infected patients receiving zidovudine and other NRTIs and found significant decreases in both body fat and BMD [34]. In addition, the recent analysis by Brown and Qaqish [35] also reported 2.5-fold increased odds of reduced BMD in ART-treated patients compared with ART-naive patients (95% CI 1.8, 3.7). However, most studies are in agreement that traditional risk factors for osteoporosis, such as ethnic variations, female sex, increasing age, low body mass index, and time since menopause, are all independent predictors of osteopenia/osteoporosis [36–40].

In addition, it has been noted that HIV-infected patients have an increased risk for osteonecrosis of the hip [41]. Keruly et al. reported 15 cases of avascular hip necrosis in HIV-infected patients and suggested that the incidence of osteonecrosis in HIV-infected patients was higher than the general HIV-negative population [42]. It is not known whether this phenomenon is attributable to HIV-1 infection itself, HAART, or other HIV-associated complications.

The mechanisms by which either HIV-1 or its treatment induces reduced BMD are as yet unclear, and several researchers have suggested that reduced vitamin D levels observed in HIV-1-infected patients, and particularly the reduced levels of the biologically active metabolite $1,25(OH)_2D$ (which is the natural ligand for the vitamin D receptor (VDR)), may contribute to reduced BMD [43]. Studies have demonstrated that the level of $1,25(OH)_2D$ in HIV-1-infected patients is between 5 and 50% lower than that in infected patients [24, 44, 45]. In addition, studies have indicated that patients receiving treatment are more likely to have greater reductions in $1,25(OH)_2D$, with a recent Dutch study suggesting that NNRTI treatment may increase the risk of vitamin D deficiency [46, 47]. In addition, the latter study demonstrated that patients receiving treatment also have increased parathyroid hormone (PTH) levels, increasing the potential risk of reduced bone mass.

In short, HIV-1-associated bone disorders are a significant and increasingly well-defined clinical issue. However, the molecular basis underpinning these clinical observations remains to be fully explained.

3. PPARγ: Mediator of Development and Disease in Bone Biology

As discussed previously, maintenance of bone homeostasis is mediated through a balance of osteoblast-mediated bone deposition and osteoclast-mediated bone resorption. The continued production of these cells from stromal (mesenchymal) and hematopoietic (monocyte) precursors, respectively, is an essential component in the maintenance of BMD. Stromal progenitor or mesencymal stem cells are multipotent cells, capable of producing cells of a number of different lineages, including osteoblasts and adipocytes [47–49].

Since the early 1990s, researchers have hypothesized that a "see-saw" relationship exists in the bone marrow cavity, where production of adipocytes from stromal precursors is at the expense of osteoblast production and vice versa [50, 51]. This theory is born out by a clinically observed phenomenon, such as the increased adipocyte content of osteoporotic and aging bone [51–53] as well as studies where agents inducing adipocyte production reduced osteoblast number [49, 50]. Likewise, treatment of bone marrow stromal cells with bone morphogenic proteins (BMPs) resulted in reduced formation of adipocytes [53]. Adipocytes can also produce secreted factors such leptin and estrogen, which can positively regulate bone mass [13, 54, 55], further underlining the interrelated nature of bone and fat development (see Figure 1).

PPARs are ligand-activated nuclear hormone receptors which stimulate expression of genes containing peroxisome proliferator response elements (PPREs) [53, 54]. There are three principal members of this family, PPARα, PPARδ, and PPARγ, activation of which stimulates genes involved in fatty acid oxidation, uncoupling of respiration toward heat production (thermoregulation) and terminal adipocyte differentiation (including intracellular lipid accumulation), respectively, (see Table 1) [49, 50, 55–59].

The activity of PPARγ and RUNX-2 is a key to our understanding of the relationship between fat and bone. Activity of the RUNX-2 transcription factor is not only essential for maintenance of osteoblast phenotype, but it is also involved in driving the differentiation of osteoblasts from mesenchymal stem cells [11–14], while activity of PPARγ in mesenchymal stem cells induces differentiation into adipocytes. The eventual phenotype of the differentiating cell is generally considered to be controlled by an antagonistic balance between RUNX-2 and PPARγ [13, 14]. Studies have demonstrated, for example, that activation of PPARγ using pharmacological agents can lead to decreased bone mass in vivo, while mice lacking the PPARγ gene display increased bone mass and an inability to develop adipocytes [59–61]. Indeed, even in the eventual mature cell, the function can be altered by dysregulating this balance, with in vivo studies using a mouse model demonstrating reduced bone formation rate and suppression of RUNX-2 in osteoblasts in which PPARγ had been activated [61], while Kim et al. have demonstrated that activation of PPARγ induces death through a MAPK-dependant mechanism in osteoblastic cells [62].

PPARγ deficient mice (having a mutation in the PPARγ2 locus) have been generated and display a "lipodystrophic" phenotype, which occurs concurrently with increased bone mass, to the point where the bone marrow is almost completely occluded and hematopoiesis moves to extramedullary sites, such as the spleen [61, 63]. Recently, our understanding of the roles of PPARγ in numerous physiologic processes, including the bone/fat paradigm, has been furthered by the development of the thiazolidinedione (TDZ) family of

Table 1: PPARγ-regulated genes involved in adiogenesis, glucose uptake, and thermoregulation (↑ positive regulation; ↓ negative regulation).

PPARγ-regulated genes		
Gene	Tissue/cell type	Function
CCAT enhancer binding protein α (CEBPα) [7]↑	Adipose/preadipose tissue	Transcription factor. CDK2/4 inhibition-cell cycle arrest
Adipose differentiation related protein (ADRP) [50]↑	Adipose/preadipose tissue	Associated with globule membrane, early marker of adipocyte differentiation
Lipoprotein lipase [49]↑	Vascular endothelium, heart, muscle, adipose	Lipid hydrolysis from lipoproteins
Adiponectin [49]↑	Adipose tissue (secreted)	Fatty acid catabolism
Adipocyte protein 2 (aP2/FABP4) [49]↑	Adipocytes/macrophages	Intercellular lipid transport
Tumour suppressor candidate 5 (TUSC 5) [56]↑	Preadipose/adipose tissue	Associated with entry into the later stages of adipogenesis
Glucose transporters 4 (GLUT) 4 [57]↑	Wide tissue distribution	Insulin stimulated glucose uptake
Uncoupling proteins 1-3 (UCP 1-3) [58]↑	Adipose tissue, skeletal muscle, liver	Thermogenesis/thermoregulation

PPARγ ligands, such as netoglitazone, pioglitazone, rosiglitazone, and GW0072 [64–67]. Studies have demonstrated that treatment of murine osteoblasts with netoglitazone and GW0072 can block osteoblast differentiation, without inducing adipogenesis [62, 64], while in vivo studies have demonstrated that rosiglitazone, a ligand with higher affinity for PPARγ, decreased bone mineral density, bone formation rate, and trabecular bone volume, while increasing adipogenesis [65, 67]. Further studies on ovariectomized rats revealed that these effects are mediated in part by the suppression of the RUNX-2 transcription factor [67], giving further strength to the argument that an antagonistic relationship between PPARγ and RUNX-2 governs bone and fat formation. Indeed, Hong et al. have demonstrated that shared coactivator protein, TAZ, accounts in some part for this relationship, in that it coactivates RUNX-2 and bone formation, while suppressing PPARγ [68].

3.1. PPARγ in HIV-1-Associated Lipodystrophy. ART is associated with changes in fat metabolism, broadly termed lipodystrophy (changes in fat distribution) or lipoatrophy (atrophy of adipose tissue). Severe forms of lipodystrophy are a major cosmetic concern, and can lead to suboptimal adherence to therapy. In addition, lipodystrophy is associated with markers of cardiovascular risk, such as insulin resistance and dyslipidemia [5].

In vitro, expression of PPARγ is decreased by exposure to anti-HIV-1 PI and NRTI drugs. In differentiating adipocytes, exposure to nelfinavir, saquinavir, and ritonavir at 10 μM concentrations resulted in decreased adipogenesis and expression of the PPARγ-mediated mRNA encoding aP2 and lipoprotein lipase (LPL) [10]. Similar effects on PPARγ expression were observed in 3T3-F442A adipocyte cells exposed to 10–50 μM indinavir [69], while studies by the same group have also demonstrated that the nuclear association of the PPARγ regulator SREBP-1 is reduced by treatment with indinavir [70]. In mature adipocytes, inhibition of PPARγ function by expression of a dominant negative PPARγ isoform results in decreased accumulation of intracellular triglyceride, decreased cell size, and decreased expression of genes involved in both fatty acid and glucose metabolism, including the glucose transporter GLUT-4 [71]. In lipoatrophic mice, ablation of PPARγ activity in liver resulted in hepatic steatosis, hypertriglyceridemia, and muscle insulin resistance [72]. Many of these features are shared by PI-treated patients with HIV-1-associated lipodystrophy.

In vivo, patients with lipodystrophy had lower adipose tissue expression of both PPARδ and PPARγ than those without lipodystrophy. This was accompanied by decreases in a number of PPARγ-responsive downstream genes including LPL and GLUT-4 [73, 74]. In studies by Mallon, NRTI treatment of non-HIV-1-infected subjects (either stavudine/lamivudine or zidovudine/lamivudine for six weeks) resulted in reduced PPARγ expression in adipose tissue (alongside alterations in transcription of mitochondrial DNA, and upregulation of genes associated with mitochondrial transcriptional regulation), although in this study the effects on overall fat mass were not determined [9].

In patients with type 2 diabetes, exposure to TZD, which act as PPARγ ligands, resulted in increased expression of PPARγ-target genes such as LPL and fatty acid synthase (FAS) in subcutaneous adipose tissue biopsies, without increasing expression of PPARγ itself [71]. However, studies utilizing TZD to treat lipodystrophy have produced variable, and at best, modest results [75–78]. More recently, van Wijk et al. demonstrated that rosiglitazone treatment, compared to treatment with metformin, increased subcutaneous abdominal and visceral abdominal fat in lipodystrophy, however this was a small study ($n = 39$), was not blinded or placebo controlled, and did not measure clinical outcomes [79].

The weight clinical and scientific evidence suggests that HIV-1/ART-associated lipid abnormalities occur largely as

a result of treatment rather than infection. However, a recent study raised the possibility that there may also be a viral component; Shrivastav et al. [80] demonstrated that treatment with the HIV-1 accessory viral protein R (Vpr) could suppress PPARγ-induced transactivation in 3T3-L1 murine adipocyte cells, with a consequent inhibition of adipocyte differentiation. Vpr is a 96-amino-acid accessory protein, which is packaged in the viral capsid, and is found in the nucleus early after cell infection [81, 82]. Among the functions of Vpr is its ability to act as a transcriptional activator of viral and cellular promoters [83–86]. Vpr enhances the activity of steroid hormone receptors, including the gluticorticoid receptor (GR), which Vpr can bind via its LXXLL motif [85]. Studies involving cotransfection with constructs expressing wild type and mutant (LXXLL null) Vpr constructs with reporter constructs containing the PPRE demonstrated that this phenomenon was dependent on the LXXLL motif. Further experiments demonstrated that the GR did not play a role, and that Vpr and PPARγ interacted directly in living cells. The authors of this study hypothesize that in vivo circulating Vpr, or Vpr produced as a result of direct infection of adipocytes, could suppress differentiation of preadipocytes in a PPARγ dependent manner with obvious consequences for the development of lipodystrophy and insulin resistance [80].

3.2. PPARγ in HIV-1-Associated Bone Disease. In contrast to the clearly defined role for PPARγ in HIV-1/ART-associated lipid abnormalities, few studies have focused on its potential impact in HIV-1/ART-associated bone abnormalities.

To date, studies into mechanism of reduced bone density have been understandably focused on two distinct strands, namely, the effects on osteoblast and osteoclast number and function. In the case of OC research, several studies have demonstrated that osteoclast function can be altered in vitro by treatment with both ritonavir and HIV-1 gp120 [87, 88]. Jain et al. demonstrated that osteoclast activity, measured using a rat neonatal calvaria assay, increased in the presence of nelfinavir, indinavir, saquinavir, or ritonavir, while lopinavir and amprenavir did not increase osteoclast activity. In addition, Pan et al. reported a significant increase in markers of osteoclastogenesis (namely, the activity of the tartaric acid phosphatase (TRAP) promoter and the NF-κb transcription factor) in RAW264.7 (mouse leukemic monocyte macrophage cell line cells) and primary mouse osteoclast precursors treated with the NRTI zidovudine [89]. This same group has more recently reported that the NRTIs ddi and lamiduvine also induced osteoclastogenesis in vitro and osteopenia in an in vivo mouse model [90].

Similarly, osteoblast-based studies have produced some interesting data. Clinically, Serrano et al. reported reduced numbers of osteoclasts in HIV patients; a phenomenon occurring along side-reduced serum osteocalcin levels and bone formation rate [19]. Previous and ongoing in vitro studies by our own group have demonstrated that osteoblast activity (as measured by calcium deposition and alkaline phosphatase activity) can be reduced by a number of antiretroviral drugs (including both nelfinavir and indinavir). In addition, these studies identified tissue inhibitor of metalloproteinase-3 (TIMP-3) as a mechanism for this observed loss in osteoblast function [91]. Further studies by our group demonstrated that treatment with the HIV-1 proteins p55-gag and gp120 reduced osteoblast activity in conjunction with reduction RUNX-2 transcription factor activity [92]. Interestingly, gp120 both decreased RUNX-2 activity and increased PPARγ. Furthermore, our studies investigating the effect of HIV-1 proteins on mesenchymal stem cell differentiation have suggested that the proteins p55 and REV alter both mesenchymal stem cell osteoblastic differentiation and RUNX-2/PPARγ signalling in nondifferentiating mesenchymal stem cells [93].

Although these studies used a somewhat simplistic model of HIV-1 exposure, given the evidence of the impact of PPARγ on normal bone biology, and the observation that it can be perturbed in HIV-1-associated lipodystrophy, it is tempting to interpret these results as being suggestive of PPARγ playing a role in HIV-1-mediated bone disease. However, there is an obvious stumbling block for this hypothesis, namely, that if increased PPARγ activity in mesenchymal stem cell and osteoblasts could result in reduced bone mass, it would surely also increase fat mass. This picture is further complicated, as previously discussed studies have demonstrated that treatment of non-HIV-1-infected subjects with NNRTIs resulted in reduced PPARγ expression in adipose tissue [9], while in vitro studies with 3T3-F442A cells have demonstrated that both PPARγ expression and its association with SREBP-1 are reduced by treatment with indinavir [69, 70]. However, different processes may govern fat redistribution in different tissues, with gain in visceral fat and loss of subcutaneous fat. In addition, at least one ex vivo study suggests that both markers of adipocyte and osteoblastic differentiation are significantly reduced in human mesenchymal stem cells treated with a subset of protease inhibitors (particularly nelfinavir and saquinavir) [94], while HIV-1 patients receiving the NRTI zidovudine were shown to have reduced both BMD and whole body fat [33]. Could it be that contributing to both HIV-1/ART-associated bone and lipid disorders is an underlying disregulation of mesenchymal stem cell function combined with separate effects on adult or partially differentiated cells?

4. Conclusion

The importance of PPARγ in both bone and fat metabolism has been clearly demonstrated, and while a role for PPARγ in the lipid abnormalities associated with HIV-1 and its treatment is emerging, its involvement in HIV-1-associated bone disease remains unclear. Given the common origin of both adipocytes and osteoblasts from mesenchymal stem cell, and the demonstrated effect of increased PPARγ expression on bone in vitro and in vivo, we hypothesize a potential role for PPARγ in the reduced bone mass associated with HIV-1 infection and treatment. It may be possible that HIV-1 infection and/or treatment, through dysregulating PPARγ (and possibly also RUNX-2) activity in undifferentiated stromal cells, or in partially differentiated preosteoblast

and preadipocyte cells, can reduce the eventual number or functional capacity of the adult cell types.

In order to further investigate this hypothesis, it may be worthwhile to conduct ex vivo experiment on primary mesenchymal stem cells collected from HIV-1 patients. The expression and activity of PPARγ and differentiation potential of these cells could be assessed and compared to those of cells harvested from uninfected individuals, and the data gathered used to generate a new model of HIV-1/PPARγ/mesenchymal stem cell interactions.

It is clear that further studies are necessary to more fully describe the role of PPARγ in the setting of HIV-1-associated bone disease and its interplay with vascular and fat disorders.

