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## Metabolic dysregulation in diabetes and HIV-associated insulin resistance: from fatty acids to fat distribution

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### ABSTRACT

Insulin resistance is one of the most important aspects of the metabolic dysregulation in diabetes and the metabolic syndrome. While most experts agree on the disturbed glucose-insulin axis in insulin resistance, the effects on lipid metabolism have received much less attention. Generally, fasting plasma triglycerides are slightly elevated and HDL cholesterol is diminished in the insulin resistance syndromes. Especially, the postprandial state seems to be one of the most important phases in which all the metabolic dysregulations come