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Review Article

The Effects of Thiazolidinediones on Metabolic Complications and Lipodystrophy in HIV-Infected Patients

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Highly active antiretroviral therapy (HAART)-associated metabolic complications include lipoatrophy (loss of subcutaneous adipose tissue (SAT)) and insulin resistance. Thiazolidinediones are insulin-sensitizing antidiabetic agents which—as an untoward side effect in obese diabetic patients—increase SAT. Furthermore, troglitazone has improved lipoatrophy and glycemic control in non-HIV patients with various forms of lipodystrophy. These data have led to 14 clinical trials to examine whether thiazolidinediones could be useful in the treatment of HAART-associated metabolic complications. The results of these studies indicate very modest, if any, effect on lipoatrophic SAT, probably due to ongoing HAART negating the beneficial effect. The benefit might be more prominent in patients not taking thymidine analoges. Despite the poor effect on lipoatrophy, thiazolidinediones improved insulin sensitivity. However, especially rosiglitazone induced harmful effects on blood lipids. Current data do not provide evidence for the use of thiazolidinediones in the treatment of HAART-associated lipoatrophy, but treatment of lipoatrophy-associated diabetes may be warranted. The role of thiazolidinediones for novel indications, such as hepatosteatosis, should be studied in these patients.

1. Introduction

The prognosis of HIV-infection has drastically changed after the introduction of combination antiretroviral therapy [1] often referred to as highly active antiretroviral therapy (HAART). Since the eradication of the virus is impossible with current medicines [2] and since periodic treatment with HAART can be harmful when compared to continuous therapy [3], patients need to continue therapy uninterruptedly and permanently.

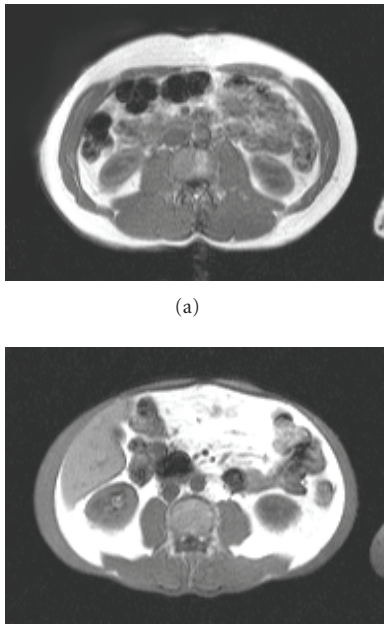
Lifelong exposure to HAART puts patients at a significant risk for long-term metabolic adverse effects including lipodystrophy, insulin resistance, hyperlipidemia, and increased cardiovascular morbidity [4, 5]. The most characteristic component of HAART-associated lipodystrophy is the loss of subcutaneous adipose tissue [6] which has proven to be very difficult to treat (Figure 1).

Thiazolidinediones (glitazones) are oral insulin-sensitizing antidiabetic agents. As an untoward side effect, glitazones increase subcutaneous fat mass in patients with type 2 diabetes [7–9]. Additionally, in non-HIV infected patients with various forms of lipodystrophy, troglitazone has improved metabolic control and subcutaneous lipoatrophy [10]. These insulin-sensitizing and fat-inducing effects of glitazones have lead to several clinical trials examining whether these drugs could reverse lipoatrophy and insulin resistance in patients with HAART-associated lipodystrophy.

The ensuing review is focused on summarizing the currently available clinical data on the use of glitazones in patients with HAART-associated lipodystrophy

2. Thiazolidinediones

Thiazolidinediones are synthetic ligands for peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ is a nuclear receptor which alters expression of multiple genes including those regulating lipid and glucose metabolism [11]. PPARγ is expressed mainly in adipose tissue and is also found in pancreatic beta cells, vascular endothelium, and macrophages, and in low quantities in other tissues such



(a)

(b)

Figure 1: Abdominal magnetic resonance image (MRI) of a HAART-treated patient with normal fat distribution (a) and a patient with severe HAART-associated lipodystrophy with complete loss of subcutaneous fat and accumulation of intra-abdominal fat (b). Fat is shown white in these MRI images.

as the liver, skeletal muscle, and the heart [12, 13]. The activation of PPARγ is critical in adipocyte differentiation, fatty acid uptake, and storage in the adipocytes [14, 15].

Glitazone-induced activation of PPARγ in adipose tissue may improve the whole body insulin sensitivity by keeping fatty acids within adipocytes and hence protecting other tissues (liver, skeletal muscle, and pancreatic beta cells) from the "toxic" effects of the high-circulating concentration of free fatty acids [12]. Alternatively or additionally, glitazones may affect whole body insulin sensitivity by altering adipokine release from adipose tissue [12].

The potential role of PPARγ in the pathogenesis of some human lipodystrophies has been demonstrated in recent studies finding dominant negative and loss-of-function mutations to affect the ligand-binding domain of PPARγ in non-HIV patients with partial lipodystrophy, hepatic steatosis, dyslipidemia, and insulin resistance [13]. Furthermore, trogilitazone was shown to improve subcutaneous lipoatrophy in HIV-negative patients with various forms of lipodystrophic/lipoatrophic syndromes [10]. Taken together, the available data make glitazones an interesting therapy option for HAART-associated lipodystrophy.

3. HAART-Associated Lipodystrophy

The prevalence of HAART-associated lipodystrophy has varied from as low as 2% [16] up to 83% [17] in HAART-treated patients. This huge variation is explained by the lack of uniformly accepted definition of lipodystrophy, and the variable combination and duration of HAART in different studies. Estimates from large surveys indicate a prevalence of 50% of at least one physical abnormality after 12–18 months of therapy [18, 19]. Most of these prevalence data arise from patients taking mainly the older and metabolically more toxic antiretroviral regimens. Although there are accumulating data demonstrating a significantly decreased risk for lipodystrophy in patients taking newer antiretroviral agents [20, 21], lipodystrophy still remains a significant clinical problem.

Lipoatrophy, that is, a decrease in subcutaneous adipose tissue (SAT) mass, has mainly been attributed to the use of nucleoside reverse transcriptase inhibitors (NRTIs) and thymidine analoges (tNRTI) in particular [22–25]. The tNRTIs stavudine and more recently zidovudine gradually decrease SAT mass. Typically, SAT initially increases during the first 4–8 months of therapy, but thereafter, a 19% decrease in limb fat per year has been described with stavudine and didanosine containing regimens as compared to a decrease of 1.7% per year with zidovudine and lamivudine [26]. Further evidence demonstrating the deleterious effects of tNRTIs on SAT arises from the so called "switch" studies. Replacing tNRTI by abacavir or tenofovir has in several studies lead to an increase of 300–500 g of limb fat during the first 6–12 months after the switch [27–29]. Although most data indicate a major role of tNRTIs in the development of lipoatrophy, other drug classes may be involved. Irrespective of the NRTI backbone, nelfinavir (a protease inhibitor) was associated with more severe fat loss than efavirenz (nonnucleoside reverse transcriptase inhibitor) [30], whereas in another study efavirenz caused more fat loss than lopinavir/ritonavir (protease inhibitor) [31]. The less significant role of protease inhibitors (PIs) for lipoatrophy is demonstrated in the switch studies. In contrast to the beneficial effects of switching away from tNRTIs, the effects of switching away from PIs have been disappointing regarding lipoatrophy [32].

The potential pathophysiological mechanisms leading to lipoatrophy include NRTI-induced inhibition of mitochondrial (mt) DNA polymerase gamma through several different mechanisms [33, 34]. This inhibition would lead to decreased mtDNA content which consequently would result in depletion of proteins encoded by mtDNA and dysfunctional mitochondria. Additionally, also genes encoded by nuclear DNA are affected by NRTIs, and these drugs promote apoptosis in adipocyte cell modes in vitro [35].

In keeping with these in vitro data, human studies have shown decreased mtDNA content in adipose tissue of patients with HAART-associated lipodystrophy when compared to HIV-negative subjects, HIV-infected patients not taking HAART, or to HAART-treated patients without lipodystrophy [36–39]. However, studies in healthy subjects have shown that a 2-week exposure to NRTIs leads to a decrease in mtRNA and alters expression of several nuclear genes without a significant change in the mtDNA content [40]. Various studies have shown multiple alterations in gene expression in lipoatrophic adipose tissue such as decreased expression of several transcription factors (PPARγ, SREBP-1c (sterol regulatory element-binding protein), PPARδ,

C/EBPα, and β (CCAAT/enhancer-binding protein)) [41, 42]. Alterations have also been described in the expression of several genes involved in lipogenesis, fatty acid, and glucose metabolism, for example, the expressions of acyl coenzyme A synthase, lipoprotein lipase, and glucose transport protein 4 are decreased in patients with HAART-associated lipodystrophy [42]. Several markers of inflammation such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNFα), CD45, and CD68 have been shown to be increased in lipoatrophic adipose tissue [41–44]. Of the adipokines, the expression of adiponectin in adipose tissue and its circulating concentration have been shown to be decreased in several studies [44–47], whereas serum concentrations of leptin have been either decreased [48, 49], unchanged [46, 50, 51], or increased [52] in lipoatrophic patients. In addition to these findings implying severe adipose tissue dysfunction, increased rate of apoptosis has also been described in the SAT of these patients [43, 53].

Although multiple alterations have been described in lipoatrophic adipose tissue, the role and sequence of each critical abnormality eventually leading to loss of SAT still remain elusive. It also remains unknown which critical abnormalities should be counteracted in order to reverse HAART-associated abnormalities in adipose tissue of these patients, and whether thiazolidinediones would have this potential.

4. Thiazolidinediones for HAART-Induced Metabolic Adverse Effects

Thiazolidinediones have been used in 14 clinical trials in HIV-infected, HAART-treated patients [54–67]. The basic characteristics of these trials are given in Table 1. In total, 281 patients have used rosiglitazone, 82 patients pioglitazone, and 6 patients troglitazone in these trials. Four of these trials were open label-uncontrolled studies [54, 59, 64, 67], 6 were randomized placebo-controlled studies [55–57, 61, 62, 66], and 4 had a comparison arm with another active agent (metformin or fenofibrate) [58, 60, 63, 65]. Followup times varied from 6 weeks to 12 months.

The presence of lipoatrophy without reference to insulin resistance was the inclusion criteria in five studies [55, 56, 58, 64, 66], one additional study required the presence of at least one feature of lipodystrophy (but not necessarily lipoatrophy) without reference to insulin resistance [62]. Insulin resistance (defined by fasting insulin concentration, oral glucose tolerance test, or clamp studies) without reference to lipodystrophy was the inclusion criteria in three studies [54, 63, 65]. One study required the presence of both lipoatrophy and insulin resistance [57], and another study included patients with changes in body fat (but not necessarily lipoatrophy) together with insulin resistance [60]. The troglitazone study included patients with newly diagnosed diabetes with lipodystrophy and dyslipidemia [67], and two studies did not specify any metabolic abnormalities in the inclusion criteria [59, 61]. The exclusion criteria were variable, but often included liver function tests >2-3 times upper limit of normal, serum creatinine >1-2 times upper limit of normal, haemoglobin <90–95 g/L, serum triglycerides >10–15 mmol/L, presence of heart failure, and pregnancy.

4.1. Effects on Body Composition. Body composition data from the eight studies which included a control arm and an objective measurement of subcutaneous fat (dual-energy X-ray absorptiometry [DEXA], computed tomography [CT], magnetic resonance imaging [MRI]) are included in Table 2. No significant changes in the amount of subcutaneous fat could be detected in four studies: two of these studies used a single method to measure adipose tissue volume (one with MRI [55], one with DEXA [62]), and the other two studies measured fat volume using both DEXA and CT [56, 65]. In contrast to these four studies, three other studies reported statistically significant increases in SAT in patients taking either rosiglitazone [57, 60] or pioglitazone [66] as compared to placebo. SAT was quantified by both DEXA and CT scan in all these three studies, but none of the studies could confirm the statistically significant increase in SAT versus placebo by the second method in the same study. The absolute changes in SAT in the thiazolidinedione arm were reported in two studies: an increase of 50 g in leg fat mass after 12-week treatment with rosiglitazone [57], and 380 g increase in limb fat mass in the pioglitazone arm after 48 weeks of therapy [66]. In the study by van Wijk et al. rosiglitazone was compared to metformin without a placebo arm [58]. In this study, there was a statistically significant increase in SAT measured by CT scan in the rosiglitazone arm versus baseline, and also relative to metformin. DEXA scanning was not performed in this study.

One study found a statistically almost significant increase in visceral adipose tissue (VAT) in the rosiglitazone group when compared to placebo [60], while there were no significant changes in VAT between the glitazone and placebo arms in the other studies. None of the placebo-controlled trials reported significant changes in BMI either within the glitazone arm or between the placebo and glitazone group, although the difference approached statistical significance in the pioglitazone trial by Slama et al. [66]. In the study comparing rosiglitazone and metformin, there was a significant increase in body mass index (BMI) within the rosiglitazone arm from baseline, and also the change between the rosiglitazone and metformin arm was statistically significant [58]. The studies finding no significant increase in SAT reported changes in body weight ranging from a loss of 3.0 kg to the gain of 3.8 kg in the thiazolidine arm [55, 56, 62, 65]. In contrast to variable effects seen on body weight with glitazones, all three studies having a metformin arm [58, 60, 63] reported a decrease in body weight from 1.2 to 2.2 kg in patients using metformin.

The effects of thiazolidinediones on body composition in HAART-treated patients contrast data from HIV-negative diabetic patients. In these patients, thiazolidinediones have increased body weight constantly by 2–4 kg after 16–26 weeks of therapy [12]. This increase has been attributed mainly to an increase in the fat mass and in some patients to edema [12]. The increase in total fat mass has been in the order of 1.5–4 kg after 3-4 months of rosiglitazone therapy [9, 68, 69],

Table 1: The basic characteristics of the studies with thiazolidinediones in HIV-infected, HAART-treated patients. HAART = highly active antiretroviral therapy, IR = insulin resistance, LA = lipoatrophy, OGTT = oral glucose tolerance test, LD = lipodystrophy.

Number of subjects	Study design	Study groups	Duration	Inclusion criteria	Reference
		Rosiglitazone studies			
8	Open label, uncontrolled	Rosiglitazone 8 mg/d	6–12 weeks	IR (defined by clamp)	Gelato et al. [54]
30	Randomized, double blind	Rosiglitazone 8 mg/d, or placebo	24 weeks	LA (clinical definition)	Sutinen et al. [55]
108	Randomized, double blind	Rosiglitazone 8 mg/d, or placebo	48 weeks	LA (limb fat% <20%, or limb fat% at least 10% less than truncal fat%)	Carr et al. [56]
28	Randomized, double blind	Rosiglitazone 4 mg/d, or placebo	3 months	LA (clinical definition) and IR (fasting insulin >15 μIU/ml, or 2 h insulin [OGTT] >75 μIU/ml)	Hadigan et al. [57]
39	Randomized, open label	Rosiglitazone 8 mg/d, or metformin 2 g/d	26 weeks	LA (clinical definition)	van Wijk et al. [58]
20	Open label, uncontrolled	Rosiglitazone 4 mg/d	24 weeks	No LD or IR requirements	Feldt et al. [59]
105	Randomized, double blind	Rosiglitazone 4 mg/d, or metformin 2 g/d, or rosiglitazone+metformin, or placebo	16 weeks	IR (fasting insulin >15 μIU/ml, or 2 h insulin [OGTT] >75 μIU/ml, or 2 h glucose [OGTT] >7.7 mmol/L and fasting insulin >10 μIU/ml) and self-reported changes in body fat (including increased waist-to-hip ratio or waist circumference)	Mulligan et al. [60]
37	Randomized, double blind	Rosiglitazone 8 mg/d, or placebo	6 months	Body mass index 19–24 kg/m^2, no requirements on LD or IR	Haider et al. [61]
96	Randomized, double blind	Rosiglitazone 4 mg/d, or placebo	24 weeks	LD (clinical definition)	Cavalcanti et al. [62]
90	Randomized, open label	Rosiglitazone 4 mg/d, or metformin 1 g/d, or no treatment	48 weeks	IR (impaired fasting glucose or impaired glucose tolerance [OGTT], with fasting insulin >20 μIU/ml)	Silič et al. [63]
		Pioglitazone studies			
11	Open label, uncontrolled	Pioglitazone 45 mg/d	6 months	LA (clinical definition)	Calmy et al. [64]
14	Randomized, double blind (2 × 2 factorial)	Pioglitazone 30–45 mg/d, or fenofibrate 200 mg/d, or pioglitazone + fenofibrate, or placebo	12 months	IR (impaired glucose tolerance [OGTT], or diabetes, or fasting insulin >20 μIU/ml) and dyslipidemia	Gavrila et al. [65]
130	Randomized, double blind	Pioglitazone 30 mg/d, or placebo	48 weeks	LA (clinical definition)	Slama et al. [66]
		Troglitazone study			
6	Open label, uncontrolled	Troglitazone 400 mg/d	3 months	LD and newly diagnosed diabetes	Walli et al. [67]

and it consists almost exclusively of the increase in the subcutaneous fat depot [7, 8, 69, 70]. Similar effects on body fat have also been described in nondiabetic patients treated with pioglitazone for insulin resistance [71] or nonalcoholic steatohepatitis [72].

Table 3 lists some confounding factors that possibly could explain the conflicting results of thiazolidinediones on SAT in different trials with HIV-infected, HAART-treated patients. The drug dose, study duration, inclusion criteria and baseline BMI appeared to be similar between studies showing a statistically significant increase versus those not showing a change in SAT. The prevalence of concomitant use of stavudine, the NRTI most strongly associated with fat loss, may explain some of the discrepancies, since the studies reporting an increase in SAT were those with least frequent use of stavudine [57, 58, 66]. Also in the study by Carr et al., after 24 weeks of treatment with rosiglitazone, there was a statistically almost significant increase in SAT in those patients not taking stavudine or zidovudine when compared to the placebo group (+480 g versus 190 g, $P = .06$), but this difference was not maintained at week 48 [56]. Similarly, in the study by Slama et al., patients not taking stavudine at baseline had a mean increase of limb fat mass of 450 g in the pioglitazone group versus 40 g increase in the placebo group ($P = .013$) [66]. Based on these data one can hypothesize that thiazolidinediones may have a fat-inducing

Table 2: Body composition data from thiazolidinedione studies which included a control arm and an objective measurement of body composition in HIV-infected, HAART-treated patients. HAART = highly active antiretroviral therapy, SAT = subcutaneous adipose tissue, NS = nonsignificant, MRI = magnetic resonance imaging, DEXA = dual-energy X-ray absorptiometry, CT = computed tomography, s.c. = subcutaneous, ND = not done, CI = confidence interval.

	N	Drug	Duration	Subcutaneous adipose tissue	Visceral adipose tissue	Body mass index (kg/m^2)	Reference
No change in SAT	30	Rosi versus placebo	24 weeks	MRI: NS	MRI: NS	NS	Sutinen et al. [55]
	108	Rosi versus placebo	48 weeks	DEXA limb fat: NS CT thigh: NS CT s.c. abdomen: NS	CT: NS	NS	Carr et al. [56]
	14	Pio versus feno versus pio + feno versus placebo	12 months	DEXA upper limb fat: NS DEXA lower limb fat: NS CT s.c. abdomen: NS	CT: NS	NS	Gavrila et al. [65]
	96	Rosi versus placebo	24 weeks	DEXA limb fat: NS DEXA arm fat: NS DEXA leg fat: NS	ND	NS	Cavalcanti et al. [62]
Increase in SAT	28	Rosi versus placebo	3 months	CT thigh (cm^2): rosi: + 2.3 versus pla −0.9, Δ rosi versus pla, $P = .002$ CT s.c. abdomen: NS DEXA leg: NS (rosi +50 g versus pla −80 g, Δ rosi versus pla $P = .08$)	CT: NS	NS	Hadigan et al. [57]
	105	Rosi versus metformin versus rosi + met versus placebo	16 weeks	DEXA leg fat (%): rosi: +4.8, *NS; met: −3.6, *NS; rosi + met: −0.5, *NS; pla: −8.3%, *NS Δ rosi vs pla $P = .03$, other groups versus pla, NS DEXA arm fat: NS DEXA limb fat: NS CT s.c. abdomen: NS	CT (%): rosi: 0.0, *NS; met: −0.6,*NS; rosi + met: −7.9, *NS; pla: −7.2, *NS Δ rosi versus pla, $P = .08$, other groups versus pla, NS	Body mass index ND, body weight (kg): rosi: 0.0, *NS; met: −2.0, $*P < .001$; rosi + met: −1.5, $*P < .01$; pla: −0.05, *NS Δ met versus pla, $P = .03$; Δ met + rosi versus pla, $P = .06$	Mulligan et al. [60]
	130	Pio versus placebo	48 weeks	DEXA limb fat (g): pio: +380 g; pla: +50 g Δ pio versus pla $P = .051$ CT s.c. abdomen: NS	CT: NS	Pio: +0.9; pla: +0.3 Δ pio versus pla $P = .07$	Slama et al. [66]
	39	Rosi versus metformin	26 weeks	CT s.c. abdomen (cm^2) rosi: +16, $*P < .05$; met: −11, $*P < .05$ Δ rosi versus met 27cm^2 (95% CI, 7 to 46)	CT (cm^2) rosi: −1, *NS; met: −25, $*P < .05$ Δ rosi versus met 24 cm^2 (95% CI, 6 to 51)	Rosi: +0.4, $*P < .05$; met: −0.4, $*P < .05$ Δ rosi versus met 0.7 (95% CI, 0.5 to 1.6)	van Wijk et al. [58]

∗ denotes significance within the study group compared to baseline value, Δ denotes comparison of the change between respective study groups.

effect in lipoatrophic SAT, but the ongoing stavudine (and zidovudine) treatment may negate this beneficial effect.

In addition to quantifying adipose tissue compartments, liver fat content was measured in one study [55]. Liver fat decreased with rosiglitazone and increased with placebo (−2.1% versus +2.1% in the rosiglitazone versus placebo, $P < .05$) [55]. Serum alanine aminotransferase (ALT) concentrations were reported in 5 rosiglitazone studies [55–58, 60]. In three of these studies, there was a statistically significant decrease in ALT concentration in the rosiglitazone arm compared either to the baseline value or to the placebo arm possibly suggesting a decrease in liver fat content [55, 56, 58].

Outside these trials, a single case report describes development of several dozen lipomas in a patient with HAART-associated lipoatrophy during 3-month therapy with rosiglitazone. After rosiglitazone was discontinued, all but 5 lipomas resolved completely [73].

4.2. Effects on Insulin Resistance and Blood Lipids. The effects of thiazolidinediones on glycemic indeces in the ten comparative studies reporting data on glucose and insulin

Table 3: Comparison of the baseline characteristics of the thiazolidinedione arms of the studies showing versus not showing an increase in subcutaneous fat mass in HIV-infected HAART-treated patients. HAART = highly active antiretroviral therapy, SAT = subcutaneous adipose tissue, NR = not reported.

	Drug (dose/d)	Duration	Inclusion criteria	BMI (kg/m^2)	% taking stavudine	Reference
No change in SAT	Rosi 8 mg	24 weeks	Lipoatrophy	24	67	Sutinen et al. [55]
	Rosi 8 mg	48 weeks	Lipoatrophy	23	49	Carr et al. [56]
	Pio 30–45 mg	12 months	Insulin resistance and dyslipidemia	26	NR	Gavrila et al. [65]
	Rosi 4 mg	24 weeks	Lipodystrophy	25	NR	Cavalcanti et al. [62]
Increase in SAT	Rosi 4 mg	3 months	Lipoatrophy and insulin resistance	26	44	Hadigan et al. [57]
	Rosi 4 mg	16 weeks	Lipodystrophy and insulin resistance	Body weight 80 kg	NR	Mulligan et al. [60]
	Pio 30 mg	48 weeks	Lipoatrophy	22	25	Slama et al. [66]
	Rosi 8 mg	26 weeks	Lipoatrophy	24	21	van Wijk et al. [58]

are shown in Table 4. In contrast to the very modest effects on SAT described above, eight [55–58, 60, 61, 63, 65] out of 10 studies showed improvements in insulin resistance in the thiazolidinedione arm when compared to the baseline value or to the placebo arm. One study reported a significant positive correlation between the change in fasting insulin concentration and the change in liver fat content [55]. Only two studies did not show significant improvements in insulin sensitivity [62, 66]. Of note, insulin resistance was an inclusion criteria in only four [57, 60, 63, 65] out of these 10 studies.

Since none of the comparative studies recruited patients with type 2 diabetes, it is difficult to compare these effects on glycemic indeces in HAART-treated patients to those in HIV-negative diabetic patients treated with glitazones. The average decrease in haemoglobin A_{1c} has been 1–1.5% in non-HIV patients with type 2 diabetes treated with glitazones [12].

The effects of thiazolidinediones on blood lipids in the nine comparative studies reporting data on cholesterol and triglycerides are shown in Table 5. Five [55–58, 60] out of seven studies with rosiglitazone reported a statistically significant increase in total cholesterol concentration in the rosiglitazone arm when compared either to the baseline value or to the comparative arm. The absolute increases in total cholesterol concentration in the rosiglitazone arms varied from 0.4 to 1.4 mmol/L. Neither of the two pioglitazone studies reported significant changes in total cholesterol concentrations [65, 66]. HDL (high-density lipoprotein) cholesterol concentration increased statistically significantly in both studies with pioglitazone (increases of 0.09 and 0.15 mmol/L) [65, 66], but decreased significantly in two out of the seven rosiglitazone studies with absolute decreases of 0.1 and 0.15 mmol/L [58, 60]. LDL (low-density lipoprotein) cholesterol was measured in five rosiglitazone studies [56–58, 60, 62]. Four of these studies reported significant increases in LDL cholesterol concentrations when compared to the baseline value (absolute increases between 0.2–0.8 mmol/L) or to the comparative arm [56–58, 60]. The increase of 1.7 mmol/L in LDL cholesterol was statistically almost significant in one of the two pioglitazone studies [65]. Statistically significant increases in triglyceride concentrations were reported in three out of seven rosiglitazone studies (versus baseline or versus the comparative arm) [55, 56, 58]. The absolute increases were between 0.5–3.0 mmol/l. There were no significant changes in triglyceride concentrations in the two pioglitazone studies. A proatherogenic effect of rosiglitazone on blood lipids was further described by Hadigan et al. [74]. Rosiglitazone treatment increased significantly the concentration of small dense LDL cholesterol, and decreased the concentration of large HDL cholesterol and also of HDL particle size [74]. In contrast, pioglitazone treatment was associated with an increase in the LDL particle size (from 19.9 at baseline to 20.6 nm at 12 months, $P = .06$) [65].

The results of these studies with HAART-treated patients suggest pioglitazone to have a more favorable lipid profile than rosiglitazone as has been observed in patients with type 2 diabetes [12]. A striking difference in HAART-treated patients relative to HIV-negative diabetic patients was the significant increase in triglyceride concentration by 1.5 to 2.3 mmol/L in the rosiglitazone arm in some studies [55, 56]. One may hypothesize that the increases in serum triglycerides were possibly aggravated by the high prevalence of stavudine use in these studies. The ongoing stavudine treatment may have prevented the storage of circulating lipids within the adipocytes. In the study by Sutinen et al., one patient had to discontinue rosiglitazone treatment due to serum triglyceride increase up to 32.5 mmol/L [55] and another patient using rosiglitazone in the study by Cavalcanti et al. discontinued due to abnormal lipid values [62]. Carr et al. reported grade 3-4 increases in triglyceride concentrations in 57% of the participants in the rosiglitazone arm compared to 36% in the placebo arm [56]. The large study by Cavalcanti et al. did not report any deleterious effects on blood lipid concentrations by rosiglitazone, but in the same study 15% of patients in the rosiglitazone arm started lipid-lowering

Table 4: The effects of thiazolidinediones on glycemic indeces in controlled trials with HIV infected, HAART-treated patients. HAART = highly active antiretroviral therapy, HOMA = homeostasis model assessment (fasting insulin [μIU/ml] × fasting glucose [mmol/L]/22.5), OGTT = oral glucose tolerance test, NS = nonsignificant, NR = not reported, AUC = area under the curve.

N	Drug	Duration	Insulin (μIU/ml)	HOMA	OGTT	Fasting glucose (mmol/L)	Reference
30	Rosi versus placebo	24 weeks	Rosi: −3.3, $^*P < .05$; pla: +6.7, *NS Δ rosi versus pla $P < .05$	NR	NR	NS	Sutinen et al. [55]
108	Rosi versus placebo	48 weeks	Rosi: −3.5; pla: +0.7 Δ rosi versus pla $P = .02$	Rosi: −1.0; pla: +0.04 Δ rosi versus pla $P = .03$	2 h glucose: NS 2 h insulin: rosi −13.6; pla +3.9 Δ rosi versus pla $P = .09$	NS	Carr et al. [56]
14	Pio versus feno versus pio + feno versus placebo	12 months	NS	Pio: −3.8, $^*P < .05$; pla: −1.3, *NS	NR	NS	Gavrila et al. [65]
96	Rosi versus placebo	24 weeks	NS	NS	NS	NS	Cavalcanti et al. [62]
28	Rosi versus placebo	3 months	NS	NR	2 h glucose: rosi: −0.3; pla: +0.1 Δ rosi versus pla $P = .06$ 2h insulin AUC: rosi: −2.3; pla: +1.8 Δ rosi versus pla $P = .003$	NS	Hadigan et al. [57]
105	Rosi versus metformin versus rosi + met versus placebo	16 weeks	Rosi: −4, $^*P = .08$; met: −2, $^*P = .07$	NR	Insulin AUC: rosi: −26, $^*P = .012$; met: −11, $^*P = .06$; rosi + met: −18, $^*P = .002$ Δ rosi + met versus placebo $P = .03$; Δ rosi versus pla $P = .07$	NS	Mulligan et al. [60]
130	Pio versus placebo	48 weeks	NS	NS	NS	NS	Slama et al. [66]
37	Rosi versus placebo	6 months	NS	Rosi: −0.1, *NS; pla: +1.3, $^*P < .05$ At 6 months: rosi versus pla $P < .05$	NR	NS	Haider et al. [61]
90	Rosi versus metformin versus No-treatment	48 weeks	Rosi: −19.3, $^*P < .001$; met: −11.1, $^*P < .001$; No-Tx: +0.7, *NS At 48 weeks: rosi versus No-Tx $P < .001$; met versus No-Tx $P < .001$; met versus rosi $P < .001$	Rosi: −7.3, $^*P < .001$; met: −6.2, $^*P < .001$; No-Tx: +0.3, *NS At 48 weeks: rosi versus No-Tx $P < .001$; met versus No-Tx $P < .001$; rosi versus met $P < .001$	NR	Rosi: −1.9, $^*P < .001$; met: −2.2, $^*P < .001$; No-Tx: 0.0, *NS At 48 weeks: Rosi versus No-Tx $P < .001$; met versus No-Tx $P < .001$; rosi versus met $P = .015$	Silič et al. [63]

Table 4: Continued.

N	Drug	Duration	Insulin (μIU/ml)	HOMA	OGTT	Fasting glucose (mmol/L)	Reference
39	Rosi versus metformin	26	NR	NR	Glucose AUC: rosi: -1.9, $^*P = .04$; met: -1.1, $^*P = .05$ Δ rosi versus met NS Insulin AUC: rosi: -37, $^*P = .01$; met -33, $^*P = .01$ Δ rosi versus met NS	NR	van Wijk et al. [58]

* denotes significance within the study group compared to baseline value, Δ denotes comparison of the change between respective study groups.

therapy during the study compared to 5% in the placebo arm [62]. In addition to these prospective clinical trials, a small retrospective study reported effects of fenofibrate alone versus fenofibrate in combination with rosiglitazone in HIV-infected patients [75]. When fenofibrate was given alone, triglyceride concentrations decreased by 27% and HDL cholesterol increased by 19%. In contrast, when fenofibrate was combined with rosiglitazone, triglycerides increased by 48% and HDL cholesterol decreased by 33% [75].

Three of the rosiglitazone studies [57, 60, 76] also reported the effects of rosiglitazone on free fatty acid (FFA) concentrations. In two of these studies, a statistically significant decrease in FFA concentration was found in the rosiglitazone arm when compared to baseline value or to placebo [57, 76].

Serum adiponectin concentration was measured in six rosiglitazone studies [56–58, 60, 61, 76]. All these studies showed a statistically significant increase of 0.8–4.1 μg/ml in the rosiglitazone arms. One study found an inverse correlation between the change in adiponectin concentration and the change in fasting insulin concentration and liver fat content [76].

Additional findings from these clinical trials with HAART-treated patients include either a decline [77] or no change [55, 78] in leptin concentration with rosiglitazone. Circulating concentrations of inflammatory markers (TNFα, C-reactive protein, or IL-6) did not change in any study reporting these measurements [78–80]. Plasma concentrations of PAI-1 (plasminogen activator inhibitor-1) and tPA (tissue plasminogen activator) were reported to either decline [79] or remain unchanged [78] with rosiglitazone therapy. Plasma resistin concentration decreased with rosiglitazone in one study [78]. Rosiglitazone decreased systolic blood pressure, but had a nonsignificant effect on flow-mediated arterial dilatation compared to placebo in one study [77].

4.3. Thiazolidinedione-Induced Gene Expression in SAT in HAART-Treated Patients. Two studies have evaluated the effects of rosiglitazone on gene expression in SAT in HAART-treated patients [76, 81]. Sutinen et al. reported statistically significant increases in the expression of adiponectin and PPARγ coactivator 1 (PGC-1), and a decrease in IL-6 expression with rosiglitazone treatment [76]. In addition, there was a significant increase in PPARγ expression in the rosiglitazone arm relative to the placebo arm [76]. Mallon et al. studied gene expression in SAT two and 48 weeks after rosiglitazone or placebo treatment, and compared patients taking tNRTIs at baseline to those without tNRTI treatment [81]. After two weeks, only those randomized to rosiglitazone in the no-tNRTI group experienced a significant rise in PPARγ and PGC-1 expression. At 48 weeks, PPARγ expression was increased in the no-tNRTI groups when compared to tNRTI groups, but there was no difference between the rosiglitazone and placebo arms [81].

The increase in the expression of adiponectin, PPARγ (albeit limited), and that of PGC-1 are consistent with data from type 2 diabetic patients treated with glitazones [82, 83]. There are, however, also some differences in these two patient populations. In non-HIV patients, the expression of lipoprotein lipase (LPL) [82, 84] and adipocyte fatty acid binding protein (aP2) [83] increased with glitazone treatment. The expression of these genes remained unchanged in HAART-treated patients [76].

Taken together, it seems plausible that thiazolidinedione treatment had a functional effect in lipoatrophic SAT by increasing the production of adiponectin and decreasing IL-6 expression. Both of these changes may have been involved in the improvement of whole body insulin sensitivity. The correlation between the change in adiponectin concentration and the change in liver fat content implies a possibility for adiponectin to have mediated this beneficial effect on liver fat [76]. These functional changes in adipose tissue occurred despite the lack of a significant increase in fat mass. One may also hypothesize that the blunted increase in PPARγ expression, especially in patients receiving tNRTI therapy, as well as the lack of an increase in LPL and aP2 expression may all have contributed to the nonsignificant effect on SAT mass and to high-serum triglyceride concentration in these patients.

4.4. Safety. Since in vitro and animal models, as well as clinical studies clearly indicate that thiazolidinediones correct endothelial dysfunction, suppress chronic inflammatory processes, reduce fatty streak formation, and enhance plaque stabilization and regression [85], one would expect favorable effects on cardiovascular endpoints also in human studies.

Table 5: The effects of thiazolidinediones on blood lipids in comparative studies with HIV infected, HAART-treated patients. HDL = high-density lipoprotein, LDL = low-density lipoprotein, NS = nonsignificant, NR = not reported, CI = confidence interval.

N	Drug	Duration	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)	Triglycerides (mmol/L)	Reference
30	Rosi versus placebo	24 weeks	Rosi: +1.4, $^*P < .01$; pla: 0.0, *NS Δ rosi versus pla NS	NS	NR	NS At 12 weeks: rosi: +3.0, $^*P < .05$; pla: NS	Sutinen et al. [55]
108	Rosi versus placebo	48 weeks	Rosi: +0.9; pla: 0.0 Δ Rosi versus pla $P < .001$	NS	Rosi: +0.8; pla: +0.4 Δ rosi versus pla $P = .04$	Rosi: +1.5; pla: +1.3 Δ rosi versus pla $P = .04$	Carr et al. [56]
14	Pio versus feno versus pio + feno versus placebo	12 months	NS	pio: +0.15, *NS; pla: −0.20, *NS Δ pio versus pla $P = .01$	Pio: +1.7, $^*P = .07$; pla: −0.6, *NS	NS	Gavrila et al. [65]
96	Rosi versus placebo	24 weeks	NS	NS	NS	NS	Cavalcanti et al. [62]
28	Rosi versus placebo	3 months	Rosi: +0.6; pla: −0.4 Δ rosi versus pla $P = .007$	NS	Rosi: +0.4; pla: −0.4 Δ rosi versus pla $P = .01$	NS	Hadigan et al [57]
105	Rosi versus metformin versus rosi + met versus placebo	16 weeks	Rosi: +0.4, $^*P < .05$; all other groups *NS	Rosi: −0.1, $^*P < .001$; all other groups *NS Δ rosi versus pla $P = .005$; Δ rosi versus rosi + met $P = .006$	Rosi: +0.2, $^*P < .05$; all other groups *NS Δ rosi versus pla $P = .048$	NS	Mulligan et al. [60]
130	Pio versus placebo	48 weeks	NS	Pio: +0.09; pla: −0.08 Δ pio versus pla $P = .005$	NS	NS	Slama et al. [66]
37	Rosi versus placebo	6 months	NS	NR	NR	NS	Haider et al. [61]
39	Rosi versus metformin	26 weeks	Rosi: +0.4, *NS; met: −0.4, $^*P < .05$ Δ rosi versus met 0.8 (95% CI, 0.3 to 1.3)	Rosi: −0.15, $^*P < .05$; met: +0.01, *NS Δ rosi versus met 0.16 (95% CI, −0.35 to −0.02)	Rosi: +0.2, *NS: met: −0.4, $^*P < .05$ Δ rosi versus met 0.6 (95% CI, 0.2 to 1.1)	Rosi: +0.5, * versus <0.05; met: −0.6, $^*P < .05$ Δ rosi versus met 1.1 (95% CI, 0.4 to 2.6)	van Wijk et al. [58]

* denotes significance within the study group compared to baseline value, Δ denotes comparison of the change between respective study groups.

In contrast, the meta analysis by Nissen and Wolski [86] demonstrated that rosiglitazone as compared to the control group significantly increased (and not decreased) the odds ratio for myocardial infarction (OR 1.43; 95% confidence interval [CI], 1.03 to 1.98; $P = .03$), and the odds ratio for death from cardiovascular causes (OR 1.64; 95% CI, 0.98 to 2.74; $P = .06$). A more recent meta analysis confirmed the increased risk of myocardial infarction (RR 1.42; 95% CI, 1.06 to 1.91; $P = .02$) and heart failure (RR 2.09; 95% CI, 1.52 to 2.88; $P < .001$) with rosiglitazone, but found no significant increase in the risk of cardiovascular mortality (RR 0.90; 95% CI, 0.63 to 1.26; $P = .53$) [87]. In contrast to findings with rosiglitazone, a meta-analysis of pioglitazone found a decreased hazard ratio for death, myocardial infarction, or stroke in patients receiving pioglitazone when compared to those receiving control therapy (HR 0.82; 95% CI, 0.72 to 0.94; $P = .005$) [88]. However, the hazard ratio for serious heart failure was increased in patients receiving pioglitazone versus the control patients (HR, 1.41; 95% CI, 1.14 to 1.76; $P = .002$) [88]. Whether these deleterious effects of glitazones on cardiovascular morbidity would be diminished in patients receiving HAART, since patients are usually younger, fewer have diabetes, and so forth, or enhanced since HAART by itself increases risk for myocardial infarction [4], remains to be studied. None of the studies with HAART-treated patients using glitazones so far have reported any significant cardiovascular events.

In general, both rosiglitazone and pioglitazone were well tolerated in all trials with HAART-treated patients. However, the total number of HAART-treated patients taking rosiglitazone and pioglitazone was only 281 and 82, respectively, and none of the studies had followup beyond

one year. Furthermore, it is important to keep in mind that due to exclusion criteria of these trials there are basically no data on glitazones in HIV-infected patients with significantly increased liver function tests, high creatinine or triglyceride concentrations, or low hemoglobin at baseline; all these laboratory abnormalities are relatively common in HAART-treated patients.

Regarding the known adverse effect profile of thiazolidinediones in non-HIV infected patients, it was reassuring that no cases of clinically significant oedema, heart failure or other cardiovascular events were reported. A decrease in haemoglobin concentration is another known side effect with all glitazones, which is not explained by hemodilution but possibly caused by mild suppressive effect on bone marrow [89, 90]. This might be of significance in HAART-treated patients, since both HIV per se and antiretroviral agents may cause bone marrow suppression [91]. A statistically, but not clinically significant decrease in haemoglobin concentration in the rosiglitazone arm was reported in one study, possibly also reflecting good adherence to study medication [55]. A single case with a decrease of haemoglobin concentration to less than 110 g/L was reported by Hadigan et al. [57]. Given the concerns for severe liver toxicity induced by troglitazone [92], liver function tests were carefully monitored in these patients receiving polypharmacy. A single participant in a pioglitazone trial discontinued the study due to an increase in liver function tests >3 times upper limit of normal [65]. In contrast, three studies observed significant decreases in ALT concentrations either within the rosiglitazone arm or when compared to placebo [55, 56, 58]. Adverse effects on blood lipids were already discussed earlier; two patients had to discontinue rosiglitazone due to abnormal lipid values [55, 62].

Harmful effects of thiazolidinediones on bone metabolism have recently been discussed in patients with type 2 diabetes. A recent analysis of the data from five glitazone studies suggests that treatment with thiazolidinediones, primarily rosiglitazone, contributes to bone loss [93]. This effect appears to be most prominent in postmenopausal women [93]. The effect on bone density may have special relevance in HAART-treated patients, since both HIV-infection as such and also antiretroviral therapy have been associated with decreased bone mineral density [94]. None of the studies using glitazones in HAART-treated patients have reported effects on bone density.

Potential for drug-drug interactions must always be considered, when new medications are combined with HAART. Most PIs and nonnucleoside reverse transcriptase inhibitors (NNRTIs) are not only metabolized by CYP450 3A4 but are also either inhibitors (PIs, ritonavir in particular) or inducers (NNRTIs) of the same enzyme and to lesser extent of other isoforms of CYP450 [95]. Both rosiglitazone and pioglitazone are predominantly metabolized by CYP450 2C8 (http://www.emea.europa.eu/). Rosiglitazone is not an inducer of any tested human CYP450 isoforms, but has shown moderate inhibition of 2C8 and low inhibition of 2C9 (http://www.emea.europa.eu/). There is no in vitro evidence that pioglitazone would either inhibit or induce any of the human CYP450 isoforms (http://www.emea.europa.eu/). Interaction studies have not shown clinically significant interactions with rosiglitazone and substrates for CYP450 3A4. These interactions are not expected with pioglitazone either (http://www.emea.europa.eu/).

There are currently very limited pharmacological data on the concomitant use of thiazolidinediones and antiretroviral drugs. Data from a limited number of patients by Oette et al. suggest that rosiglitazone could be safely administered together with either lopinavir or efavirenz [96]. Rosiglitazone, however, seemed to decrease nevirapine concentrations and the authors recommend to monitor nevirapine serum concentrations if these drugs are used concomitantly [96]. Serum PI concentrations were measured in one study with rosiglitazone [55] and both PI and NNRTI concentrations were measured in one pioglitazone study [66]. Neither study observed any significant change in the serum concentrations of these antiretroviral drugs during the study period. None of the studies reported any statistically significant changes in either HIV viral load or CD4 count within the glitazone arm or between different study arms. Nevertheless, six patients in the pioglitazone arm versus two in the placebo arm ($P = .1$) experienced viral breakthrough (>400 copies/ml) during a pioglitazone trial [66]. It is not reported if these patients were possibly taking nevirapine-based HAART (potential interaction between nevirapine and rosiglitazone, see above). Interestingly, in vitro both PPARγ (rosiglitazone, ciglitazone, troglitazone) and PPARα (fenofibrate) agonists have been shown to inhibit HIV replication [97, 98].

5. Conclusions Regarding the Role of Thiazolidinediones in the Treatment of HAART-Associated Metabolic Complications

The available evidence does not support the use of thiazolidinediones for HAART-associated lipoatrophy although they may have beneficial effects in subgroups of patients such as those that do not receive concomitant tNRTI therapy. The lack of effect may at least partially be explained by the decreased expression of PPARγ which has been demonstrated not only in SAT of lipoatrophic HAART-treated patients [41, 42], but also in SAT of healthy volunteers after only a 2-week exposure to NRTIs including a thymidine analog [40]. However, if a thiazolidinedione is used for lipoatrophy, pioglitazone should perhaps be preferred because of its more favorable effects on serum lipids. Currently, the best treatment option for HAART-associated lipoatrophy is to replace tNRTIs by abacavir or tenofovir [27–29] or possibly to avoid use of NRTI altogether [99]. However, if this causal treatment of lipoatrophy is not feasible due to HIV resistance profile or intolerance to other antiretroviral agents, one may consider using uridine, which showed a significant increase in SAT in a small randomized, placebo-controlled trial in lipoatrophic patients continuing tNRTI-based HAART [100]. Pravastatin has also been shown to increase SAT in HAART-treated patients in a single small trial [101]. However, patients in this trial were not recruited for lipoatrophy but hyperlipidemia and hence the effect of pravastatin in severely lipoatrophic patients remains to be

studied. Finally and most importantly, one should aim to prevent lipoatrophy altogether by avoiding the use of tNRTIs in the primary combinations of HAART as has recently been suggested [102].

Glitazones, pioglitazone in particular, could however, be used for the treatment of HAART-associated diabetes, especially in patients with reduced amount of SAT. The consistent data on the beneficial effects on insulin sensitivity in HAART-treated patients support this, albeit none of these studies recruited diabetic patients. The direct evidence of the benefits in diabetic HAART-treated patients is therefore still lacking. When using glitazones for diabetes in HAART-treated patients, one must keep in mind the potential risk for heart failure associated with both glitazones and increased risk for myocardial infarction with rosiglitazone. Nevertheless, glitazones might be the preferred choice, since metformin has been associated with further loss of SAT in HAART-treated lipodystrophic patients [58, 103] and there are no clinical data at all on other oral antidiabetic agents in this patient population.

Finally, the potential therapeutic role of thiazolidinediones on liver-related morbidity in HAART-treated patients should be evaluated. Patients who are infected with both hepatitis C (HCV) and HIV seem to have higher liver fat content than those having HCV monoinfection [104, 105] although some controversy exists [106]. Among HIV-infected patients without chronic HCV infection, those with lipodystrophy have increased liver fat content when compared to age and BMI-matched HIV-negative subjects or HAART-treated nonlipodystrophic patients [48]. It, therefore, appears that both prevalent coinfection with HCV and HAART-induced metabolic complications put HIV-infected patients at increased risk for liver steatosis.

In HIV-negative patients with nonalcohol hepatic steatosis, 6-month treatment with pioglitazone decreased liver fat content, increased hepatic insulin sensitivity, and improved histologic findings with regards to liver steatosis, ballooning necrosis and inflammation [107]. In the study by Sutinen et al., treatment with rosiglitazone decreased significantly liver fat content when compared to placebo in HAART-treated patients, although patients were not recruited for increased liver fat but for the presence of lipoatrophy [55]. Two additional trials reported significant decreases in liver function tests in the glitazone arms [56, 58]. Although promising, it remains unknown whether glitazones would also improve inflammatory changes in the liver in these patients, since no biopsy data are so far available.

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Review Article

PPAR and Liver Injury in HIV-Infected Patients

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Due to the introduction of active HIV antiretroviral treatment, AIDS-related morbidity and mortality have markedly decreased and liver diseases are now a major cause of morbidity and mortality in HIV-infected patients. Chronic liver injury encompasses a wide spectrum of diseases due to HCV and HBV coinfection, drug-related toxicity, and NASH. HIV-infected patients who are receiving treatment present with a high prevalence of metabolic complications and lipodystrophy. Those patients are at high risk of nonalcoholic fatty liver disease, the liver feature of the metabolic syndrome. This review will focus on (1) the liver injuries in HIV-infected patients; (2) both the current experimental and human data regarding PPAR and liver diseases; (3) the interactions between HIV and PPAR; (4) the potential use of PPAR agonists for the management of HIV-related liver diseases.

1. Introduction

The efficacy of highly active antiretroviral treatment (HAART) has resulted in a considerable improvement in the life expectancy of HIV-infected patients. As a consequence, liver diseases have emerged as a key issue in the management of HIV-infected patients, and they are now a major cause of morbidity and mortality [1]. Chronic liver injuries in HIV-infected patients encompasses a wide spectrum of liver diseases mostly consisting of coinfection with hepatitis B and C viruses, excessive alcohol consumption, drug-related toxicity, and more recently identified fatty liver disease. Over the last few years, a set of metabolic alterations has also emerged in HIV-infected patients treated with HAART, including the lipodystrophy syndrome, which is closely associated with insulin resistance. The syndrome typically associates visceral fat hypertrophy, limb lipoatrophy, dyslipidemia, and insulin-resistance, thereby resembling a caricature of the metabolic syndrome. Those patients are at high risk of nonalcoholic fatty liver disease, the liver feature of the metabolic syndrome.

Peroxisome proliferator-activated receptors (PPAR) constitute a family of nuclear receptors, some of them being expressed in the liver. They are involved in glucose and lipid metabolism, in insulin sensitivity [2] and in many other physiological processes, particularly inflammation fibrogenesis [3] and carcinogenesis. Experimental and human data have portrayed PPAR as potential molecular players in chronic liver diseases. Interactions between some HIV proteins and PPAR reinforce the potential role of these nuclear receptors in the development of HIV-associated liver injuries. In addition, the development of new nonhepatotoxic ligands made it possible to use PPAR agonists as new therapeutic targets in liver diseases. For these reasons, studies in the field of PPAR are of high concern, not only to basic researchers but also to clinicians for the management of liver diseases. This review will focus on (1) the liver injuries in HIV-infected patients; (2) both the current experimental and human data regarding PPAR and liver diseases; (3) the interactions between HIV and PPAR; (4) the potential use of PPAR agonists for the management of HIV-related liver diseases.

2. Liver Injuries in HIV-Infected Patients: Which Disease Has Which Prevalence?

2.1. Coinfection with Hepatitis B and C. Due to their common routes of transmission, chronic hepatitis B and C

viruses are often associated with HIV infection and are present in 10% and 30% of HIV-infected patients, respectively.

It has been clearly established that HIV infection significantly changes the natural history of HBV and HCV by enhancing their viremia levels [4]. HIV also worsens the histological course of HBV and HCV by increasing the severity of fibrosis and accelerating the risk of cirrhosis and hepatocellular carcinoma [5, 6].

2.2. Excessive Alcohol Consumption and Drug-Related Toxicity. Excessive alcohol consumption has been observed in one-third of HIV-infected patients [5, 7]. In addition, alcoholic hepatitis is more frequent and more severe, suggesting a specific sensitivity to alcohol in HIV-infected patients [8].

All classes of antiretroviral drug have been associated with liver toxicity. Drug-related toxicity is more frequent in patients treated with some nonnucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs) and in patients with preexisting liver injuries due to alcohol, HBV, or HCV [9]. Some nucleoside reverse transcriptase inhibitors (NRTIs) are able to induce microvesicular steatosis with lactic acidosis, potentially leading to hepatic failure [10].

2.3. Nonalcoholic Fatty Liver Disease: An Underestimated Cause of Liver Injury in HIV-Monoinfected Patients. Steatosis, the main feature of fatty liver, is defined as abnormal fat accumulation in hepatocytes related to metabolic abnormalities and insulin resistance, toxic injuries (alcohol, drugs), or viral infections, in particular with HCV. Nonalcoholic fatty liver disease (NAFLD,) also called metabolic liver disease, has become the most common cause of chronic liver injury in HIV-uninfected patients [11], with an estimated prevalence in the general population ranging from 14% to 31% [12–14]. One-third of those patients have histologic signs of fibrosis and necroinflammation, indicating the presence of nonalcoholic steatohepatitis (NASH) [11]. Such liver injuries are likely to lead to cirrhosis, liver, failure and hepatocellular carcinoma. Insulin resistance plays a central role in the development of liver steatosis, but the precise molecular mechanisms leading to steatohepatitis and fibrosis remain undefined.

Lipodystrophy is a frequent long-term side effect of antiretroviral therapy, being reported in 40 to 50% of HIV-infected patients receiving HAART [15]. As a result of insulin resistance and/or visceral fat hypertrophy, HIV patients with HAART-related lipodystrophy are considered at risk of NAFLD. In addition, NAFLD has been recently demonstrated to be an early marker of cardiovascular disease, which has also become an emergent issue in HIV-treated patients with HAART over the last decade [16]. These data underscore the importance of assessing the presence of NAFLD in HIV-infected patients with antiretroviral treatment who are particularly at risk of metabolic disorders.

In HIV-monoinfected patients with HAART-related lipodystrophy, few data are available on the true incidence of NAFLD and most of the studies used indirect tools for the diagnosis of steatosis, whereas the benchmark is histologic assessment of liver fat content through the use of biopsy [13]. Indirect evidence of fatty liver has been suggested in HIV patients with lipodystrophy by demonstration of a significant correlation between alanine aminotransferase (ALT) serum levels and insulin resistance [17]. However, it is thought that liver injury is poorly correlated with liver enzyme serum levels in patients with NAFLD [18]. By using proton spectroscopy, Sutinen et al. found increased liver fat related to the severity of insulin resistance in 25 HIV patients with lipodystrophy [19]. Moreno-Torres et al. found intrahepatic triglycerides deposits in 17 of 29 HAART recipients, 4 of whom (13.8%) had liver fat contents compatible with the diagnosis of liver steatosis [20]. More recently, Hadigan et al. identified hepatic steatosis in 42% of their patients [21]. Mohammed et al. demonstrated that HIV-infected patients with NAFLD had lower body mass indices than HIV-seronegative patients [22], suggesting that NAFLD may be associated with factors other than those classically observed in obesity, including direct HIV infection and antiretroviral therapy. Among 225 HIV-infected patients enrolled in a recent study, 83 (36.9%) were diagnosed with NAFLD using tomodensitometry [23]. Two studies used liver biopsy for the diagnosis of unexplained chronic transaminase elevation and NASH was observed in over half of HIV-infected patients in the absence of other causes of chronic liver diseases, with a close association with insulin resistance [24, 25]. Guaraldi et al. [23] showed that NAFLD was associated with lipodystrophy and waist size, suggesting a relationship between adipose tissue, insulin resistance, and fatty liver. In light of these studies, NAFLD may be considered a common liver disease in HIV-monoinfected patients. HAART-related insulin resistance and lipodystrophy likely play a major etiologic role but their mechanisms remain to be determined. Some PPARs are expressed in the liver, the central organ in the balance of glucose and lipid metabolism, and as a result they could be involved in metabolic-related liver injuries.

2.4. PPAR and Liver Injury. PPAR are transcription factors belonging to the family of nuclear receptors [26]. When activated by ligand binding, PPAR are able to activate promoters and to modulate the expression of target genes. They exist in three isoforms, PPARα, PPARβ/δ, and PPARγ. This review will focus on PPARα and PPARγ, as the functions of PPARβ/δ in glucose and lipid metabolism are less established.

PPARα is highly expressed in the liver, and its functions are better documented than those of PPARγ which are synthesized at lower levels. PPARα is the most abundant nuclear receptor in the liver and is mainly expressed by the hepatocytes [27] though also by the stellate cells, biliary cells, endothelial cells, and Kupffer cells [3, 28–30].

These nuclear receptors are involved in glucose and lipid metabolism and also in nonmetabolic functions including inflammation, tissue repair, cell proliferation, differentiation, carcinogenesis, and fibrosis [3, 31, 32]. Several studies mostly conducted in vitro and in animal models have suggested that these nuclear receptors could be involved in the development of liver injuries including steatosis,

inflammatory injuries, and fibrosis. There is very little data available on PPAR expression and functions in HIV-infected livers. As no data have been published in HIV/HCV or HIV/HBV coinfected patients, this review will focus on HIV-monoinfected patients, particularly those with metabolic disorders.

2.5. PPAR and Steatosis. Steatosis is closely associated with insulin resistance and impairment of glucose and lipid homeostasis. Activation of PPARα directly regulates genes involved in fatty acid uptake by increasing the expression of the fatty acid transport protein (FATP) and the fatty acid translocase (FAT) [33]. PPARα also promotes fatty acid oxidation in the peroxisomes, as well as in the mitochondria, reducing the fatty acid pool available to the liver for triglyceride synthesis [34]. In addition, PPARα activation decreases triglyceride levels by enhancing lipoprotein lipase (LPL) expression [35] and by inhibiting apolipoprotein C-III in the liver [36]. Activators of PPARα include diverse chemicals such as endogenous molecules (fatty acids/steroids) and xenobiotics (fibrate lipid-lowering drugs) [37–39].

The potential role of PPARα in the development of liver steatosis is mostly based on experimental data conducted in murine lacking PPARα. PPAR$\alpha-/-$ mice display obesity and serious liver steatosis without excessive food intake and PPARα activation by fibrates reverses insulin resistance and reduces weight [40]. There is a little data available in the human liver on PPARα liver expression and its role in liver diseases. In chronic hepatitis C with steatosis, reduced levels of PPARα mRNA have been demonstrated [41]. In HIV-infected patients with HAART-related lipodystrophy and NAFLD, Lemoine et al. did not observe changes in the liver expression of PPARα mRNA compared to NASH patients without HIV and to normal liver controls [24]. No additional data have been published on human liver disease to date.

PPARγ is a transcription factor that regulates the gene expression involved in lipid metabolism and in adipocyte differentiation [42]. PPARγ is highly expressed in the adipose tissue under two isoforms (PPARγ1 and PPARγ2) that are generated by the same gene through altering splicing [43]. Whereas the functions of PPARγ are well established in the adipose tissue, they remain hypothetical in the liver.

PPARγ activation plays a role in various physiological and pathological events, including adipocyte differentiation, insulin sensitivity and regulation of lipid metabolism. The natural ligands of PPARγ remain unknown. There is evidence that small lipophilic compounds, such as polyunsaturated fatty acids and fatty acid derivatives (eiocosanoids), bind and activate PPARγ [44]. Thiazolinediones are synthetic ligands used as antidiabetic drugs, as they have been reported to enhance insulin sensitivity [45]. In animal as well as in human livers, PPARγ levels are much lower than those of PPARα. Several murine models of obesity and diabetes, including ob/ob, A-ZIP, aP2/DTA, and KKAy have been shown to develop fatty livers that express high levels of liver PPARγ mRNA [46, 47]. In addition, mice lacking PPAR$\alpha-/-$, which develop liver steatosis, also expressed high hepatic PPARγ mRNA [40]. These results suggest a potential etiologic role of PPARγ in fatty liver disease. Few studies have been conducted in human livers. PPARγ is expressed at low levels and its expression is decreased in HCV-monoinfected patients [41] and HIV-infected patients [24].

2.6. PPAR and Inflammation. Many experimental data are in favor of anti-inflammatory activities of PPAR. PPARα and PPARγ have been shown to downregulate inflammatory response genes by inhibiting the STAT, AP-1, and NF-κB transcriptional pathways in human hepatocytes and monocytes [31, 48, 49]. In primary human hepatocytes, Delerive et al. suggested the anti-inflammatory effects of fibrates, which are PPARα agonists, by increasing IκB expression and antagonizing NF-κB activation [49]. By regulating antioxidant enzyme activities, such as catalase, PPARα agonists may also reduce the oxidative stress [50].

These anti-inflammatory effects have also been validated in vivo. Acute hepatitis induced in PPAR$\alpha-/-$ or in PPAR$\gamma+/-$ animals is particularly severe with exacerbation of liver inflammatory injury [51, 52]. One of the suggested mechanisms is the ability of PPARα activation to inhibit the nuclear translocation of NF-κB [49]. In livers of PPARα wild-type mice, treatment with fibrates resulted in IκB induction, whereas no increase was observed in PPARα null mice [49].

Not only in alcoholic models of rats and mice but also in hepatocytes treated with ethanol and acetaldehyde, Lee et al. recently showed a downregulation of PPARα mRNA [53]. In humans treated with fenofibrate, a decreased level of blood cytokines (TNFα, IL-6, IFNγ) was found [54]. No relationship has been demonstrated between the extent of PPAR expression and liver inflammation in human livers.

2.7. PPAR and Liver Fibrosis. PPARγ has been shown to be expressed in quiescent hepatic stellate cells (HSCs) and its expression and activity are decreased during HSC activation in rats in vitro and in vivo [3, 28, 55]. The treatment of rat HSC with PPARγ ligands prevents their activation in vitro. In addition, the in vivo treatment of rats with synthetic PPARγ agonists prevents fibrosis induced by toxic or bile duct ligation [3, 56]. However these effects remain controversial considering the results of contradictory studies [57, 58]. A human study has suggested an antifibrogenic effect induced by a synthetic agonist (pioglitazone) [59]. In HIV-infected patients with NAFLD, it has been shown that hepatic PPARγ expression was significantly decreased compared to controls with normal liver histology and was inversely related to fibrosis [24]. In light of those results, PPARγ agonists appear to be compelling drugs for the prevention of liver injury related to insulin resistance. PPARα could be involved in fibrogenesis through adiponectin activation, as this adipokine could have antifibrogenic properties. Adiponectin has been shown to enhance PPARα activation through the AMP-kinase pathway in animal models of cardiac fibrosis [60]. In HIV patients, a hepatic decrease of PPARγ mRNA has been shown to be related to the presence and the severity of liver fibrosis [24].

3. Interactions between HIV and PPAR

3.1. Effects of HIV Infection on PPAR Activity. The possibility that HIV-1 infection could influence PPARγ activity has been established through in vitro studies.

In a recent study, HIV-1 viral protein r (Vpr) was shown to suppress the transcriptional activity of PPARγ in mouse adipocytes. HIV could directly alter insulin sensitivity by suppressing PPARγ activity [61]. The HIV Nef protein involved in viral replication has been shown to suppress PPARγ expression [62].

3.2. Effects of PPAR on HIV. The ability of nuclear receptors to interact with the long-terminal of HIV-1 was recognized several years ago [63]. RXR and PPARα were seen to bind a region between -356 to -320 in the long terminal repeat. In addition, PPARα agonists such as clofibrate have been shown to activate HIV-1 transcription [64]. Fenofibrate, another PPARα agonist also has properties that inhibit HIV replication and TNFα production in alveolar macrophages of HIV-infected patients [65].

In HIV-infected macrophages, natural and synthetic PPARγ agonists inhibit HIV replication [60, 66]. More recently, it has been reported that HIV-1 replication was inhibited by ciglitazone, a PPARγ agonist, in a dose-dependent manner in acutely infected human monocyte-derived macrophages and in latently infected and viral entry-independent U1 cells, suggesting an effect at the level of HIV-1 gene expression [66]. Cotransfection of PPARγ wild-type vectors and treatment with PPARγ agonists inhibited HIV-1 promoter activity in U937 cells, and activation of PPARγ also decreased HIV-1 mRNA stability following actinomycin D treatment [67]. Similar results were observed by Skolnik et al. In this study, PPARα and PPARγ agonists decreased HIV-1 replication in peripheral blood monocular cells infected with HIV-1, in chronically infected monoblastoid cells and in alveolar macrophages from HIV-1 patients and controls [65]. The mechanisms of action by which PPAR acts on HIV-1 remain unknown. A direct effect on specific regions on HIV-1 and indirect effects via NF-κB has been proposed [68].

3.3. PPAR Agonists as a New Therapeutic Path. Management of liver damage in HIV-infected patients requires increasing attention in regard to the growing liver-related mortality in these patients. The cofactors that are likely to worsen liver injuries need to be eradicated when possible: viral infection, alcohol, drug toxicity, overweight, and metabolic abnormalities. Lipodystrophy syndrome associated with insulin resistance plays a role in the development of fatty liver disease. By enhancing insulin sensitivity, PPARγ agonists or thiazolinediones are used for the treatment of type 2 diabetes. In addition, benefits of these drugs have been suggested from experimental data conducted in vitro with PI-treated adipocytes [69]. As a consequence, several studies have been performed to evaluate the benefits of thiazolinediones in lipodystrophic HIV-infected patients. A recent study has evaluated the benefits of a treatment with pioglitazone (30 mg/d) compared to placebo among 130 HIV-infected patients with lipoatrophy. There was an increased amount of limb fat, but no significant difference in visceral abdominal fat, and the lipid profile (increased high-density lipoprotein) was improved [70]. Recent studies have suggested positive effects of thiazolinediones on steatosis and possibly fibrosis in NAFLD patients without HIV infection [59, 71]. Assessment of the benefits of pioglitazone on insulin resistance and liver injuries in HCV-infected patients is in progress (http://clinicaltrials.gov/show/NCT001891633). The main obstacle to using thiazolinediones is their cardiovascular side-effects, particularly in patients with increased cardiovascular risks, such as the HIV-infected patients treated with HAART. Such benefits need to be assessed in monoinfected and coinfected HIV-infected patients with fatty liver disease.

Fibrates are synthetic ligands of PPARα, and they have been used for years in the treatment of lipid disorders. In HIV-infected patients, fibrates are efficient and safe in diet-resistant hyperlipidemia [72]. In a small cohort of uninfected patients with NAFLD, fenofibrate has been demonstrated to improve metabolic syndrome and liver tests without significant effects on liver histology [73].

The recently discovered endocannabinoid system contributes to the physiological regulation of food intake and glucose and lipid balance and is overactive in obese subjects. Two types of receptors have been described, CB1 and CB2, and are expressed in numerous tissues including the liver. An antagonist of CB1, called rimonabant, has been shown to induce weight loss and improve metabolic disorders in animals and humans [74]. In addition, this treatment could have antifibrogenic effects [75]. Interactions between the endocannabinoid system and PPARγ ligands have been established, opening a new path for the management of metabolic-related liver injuries [76].

4. Conclusions

Liver diseases in HIV-infected patients both with and without viral hepatitis coinfection have received increasing attention in recent years. Metabolic disorders including insulin resistance, lipodystrophy, and NAFLD are long-term side effects that are frequently observed in HIV-infected patients receiving HAART.

Since the discovery of PPAR in 1990, significant progress has been made in understanding their effects and their potential roles in human disease and in metabolism alterations in particular. Animal and human experimental data have provided strong evidence for establishing a pathophysiological link between PPAR and NAFLD. Although the molecular mechanisms remain unclearly defined, direct reciprocal interactions between the virus itself and PPAR reinforce the hypothesis for the role of these transcription factors in the control of liver injury, particularly in steatosis, inflammation, and fibrosis. The existence of natural and synthetic ligands of PPAR opens new therapeutic options for the management of metabolic disturbances in HIV-infected patients with HAART-associated lipodystrophy, often associated with liver diseases.

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Review Article

Peroxisome Proliferator-Activated Receptors and Hepatitis C Virus-Induced Insulin Resistance

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Insulin resistance and type 2 diabetes are associated with hepatitis C virus infection. A wealth of clinical and experimental data suggests that the virus is directly interfering with the insulin signalling in hepatocytes. In the case of at least one viral genotype (the type 3a), insulin resistance seems to be directly mediated by the downregulation of the peroxisome proliferator-activated receptor γ. Whether and how this interaction may be manipulated pharmacologically, in order to improve the responsiveness to antivirals of insulin resistant chronic hepatitis C, patients remain to be fully explored.

1. Introduction

Hepatitis C virus (HCV) infection is associated with an increased risk of developing glucose intolerance and diabetes. This is in part due to a direct interference of HCV with the insulin signalling pathway. The mechanisms involved seem to be HCV genotype-specific, and this suggests that HCV may profit from the insulin resistant phenotype to establish and/or maintain a persistent infection. Since the peroxisome proliferator-activated receptors (PPARs) are nuclear factors involved—among others—in the regulation of glucose homeostasis, the relationship between HCV replication and protein expression and PPARs has been the focus of some recent studies. However, the data available so far are quite scanty and concern only the HCV genotype 3a. In fact, although most viral genotypes seem to activate members of the suppressors of cytokine signalling (SOCS) family in order to inhibit insulin signalling, in the case of genotype 3a, some in vitro observations are consistent with a downregulation of the PPARγ. If confirmed, these observations may be relevant to the treatment of chronic hepatitis C, since insulin resistance is a factor of poor response to antivirals. This article will briefly review the role of PPARs in insulin resistance, the interactions between HCV and PPARs, and their clinical significance.

2. PPARs in Insulin Resistance and Diabetes

PPARs are the established targets of several classes of drugs used in the management of the metabolic syndrome, like the fibrates and the thiazolidindiones. A new class of pan-PPAR agonists, the glitazars, is presently under investigation. This article will describe the role and significance of PPARα and γ in glucose metabolism, since the few data from HCV models have addressed the deregulation of only these two factors. No data are currently available about the involvement of PPARδ in the HCV-associated insulin resistance.

PPARα is principally expressed in tissues exhibiting high rates of β-oxidation such as liver, kidney, heart, and muscle, and can be activated by dietary fatty acids and eicosanoids or by specific drugs such as fibrates. Activation of PPARα results in increased fatty acid β-oxidation in the liver [1], increased expression of HDL apolipoproteins Apo A-I and Apo A-II [2], inhibited expression of Apo C-III [3], increased lipoprotein lipase activity [4], and, therefore, increased VLDL and remnants clearance. As to insulin resistance, PPARα seems to improve—indirectly—insulin sensitivity by increasing hepatic catabolism of lipids, and thus reducing lipid supply to skeletal muscle [5].

PPARγ is expressed at very high levels in adipose tissue, and much less in the liver and other organs. Apart from its

natural ligands, that is, fatty acids, it is activated by drugs of the class of thiazolidindiones such as rosiglitazone and pioglitazone. Hyperglycaemia and hyperlipidaemia in obese and diabetic animals are improved by pioglitazone through reduction of both peripheral and hepatic insulin resistance [6]. In the animal model, treatment with PPARγ agonists lowers plasma level of free fatty acids and insulin, and increase the phosphorylation level of Akt at both threonine 308 and serine 473 in the liver and both the adipose and muscle tissues [7]. This, in turn, is correlated with tyrosine phosphorylation of insulin receptor β subunit and insulin receptor substrate-1, and serine phosphorylation of glycogen synthase kinase-3α/β [7]. Additional hepatic effects include increased insulin sensitivity via G-protein subunits downregulation, leading to reduced glucose production by approximately 30%, accompanied by a significant increase of glucose-induced insulin suppression in β-cells [8].

In addition to the direct effects on factors involved in lipid and glucose homeostasis, PPARs may have insulin sensitizing effects via their anti-inflammatory activity. PPARγ reduces the expression of activator protein-1 and nuclear factor-κB, reduces specific subsets of lipopolysaccharide and interferon (IFN) target genes in macrophages [9, 10], and reduces tissue expression of tumor necrosis factor-α (TNFα), IFN-γ, C-reactive protein, and induction of other proinflammatory cytokines, including hepatocytes [11].

Thus, treatment with PPARs agonists results in improved insulin sensitivity via diverse mechanisms, both direct and indirect, and both at the level of the liver and at the level of extrahepatic tissues.

3. Hepatitis C Virus Infection and Insulin Resistance

Insulin resistance and type 2 diabetes are common complications of all liver diseases, especially at the advanced stage. In the case of HCV infection, however, both clinical and experimental observations suggest that HCV may directly interfere with glucose homeostasis. Cross-sectional, case-control, and longitudinal studies—performed in both large unselected cohorts and in patients with liver or kidney transplantation—have suggested that type 2 diabetes may be more prevalent in chronic hepatitis C patients, especially if aged more than 40 and if other major risk factors of glucose intolerance are present [12]. The confounding effect of liver disease stage can be eliminated if studies are conducted in patients with little or no liver fibrosis, and if—instead of looking for cases with overt diabetes—glucose homeostasis is assessed as level of insulin sensitivity. Hui et al. [13] found that 121 HCV-infected patients with stage 0 or 1 liver fibrosis had higher levels of HOMA score compared with 137 healthy volunteers matched by sex, body mass index, and waist-to-hip ratio. This work proved that HCV may induce insulin resistance at early stages of liver diseases, and provided, in addition, the first evidence that this effect may be due to genotype-specific sequences.

Some recent work suggests a trend between HCV replicative level and level of insulin resistance [14]. The low-level correlation may be due to the fact that the global level of insulin resistance is likely to depend on the contribution from the adipose tissue and the muscle, two extrahepatic compartments that are not infected by HCV.

The effect of antiviral therapy is another classical way to prove an association between infection and disease. Romero-Gómez et al. have shown that both the level of insulin resistance [15] and the incidence of ex novo glucose intolerance over time [16] are reduced after successful therapy in chronic hepatitis C patients, whereas no improvement is observed in nonresponders. However, independent confirmation of these observations is warranted.

The association between HCV and insulin resistance has noteworthy consequences, clinically and conceptually. From the clinical standpoint, insulin resistance accelerates fibrogenesis [13, 17–19] and impairs response to IFN-α-based antiviral therapy [15, 20, 21]. In addition, HCV infection is an interesting example of insulin resistance not necessarily associated with other components of the metabolic syndrome, thus providing the framework for longitudinal studies on specific risk assessment. Last, but not least, the question arises what may be the advantage for HCV to increase insulin resistance; it is apparent that all HCV genotypes studied so far induce insulin resistance, albeit to a different extent [13, 14], suggesting some evolutionary constraints aimed at maintaining the insulin-resistant phenotype despite the viral genome sequence divergence over time.

Since HCV appears to directly interfere with the glucose homeostasis, several studies have tried to analyze in detail the potential interactions between viral products and insulin signalling. Experimental data suggest a direct interference of HCV with the insulin signalling cascade via proteasome degradation of the insulin receptor substrates -1 and -2 [22, 23]. HCV may also impair insulin signalling transduction indirectly, that is, through increased levels of proinflammatory cytokines such as TNF-α [24, 25]. The interference with the insulin signalling seems to proceed via HCV genotype specific mechanisms (see below) and insulin resistance levels vary according to the infecting HCV genotype, although all genotypes induce insulin resistance. Interestingly, intracellular factors dysregulated by HCV and responsible for the insulin resistant phenotype may play promiscuous effects as they are also involved in regulating IFN-α signalling. These factors include some members of the suppressor of cytokine signalling (SOCS) family [22, 23, 26] and the protein phosphatase 2A [27]. Thus, modulating the levels and/or the activity of these factors may not only reverse hepatic insulin resistance but also help establishing the IFN-α-induced antiviral state at the site of HCV replication. This is one of the reasons for trying to restore insulin sensitivity in chronic hepatitis C patients failing to respond to therapy (see below).

4. HCV Infection and PPARs Expression

Very little data is available concerning the interaction between HCV products and PPARs expression (Figure 1). PPARγ mRNA level was measured in Huh-7 hepatoma

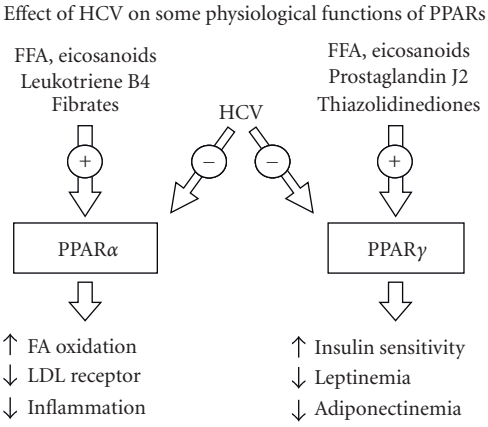

Figure 1: Reported effects of HCV on some of the relevant physiological functions of PPARα and PPARγ. The inhibitory effect on PPARγ has been described only for the genotype 3a. For further explanations, please refer to the text.

cells transfected with the HCV genotype 3 core encoding sequence, and was found significantly decreased compared to untransfected cells. No changes were observed in cells transfected with the core protein 1b [28]. Incidentally, PPARα was undetectable, even in untransfected cells, precluding further evaluations of an interaction with HCV, if any.

Cells transfected with the genotype 3a had both increased content of triglycerides [29] and reduced levels of IRS-1, leading to insulin resistance, as measured by reduced Akt phosphorylation following incubation with insulin [23]. The role of PPARγ in HCV 3a-associated insulin resistance in vitro was further assessed by treating transfected cells with a PPARγ agonist, rosiglitazone. Both IRS-1 protein and insulin-stimulated Akt phosphorylation levels increased after treatment with rosiglitazone of cells transfected with the core protein 3a. The recovery of the IRS-1 protein expression and Akt phosphorylation levels was, however, rather modest, that is, about 20% and 26%, respectively [23]. Additional recovery of both IRS-1 level and Akt activation was obtained by inhibiting SOCS-7 upregulation induced by the same viral genotype in this model [23]. Although cells expressing the HCV core 1b had reduced IRS-1 content and insulin resistance, the effect seemed to be mediated by both SOCS- and PPARγ-independent mechanisms. Thus, based on this isolated observation, it seems that PPARγ downregulation may be responsible, at least in part, for the insulin resistance observed in vitro upon expression of HCV genotype 3a core protein expression.

By analyzing total RNA extracted from the liver of chronic hepatitis C patients, de Gottardi et al. found that the transcription level of both PPARα and PPARγ was decreased in genotype 3 infection as compared to genotype 1 [28]. When patients were stratified according to the presence of steatosis, PPARα and γ mRNA were reduced only in steatotic livers from patients infected with genotype 1. In those with genotype 3, both PPAR mRNA levels were always low, independently of the presence of steatosis. In this study, the two groups of patients with genotype 3 and 1 were comparable as to gender distribution, age, BMI, liver disease activity, and fibrosis stage. However, in this study, a direct correlation between PPARs levels and IR was not evaluated.

The level of PPARα mRNA has been measured in the liver of chronic hepatitis C patients also in another study [30], and found to be profoundly suppressed (about 85% compared to control livers). The inhibition of PPARα was paralleled by a significant decrease of the carnitine palmitoyl acyl-CoA transferase 1 mRNA, a key enzyme in the mitochondrial β-oxidation of fatty acids, and confirmed in an in vitro model expressing the HCV core protein [30]. These authors focused their discussion on the role of PPARα as anti-inflammatory mediator, and on the consequences of its suppression in the pathogenesis of hepatitis C. It is important to remember that the impaired transcriptional activity of PPARα associated with HCV infection may indirectly worsen IR via increased expression of TNF-α; thus, the role of inflammation in the pathogenesis of HCV-associated IR should not be overlooked [31]. On the contrary, it is unlikely that HCV-induced steatosis, due to decreased expression of PPARα and several other mechanisms [32], may aggravate insulin resistance, since the latter has been convincingly shown to precede steatosis, and not vice versa [33].

5. Perspectives for Treatment

As said above, insulin resistance reduces the rate of response to antivirals in chronic hepatitis C [15, 20, 21]. A sustained virological response (SVR) was observed in 23 of 70 (32.8%) of patients with genotype 1 and insulin resistance (i.e., with a HOMA > 2) versus 26 of 43 (60.5%) of genotype 1 patients without insulin resistance ($P = .007$) [15]. These findings were independently confirmed [20] and extended to nonresponders with genotypes 2 and 3 [21]. Thus, it was suggested that insulin resistance should be corrected in patients with chronic hepatitis C not responding to IFN-α-based treatment, in order to improve response upon retreatment. The modalities of this intervention, however, have not been established. In addition, the optimal HOMA-IR score to be reached has not been identified.

A recent prospective, multicenter study aimed at investigating the efficacy and safety of the insulin-sensitizer pioglitazone, 15 mg QD, added to the pegylated IFN-α_{2a}, 180 μg QW/ribavirin, 1 000–1 200 mg QD combination therapy in chronic hepatitis C patients who were previously nonresponders to a pegylated IFN-α/ribavirin combination without the insulin sensitizer [34]. All patients had a baseline HOMA > 2 as additional inclusion criterion, because this was the threshold discriminating responders from nonresponders in previous works [16, 21]. Diabetic patients were however excluded. Unfortunately, none of the first five patients enrolled into the trial had a satisfactory virological response after 12 weeks of retreatment, despite the fact that in at least three of them the insulin resistance score improved, and thus the study was prematurely terminated [34].

Additional data on this topic have been presented at the 2008 annual meeting of the American Association for the Study of Liver Diseases. In an interim analysis of a clinical trial, 30 mg QD of pioglitazone were given for four weeks

as monotherapy and then added for the first four weeks of a standard therapy of treatment-naïve, nondiabetic chronic hepatitis C patients. The authors showed that the triple regimen containing pioglitazone increased significantly the rate of virological response after 4 weeks of therapy compared to pegylated IFN-α/ribavirin combination [35]. Long-term data are eagerly awaited. However, in another randomized, double-blind, placebo-controlled study, adding pioglitazone 30 mg QD simultaneously to the standard of care (i.e., without a preceding administration as monotherapy) clearly increased the on-treatment virological response, but failed to increase the sustained virological response after the end of treatment [36]. Additional studies evaluating different schedules are clearly warranted. This approach, however, should also take into consideration the known effects of PPAR agonists on serum lipid profile and their potential consequences on the HCV life cycle. HCV circulates bound to lipoproteins in complexes known as lipoviroparticles [37]. As a result, HCV entry into hepatocytes appears to be mediated and facilitated, among others, by the LDL receptor [38]. In keeping with this, at least two recent studies have suggested that baseline LDL-associated cholesterol levels may affect response to antiviral therapy [39, 40]. In fact, higher levels of cholesterol- and ApoB-rich lipoproteins could facilitate viral clearance by impeding HCV interaction with cell surface receptors. Thus, drugs like thiazolidinediones that modify the circulating lipoprotein profile may have unexpected—and potentially unwanted—effects on the HCV life cycle. On the other hand, in addition to lipid-lowering effects, PPARα agonists have been shown to decrease also the expression of the LDL receptor in experimental models [41]. This may offset the untoward effect on lipoproteins by impairing HCV entry into target cells. Although highly speculative, these hypotheses deserve being appropriately evaluated in clinical trials.

Modulating the levels and/or the activity of intracellular factors involved in HCV-induced insulin resistance may not only reverse hepatic insulin resistance but also help establishing the IFN-α-induced antiviral state at the very site of HCV replication.

However, specific inhibitors of SOCS family members and of the protein phosphatase 2A are either not suitable for in vivo administration or toxic. Alternatively, increasing insulin sensitivity may be achieved by modulating serum levels of specific cytokines, such as TNF-α, associated with insulin resistance [24, 25], but the administration of anti-TNF-α antibodies to chronic hepatitis C patients may be risky [42]. Insulin sensitizers may also inhibit HCV replication by decreasing serum free fatty acids flow to hepatocytes; saturated and monounsaturated free fatty acids have indeed been shown to stimulate HCV replication in an in vitro model [43].

Thus, although increasing insulin sensitivity may be a rational option in chronic hepatitis C patients not responding to current combination therapy, more work is warranted to identify the appropriate treatment schedule. It is not clear whether the best approach would be using a thiazolidindiones, activating PPARs, or a biguanide such as metformin, whose mechanism of action is specifically directed on the hepatic AMP-activated protein kinase [44]. Another major issue concerns the treatment schedule. It is unclear whether one should start the antiviral retreatment together with the insulin sensitizer or only once the HOMA-IR score has decreased to a level predicting a sufficient SVR rate [16]. As an alternative, higher dosages of thiazolidindiones (as compared to the above quoted study) or metformin may be used. Finally, insulin sensitizing therapy might need to be tailored according to HCV genotype, and PPARs agonists should probably be considered only in insulin-resistant patients with HCV genotype 3a [23]. Finally, interactions of insulin sensitizing agents with other drugs, most notably those taken for psychiatric comorbidities, should be considered. In the quoted study, a paradoxical increase of the HOMA score was observed in two patients during therapy, and both patients were taking drugs for their psychiatric comorbidities that may also alter insulin sensitivity, that is, zopiclone and olanzapine. Thus, the beneficial effect of pioglitazone may be nullified in patients taking drugs for psychiatric indications. In addition to the potential effects of these and other drugs, also the acute administration of IFN-α may induce some degree of insulin resistance in both healthy subjects [45] and chronic hepatitis C patients [46]. The interactions among all these drugs are, however, speculative, but require further studies, in view of the frequent use of antidepressants in chronic hepatitis C patients. Thus, further clinical trials aiming at reducing the insulin resistance in chronic hepatitis C via different pharmacological interventions are warranted.

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Review Article

Peroxisome Proliferator-Activated Receptors in HCV-Related Infection

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Recommended by Jacqueline Capeau

The topic of peroxisome proliferator-activated receptors has been developed in the field of hepatology allowing envisaging therapeutic strategies for the most frequent chronic liver diseases such as chronic infection with hepatitis C virus (HCV). PPARs contribute to wide physiological processes within the liver such as lipid/glucid metabolisms, inflammatory response, cell differentiation, and cell cycle. In vitro experiments and animal studies showed that PPARα discloses anti-inflammatory property, and PPARγ discloses anti-inflammatory, antifibrogenic, and antiproliferative properties in the liver. Experimental and human studies showed impaired PPARs expression and function during HCV infection. The available nonhepatotoxic agonists of PPARs may constitute a progress in the therapeutic management of patients chronically infected with HCV.

1. Introduction

Chronic infection with the hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide, affecting about 3% of the population [1]. The natural history of HCV infection is characterized by a high rate of progression to chronic hepatitis leading, in at least 20% of cases, to cirrhosis and ultimately to hepatocellular carcinoma [2, 3]. The precise mechanisms underlying HCV-related liver injury are not well understood but involve a cell-mediated immune response with lymphocytic infiltration leading to a chronic inflammatory response and progressive scale fibrosis which are dependent on proinflammatory and fibrogenic mediators. HCV infection is also characterized by disruption of lipid and glucid metabolisms leading to hepatocyte fat accumulation (also called hepatic steatosis) and an increase risk of diabetes [4–7]. These latter are under dependence, at least in part, of peroxisome proliferator-activated receptors functions.

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily and require heterodimerization with receptor X for retinoids (RXR) in order to function [8]. The first PPAR identified was murine PPARα [9]. Since its description in 1990, a family of homologous receptors has been identified in various species. The PPAR family includes three subtypes: PPARα (NR1C1), PPARβ (PPARδ or NR1C2), and PPARγ (NR1C3) [10]. PPARα/γ, together with their obligate partner RXR, are the three main nuclear receptors expressed in the liver. These receptors contribute to the great diversity of physiological processes in the liver, such as control of lipid and glucid metabolisms, inflammatory responses, and cellular differentiation and proliferation. PPAR activation has been associated with anti-inflammatory and antifibrotic functions in the liver. In light of their multiple activities, PPARs quickly became considered as therapeutic targets of the most widespread human metabolic diseases (obesity, diabetes, and atherosclerosis). The development of new

nonhepatotoxic ligands made it possible to use PPARs as new therapeutic targets in hepatology. For these reasons, recent discoveries in the field of PPARs are of intense interest not only to fundamental researchers but to clinicians as well. This review will first describe the main functions of PPARs within the liver. Then the interactions between PPARs and HCV will be developed to provide a precise picture of the potential role of PPARs in HCV pathophysiology and therapy.

2. Control of Lipid and Lipoprotein Metabolism

PPARα is strongly involved in the control of lipid and lipoprotein metabolisms. PPAR$\alpha^{-/-}$ mice display minimal accumulation of triglycerides in the liver under fed conditions, but manifest an exaggerated steatotic response to fasting [11]. Moreover, PPAR$\alpha^{-/-}$ mice fed a high-fat diet showed massive accumulation of lipids in the liver, highlighting its crucial role in lipid metabolism [12]. Intracellular fatty acid (FA) concentrations are controlled, in part, by regulation of the FA import and export system. PPARs control the uptake of FA in liver; for example, PPAR agonists control expression of the FA transport protein (FATP-1) responsible for FA transport across the cell membrane, FA translocase (FAT/CD36), and the hepatic cytosolic FA binding protein (L-FABP) involved in FA trafficking [13]. The absence of induction of FATP-1 and FAT/CD36 mRNA in liver by PPARα activators has been well demonstrated in PPAR$\alpha^{-/-}$ mice. PPARα and -γ also prevent the efflux of FA by promoting their activation into fatty acyl CoA thioesters by the acyl-CoA synthetase ACS [14, 15]. In addition, PPARα stimulates activated FA catabolism by the peroxisomal, microsomal, and mitochondrial β-oxidation systems. It regulates the expression of genes coding for enzymes such as acyl-CoA oxidase (AOX), the first and main enzyme in the classic peroxisomal β-oxidation system. It also activates the transcription of genes encoding for classical microsomal β-oxidation system cytochrome P450 CYP4A (A1 and A3) isoforms, and stimulates activated FA catabolism by mitochondrial β-oxidation. It is generally accepted that oxidation of long-chain FA is regulated at the level of the liver carnitine palmitoyl-transferase I (LCPT1), whose product catalyzes the initial step in long-chain FA import into mitochondria. PPARα has been demonstrated to affect FA import into mitochondria by upregulating expression of the LCPT1 gene [14]. Concerning PPARγ, treatment of HepG2 with troglitazone, a synthetic agonist, specifically increased recycling by peroxisomal β-oxidation of C18 to C16 FAs, and the interconversion of long-chain FAs was associated with reduced de novo lipogenesis. It is conceivable that these effects of troglitazone on oxidation, interconversion, and synthesis-saturated FA play a major role in cellular energy metabolism, membrane lipid composition and turnover [16].

It has been convincingly established that PPARα activation increases plasma HDL cholesterol and enhances reverse cholesterol transport via the induction of hepatic apolipoprotein A-I and apolipoprotein A-II expression in human liver. Nevertheless, PPARα activators affect HDL metabolism in an opposite manner in rodents and humans. While fibrate treatment of rats lowers plasma HDL, an increase is generally observed in humans; such an increase in HDL plasma levels is related, at least in part, to changes in apoAI and apoA-II gene expression in liver. ApoA-I and apoA-II gene transcription is upregulated in humans and repressed in rodents by PPARα activators. Treatment of human primary hepatocytes with fibrates increases mRNA levels and secretion of apoA-I. Moreover, in patients with familial combined hyperlipoproteinemia, ciprofibrate administration enhances production of apoA-I [17].

3. Anti-Inflammatory and Immunomodulatory Properties of PPAR in the Liver

PPARα exert catabolic function through peroxisomal, microsomal, and mitochondrial β-oxydation pathways, and therefore allows the use of FA by hepatocytes as energetic substrate. Thus, it degrades several lipid inflammatory mediators (prostaglandins, leukotriens) using these metabolic pathways [18, 19]. There are numerous experimental and clinical evidences in favor of anti-inflammatory activity of PPAR in the liver. PPARα and PPARγ were shown to negatively interfere with the nuclear factor (NF)-κB, signal transducers and activators of transcription (STAT), and activating protein 1 (AP-1) signalling pathways in primary hepatocyte cultures and monocytes/macrophages [20, 21]. Delerive et al. showed that fibrates, which are the main synthetic agonists of PPARα induced the expression of IκBα protein in primary human hepatocytes, whereas neither IκB kinase activity nor the degradation rate of IκBα was affected. The consequence is that the downstream inflammatory responses genes such as IL-2, IL-6, IL-8, TNFα, and metalloproteases are inhibited [20]. PPARα can also control hepatic inflammation by regulating hemostatic factors and acute-phase response proteins in hepatocytes [22].

T-lymphocytes, natural killer cells, and monocytes/macrophages express significant amount of PPARα and $\gamma$when activated, and they inhibit the production of inflammatory and immunomodulatory activity cytokines [23–28]. In lymphocytes, immunomodulatory activity is supported by a decrease in nuclear factor of activated T-cells (NFAT) activity which regulate IL-2 promoter [23, 29]. PPARγ inhibit the proliferation of T-lymphocytes and the production of IFNγ, TNFα, and IL-2 [30].

Animal studies confirmed the role of PPARα and PPARγ in inflammation control showing that PPAR$\alpha^{-/-}$ mice display a prolonged response to inflammation induced by arachidonic acid or zymosan [31–33]. Interestingly, PPAR$\alpha^{-/-}$ and PPAR$\gamma^{+/-}$ mice displayed an exacerbated sensibility to hepatitis in different models of liver injury [34, 35].

4. Antifibrogenic Activities of PPAR in the Liver

Hepatic stellate cells (HSCs) are the main cells responsible for liver modulation, by producing the protein of the extracellular matrix [36, 37]. In response to liver injury, HSC changes from a quiescent to an activated phenotype. This activation process includes a phenotypic change to a myofibroblast-like cell, an increased proliferation rate, loss of retinoid stores, and increased production of extracellular matrix proteins, chemokines, cytokines, and contractility. Interestingly, HSC activation is associated with impaired expression of PPARγ and with a decrease in binding to the peroxisome proliferator response element (PPRE) in vivo, whereas NFκB and AP-1 binding is increased. HSC activation is reversed by treatment with PPARγ agonists in vitro, suggesting antifibrogenic activity [38, 39]. PPARγ activation dose-dependently inhibited HSC proliferation and chemotaxis induced by platelet-derived growth factor. This effect was independent of changes in postreceptor signalling or expression of c-fos and c-myc, and was associated with inhibition of cell progression beyond the G1 phase. PPARγ activation also resulted in complete inhibition of the expression of monocyte chemotactic protein (MCP1), a potent chemoattractant for monocytes and T-lymphocytes [40]. All things considered, PPARγ activation in vitro resulted in decreases in HSC proliferation, migration, and chemokine expression, three pivotal actions relevant to the process of liver wound healing and fibrogenesis. A study performed in a model of liver fibrosis induced by dimethylnitrosamine or carbon tetrachloride showed that oral administration of pioglitazone or rosiglitazone reduces hepatic extracellular matrix deposition and HSC activation [41]. PPARα ligands may also have rescue effects on hepatic fibrosis. Using thioacetamide models of liver cirrhosis, animals treated with a diet containing one of the two PPARα ligands, Wy-14 643 (WY) or fenofibrate, displayed reduced hepatic fibrosis. PPARα ligands had an antifibrotic action in the thioacetamine model of liver cirrhosis, probably due to an antioxidant effect of enhanced catalase expression and activity in the liver [42].

5. Interaction between HCV and PPARs

Liver inflammation, hepatocyte fat accumulation, and diabetes are three pivotal hallmarks in the natural history of chronic HCV infection which are at least in part controlled by PPARs. Thus, it was tempting for several teams to investigate whether PPARα/γ may be impaired and/or nonfunctional, and might therefore play a role in the physiopathology of HCV infection.

Numerous studies have demonstrated that PPARα/γ were physiologically highly expressed by parenchymal hepatic cells, where they play a pivotal anti-inflammatory and metabolic role. Several teams have demonstrated, using in vitroand ex vivo approaches, a decrease expression and functional activity of PPARα in hepatocytes during HCV infection, which may contribute to the pathogenesis of the disease. (1) During chronic HCV infection in humans [43, 44], (2) in an experimental model of transgenic mouse [4], and (3) in transfected hepatocytes expressing the capsid protein of HCV [4, 43, 44]. These abnormalities may be the consequence of viral capsid inhibiting transcriptional activity of genes involved in lipid metabolism of infected host cell [4, 43, 44].

In the same line, De Gottardi et al. showed that (1) PPARγ expression was significantly lower in genotype 3 compared with genotype 1 HCV infection and (2) steatosis was associated to decreased levels of PPARγ in genotype 1 HCV infection. In this study, there was no significant relationship between PPARs mRNA levels and liver activity or fibrosis. The presences of steatosis and hepatitis C virus genotype 3 were both associated with a significant downregulation of PPARs [44].

The ways in which impaired expression of PPARα/γ contribute to the pathogenesis of HCV hepatitis are numerous. As previously described, PPARα activators play a regulatory role in the inflammatory response at the hepatic level by inhibiting cytokines and acute-phase inflammatory protein production by negatively interfering with NF-κB and AP-1 pathways. Clinical evidence for the anti-inflammatory role of PPARα in the liver was provided by the characterization of PPAR$\alpha^{-/-}$ mice which were more susceptible to various hepatotoxic compounds and had delayed liver regeneration compared to their wild-type littermates. A recent study also demonstrated that activation of PPARα limits the expression of the TH1 cytokine profile and TNFα production by human CD4-positive T-cells, a cytokine pattern known to substantially contribute to HCV pathogenesis.

Preliminary human data reinforce interest in this topic. Two pilot studies suggested therapeutic effects of bezafibrate, an agonist of PPAR$\alpha/\beta/\gamma$, during chronic HCV infection. In the first open trial, 30 patients unresponsive to interferon monotherapy were treated for 6 months with 400 mg/day [45]. At endpoint, viral load and liver enzymes were significantly decreased, suggesting anti-inflammatory and antiviral activities of bezafibrate. More recently, 7 patients received 400 mg/day of bezafibrate for 8 weeks. Once again, ALT, AST, and viral load were lowered compared to baseline [46]. Taken together, these results may argue in favor of restoration of transcriptional activity of the hepatoprotective PPARα and/or PPARγ receptors during chronic HCV infection. Pioglitazone, an agonist of PPARγ, is currently evaluated in patient with chronic HCV infection and insulin resistance (http://clinicaltrials.gov/show/NCT00189163).

6. Conclusion

Chronic infection with HCV is a major cause of chronic liver disease worldwide and is associated with chronic liver inflammation, hepatocyte fat accumulation, and diabetes. Nuclear hormone receptors PPARα and γ are involved in the transduction of metabolic and nutritional signals into transcriptional responses in order to maintain liver homeostasis. Evidence exists in favor of a decreased expression and functional activity of PPARα/γ in hepatocytes during

HCV infection related to HCV core protein, suggesting that HCV may have evolved strategies to prevent inducing, or to overcome, an efficient response by the host. This decreased expression of PPARα/γ may be involved in the pathogenesis of HCV infection through an alteration of the protective effects of these nuclear receptors against hepatic inflammation and fibrosis. Taken together, all these considerations suggest that PPARα/γ may represent new potential therapeutic targets in HCV infection.

Nomenclature:

PPAR: Peroxisome proliferator-activated receptor
RXR: Retinoid X receptor
HCV: Hepatitis C virus
FA: Fatty acid.

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Review Article

Peroxisome Proliferator-Activated Receptors in HBV-Related Infection

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Thirty years after its discovery, the hepatitis B virus (HBV) still remains a major global public health problem. Worldwide, two billion subjects have been infected, 350 million have a chronic infection and more than 600 000 die annually of HBV-related liver disease or hepatocellular carcinoma; new infections occur because of the presence of a large reservoir of chronic carriers of the virus. Since a decade several studies describe the interrelations between HBV and nuclear receptors and more particularly the peroxisome proliferator-activated receptors (PPARs). After a brief introduction, this review will make a rapid description of HBV incidence and biology. Then a report of the literature on the role of PPARs on viral transcription and replication will be developed. Finally, the role of HBV on PPARγ expression and activity will be discussed. Concluding remarks and perspectives will close this review.

1. Introduction

Hepatitis B virus (HBV) infection is a major public health problem with approximately 350 million people chronically infected but the prevalence of HBV infection and patterns of transmission vary greatly throughout the world. Fifteen percent to 40% of HBV-infected patients will develop cirrhosis, liver failure, and hepatocellular carcinoma (HCC) [1]. *Hepatitis B virus was considered to be not directly cytopathic*, and the development of HCC in individuals with chronic HBV infection is a multistage, multifactorial process including the interaction between host and environmental factors. *However, a recent study suggested that elevated serum HBV DNA level (≥10 000 copies/mL) was a risk predictor of HCC independent of hepatitis B e antigen (HBeAg), serum alanine aminotransferase level, and liver cirrhosis suggesting that HVB proteins themselves may have direct effect on cellular functions* [2].

Recent data suggested the implication of nuclear hormone receptor and especially of the retinoid X receptors (RXRs) and peroxisome proliferator-activated receptors (PPARs) in the transcription and the replication of the HBV. The peroxisome proliferator-activated receptors (PPARs) α, β/δ, and γ are members of the nuclear receptor superfamily activated by fatty acids and involved in the transduction of metabolic and nutritional signals into transcriptional responses [3, 4]. Among these transcription factors, PPARα/γ together with their obligate partner the RXR are three main nuclear receptors expressed in the liver [5–7]. However, despite strong expression in the liver, proof of an eventual role of PPARs in hepatic disease remains limited to the link between hepatic tumorigenesis and chronic administration of PPARα activators in rodents [8], the development of extensive hepatic steatosis in response to fasting and delayed liver regeneration in PPARα knockout mice [9, 10], impaired expression of PPARα in a murine model of alcoholic liver diseases [11], and impaired liver

expression of PPARα influenced by the HCV core protein during chronic hepatitis C virus infection [12].

This review will first describe the importance of HBV infection worldwide and the biology of the virus. Then the interactions between PPARs and HBV will be developed to provide a precise picture of the potential role of PPARs in HBV pathophysiology.

2. Hepatitis B Virus: Incidence and Prevalence

Approximately 2 billion people have been exposed to the HBV and 350 million people are chronically infected with the virus. Each year over 1 million people die from HBV-related liver disease. The chapter below will expose the incidence and prevalence of this huge public health problem worldwide.

The prevalence of HBV infection varies depending on the geographical area. In the Far East, the Middle East, Africa, and parts of South America, the prevalence is high, with hepatitis B surface antigens (HBsAgs) rates ranging from 8% to 15% [13]. In regions of high HBsAg endemicity, serologic evidence of prior HBV infection (anti-HBc and/or anti-HBs Ag) is almost universal in subjects without active infection. As a general rule, in these areas with high HBV endemicity the source of infection is mainly through perinatal transmission from the chronically infected mother or through infection during early childhood.

Areas of intermediate prevalence (2–7%) include Japan, parts of South America, Eastern and Southern Europe, and parts of central Asia. Areas with low HBV endemicity (prevalence of chronic infection <2%) include Northwestern Europe, North America, and Australia [14]. The source of infection in these areas is mainly through sexual contacts and needle sharing among injecting drug users, with a peak incidence in the 15–25-year-old age group.

Globally the incidence of acute HBV infection has been falling in the last decade, due to changes in behavior (e.g., increase in safe sexual practices related to HIV education efforts) and, to a lesser extent, to the introduction of effective vaccination programs [15]. Transmission of HBV via transfusion of blood and plasma-derived products has been eliminated in most countries through donor screening for HBsAg and viral inactivation procedures.

3. Viral Structure, Genomic Organization and Replication

HBV is a member of the family of the hepadnaviridae, hepatotropic DNA viruses. Characteristics of these viruses are as follows: a partially double-stranded DNA, with an outer lipoprotein envelope and an inner nucleocapsid or core bearing the viral genome; a polymerase with reverse transcription activity; the massive overproduction of viral envelope proteins (e.g., HBsAg), and a relative but not absolute hepatotropism. The following chapter will briefly describe the viral structure, genomic organization and replication mode of the HBV.

HBV virions are 42 nm double-shelled particles. The genome contains four open reading frames (ORFs) (S, P, C, and X) that encode four major proteins (surface, polymerase, core, and X protein, resp.) (Figure 1). The major abundant protein on the virus surface is the HBsAg or S protein, 24 kDa in size. In the viral envelope there are two other proteins, the L—involved in binding the virus to a receptor on the hepatocyte surface—and the M protein, whose function is unknown.

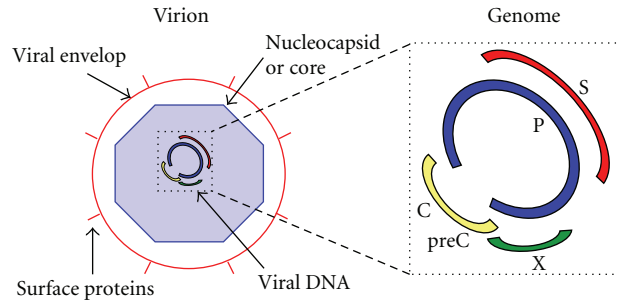

Figure 1: *HBV virion and genomic organization.* The HBV virion is composed of a viral envelop that contains the surface proteins, which are of different lengths (L, M, and S). The nucleocapsid or core wraps the viral DNA. The viral genome contains four open reading frames, the S that encodes for the surface protein (red), P that encodes for the viral polymerase (blue), preC and C that encode for the core (yellow) and X that encodes for the X protein (green).

The 27 nm nucleocapsid is an icosahedral symmetric structure containing 180 or 240 copies of the viral core (C) protein [16, 17], known as hepatitis B core antigen (HBcAg). The nucleocapsid contains the viral genome (Figure 1), a relaxed circular molecule that consists of a 3.2 kB minus strand and a smaller, complementary DNA (plus strand) of variable length. Circularity of HBV is maintained by hydrogen bonds between 250 bp at the two 50 ends of the plus and minus strands. The 50 ends of the DNA strands are each linked covalently to additional structures, essential for the initiation of DNA synthesis, that is, the polymerase and an oligo RNA. The viral polymerase is encoded by the P gene of the virus and is implicated in the synthesis of both strands of viral DNA through a reverse transcriptase (protein P) enzyme (RT). This RT shares sequence similarities with retroviral RT; the latter has been used in the development of antiviral drugs against HBV.

In addition to complete virions, HBV-infected hepatocytes produce in great excess two distinct subviral lipoprotein particles: the spheres, containing primarily the S protein, and the filaments, less numerous, rich in L protein. As these subviral particles contain only envelope glycoproteins and host-derived lipids, but not viral DNA; they are not infectious; nevertheless, they strongly stimulate the production of neutralizing anti-HBs antibodies. The overproduction of these particles makes it easy to diagnose HBV infection by the detection of the surface antigen in the blood.

Little is known about the earliest steps in the HBV life cycle. Virion binding to hepatocytes is mediated by a 180 kDa host protein identified as a member of the carboxypeptidase family [18]; antibodies against this protein block viral infection [19]. After direct membrane fusion uncoating of the virus allows the presentation of the nucleocapside to the

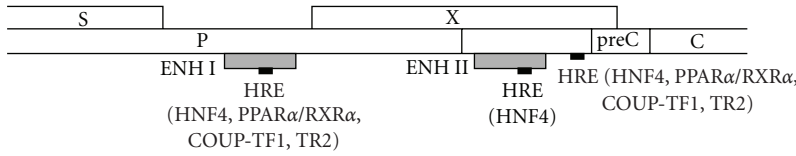

Figure 2: *NR regulatory region in HBV genome*. Schematic diagram of the HBV genome. The viral polymerase (P), surface proteins (S), precore (preC), core (C), and X protein (X) open reading frames are indicated by open rectangular boxes. Enhancers (ENHs) I and II are indicated by grey rectangular boxes. The hormone response elements (HREs) are indicated by small black rectangular boxes. Nuclear receptors that can bind these HREs are indicated into brackets.

cytosol. The naked viral core migrates to the nucleus where the viral genome is repaired to a covalently closed circular form (cccDNA). This cccDNA is transcribed by host RNA polymerase II to generate genomic and subgenomics stable RNAs. All viral RNAs are transported to the cytoplasm for translation yielding the viral envelope, core and preC, viral DNA polymerase, and X proteins. Finally, nucleocapsids are assembled in the cytosol; assembly requires the binding of viral polymerase (P) to a selective structure located at the 5′end of the genomic RNA. Once the P-RNA complex is formed, RNA packaging and reverse transcription begin. The replication of HBV requires an RNA intermediate followed by the synthesis of viral DNA by RT [20]. After replication is completed, viral cores are transported back into the nucleus, where they are either converted to cccDNA to maintain a stable intranuclear pool of transcriptional templates or more frequently, bud into the endoplasmic reticulum or Golgi apparatus; in this site nucleocapsidic particles are wrapped in the envelope proteins (surface, L, and M) and finally exported from the cell as full virions by vesicular transport [21].

4. Impact of PPAR on Viral Transcription and Replication

Studies in hepatoma cell line HepG2 and studies on a transgenic mouse model for HBV have provided evidence for a role of PPARS in controlling viral transcription and replication.

HBV has a partially double-stranded DNA genome and replicates through an RNA intermediate. After infecting host liver cells, there are four HBV transcripts from four different viral promoters: Core, SPI, SPII, and X promoter. The first studies that have linked PPAR and HBV have shown the presence of hormone response elements (HREs) in the promoters of HBV genome (Figure 2). In the dedifferentiated hepatoma cell line, HepG2, it was found that the nucleocapsid and large surface antigen promoters were transactivated in the presence of hepatocyte nuclear factor 4 (HNF4) whereas the enhancer I/X gene, nucleocapsid, and large surface antigen promoters were transactivated in the presence of RXR and PPAR [22]. Characterization of the nucleocapsid promoter region demonstrated that HNF4 is the primary transcription factor binding to the regulatory region spanning nucleotides −127 to −102 whereas HNF4, RXR-PPAR heterodimers, and chicken ovalbumin upstream promoter transcription factor 1 (COUP-TF1) bind the regulatory region spanning nucleotides −34 to −7 [22]. Modulation of the level of transcription from the nucleocapsid promoter by RXR-PPAR appears to be regulated by the regulatory sequence element spanning nucleotides −34 to −7 and the HBV enhancer I region (Figure 2). Another study demonstrated that HNF4 and testicular receptor 2 (TR2) repressed synthesis of the pre-C RNA, whereas PPAR-RXR activated synthesis of the pregenomic RNA and COUP-TF1 repressed synthesis of both the pre-C and pregenomic RNAs [23].

The regulation of HBV transcription and regulation were then explored in vivo. Using an HBV transgenic mouse model, Guidotti et al. demonstrated that activation of PPARα increased transcription and replication of HBV and suggested that even a modest alteration in transcription could have big impact on virus replication [24]. To point out the importance of nuclear receptors and specially PPARα on the HBV replication, Tang and McLachlan have shown that ectopic expression of HNF4 and PPARα was necessary and sufficient to allow HBV replication in nonhepatic cells, which is normally impossible due to the virus tropism [25].

Two studies performed in the team of McLachlan in La Jolla specified the sequences of interaction between the HBV and PPARα [26, 27]. Indeed, this team has developed a transgenic mouse for a natural hepatitis B virus (HBV) variant associated with seroconversion from HBeAg to anti-HBe antibody that contains two nucleotide substitutions (A1764T and G1766A) in the proximal nuclear hormone receptor binding site in the nucleocapsid promoter. This model suggested that peroxisome proliferators may enhance viral transcription directly in a PPARα-dependent manner through the nuclear hormone receptor recognition site in the enhancer I region of the HBV genome. Moreover, those mice transcribe very little precore RNA and secrete extremely low levels of HBe antigen compared with the wild-type HBV transgenic mice [26]. Analysis of HBV transcription and replication in nonhepatoma cells indicates that PPARα/RXRα heterodimers support higher levels of pregenomic RNA transcription from the wild-type than from the variant nucleocapsid promoter, producing higher levels of wild-type than of variant replication intermediates [27]. These observations indicate that the replication of wild-type and variant viruses can be differentially regulated by the liver-specific transcription factors that bind to the proximal nuclear hormone receptor binding site of the nucleocapsid promoter.

More recent data concern approaches to counteract this nuclear receptor-induced HBV transcription and replication.

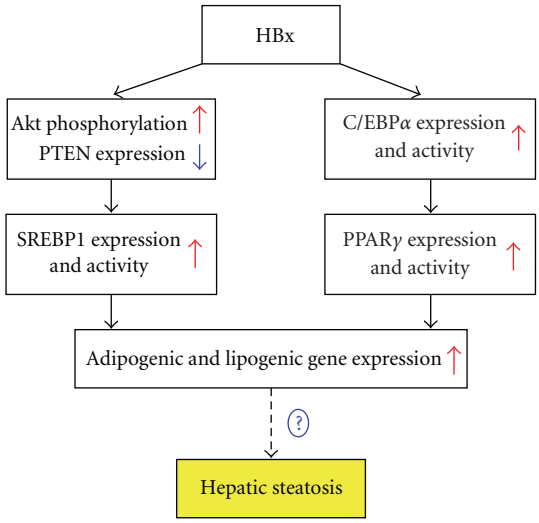

Figure 3: *HBx protein* could influence *liver steatosis through SREBP1 and PPARγ*. Protein X of the HBV (HBx) increases the kinase AKT phosphorylation and inhibits PTEN expression that leads to increased expression and activation of SREBP1 in the liver. In another way HBx enhances C/EBPα that in turn induces PPARγ expression and activation. Both pathways lead to an increased expression of adipogenic and lipogenic genes, which finally *could* contribute to liver steatosis.

Oropeza et al. showed that the nuclear receptor short heterodimer partner (SHP) inhibits the nuclear receptor-mediated HBV replication [28]. HBV replication that is dependent on HNF4 seemed considerably more sensitive to SHP-mediated inhibition than PPARα/RXRα-directed viral biosynthesis. A nonnucleosidic compound, Helioxanthin (HE-145), was found to suppress HBV gene expression and replication in HCC cells. It was found that HE-145 selectively suppresses surface antigen promoter II (SPII) and core promoter (CP) but has no effect on surface antigen promoter I (SPI) or promoter for X gene (XP). Tseng et al. showed that HE-145 acted by decreasing the DNA-binding activity of PPAR to specific cis element of HBV promoter for core antigen [29]. Taken together, all these data provide an interesting rationale for modulating the PPARα/RXRα heterodimer to control the HBV infection.

5. HBV Modulates PPARγ Expression: Role in Steatosis

Until now, two studies described a role of HBx protein on the regulation of PPARγ expression and activation and one of which suggests a role in steatosis.

In the below paragraph, we have described the role of PPAR on HBV transcription and replication. Conversely, the HBx protein of HBV modulated PPARγ by protein-protein interaction. Indeed, ligand activation of PPARγ has been reported to induce growth inhibition and apoptosis in various cancers including HCC. Choi and coll demonstrated that HBx counteracted growth inhibition caused by PPARγ ligand in HBx-associated HCC cells [30]. They found that HBx bound to DNA binding domain of PPARγ and this interaction blocked nuclear localization and binding to PPRE. HBx significantly suppressed the PPARγ mediated transactivation.

More recent report described a positive effect of HBx protein on PPARγ expression and transcriptional activity [31]. *Some* observations suggest that chronic HBV infection is associated with hepatic steatosis, which is a common histological feature of chronic infection with hepatitis C virus [32]. *Even if other report described lower frequency of steatosis in hepatitis B* [33, 34], evidence indicates that hepatic steatosis is a more vulnerable factor that leads to liver inflammation, fibrosis, and cancer. Based on these observations, Kim et al. demonstrated that overexpression of HBx induced hepatic lipid accumulation [31]. This phenomenon was accompanied by increased expression of sterol regulatory element binding protein 1 (SREBP1) and PPARγ. The authors proposed that HBx could participate to hepatic steatosis during HBV infection by regulating SREBP1 and PPARγ expression and activation (Figure 3) *but a direct proof remains to be obtained*.

6. Conclusion

HBV infection is a global health problem and recent data indicate that the HBV DNA level is a strong risk predictor of liver cirrhosis and HCC. Studies indicate the presence of hormone response elements in the promoters of HBV genome. Peroxisome proliferators may enhance HBV viral transcription directly in a PPARα-dependent manner. Conversely, HBx protein of HBV is able to induce the gene expression and transcriptional activity of SREBP1 and PPARγ, thereby causing hepatic lipid accumulation by increasing adipogenic and lipogenic gene expression. This regulation loop between PPAR and HBV may contribute to the progression of HBV-induced pathogenesis and the development of PPAR antagonist could represent a new therapeutic strategy.

Abbreviations

cccDNA:	Covalently closed circular DNA
COUP-TF1:	Chicken ovalbumin upstream promoter transcription factor 1
HBV:	Hepatitis B virus
HBcAg:	Hepatitis B core antigen
HBeAg:	Hepatitis B e antigen
HBsAg:	Hepatitis B surface antigen
HCC:	Hepatocellular carcinoma
HIV:	Human immunodeficiency virus
HNF4:	Hepatocyte nuclear factor 4
ORF:	Open reading frame
PPAR:	Peroxisome proliferator-activated receptor
RT:	Reverse transcriptase enzyme
RXR:	Retinoid X receptor
SHP:	Short heterodimer partner
SREBP1:	Sterol regulatory element binding protein 1
TR2:	Testicular receptor 2

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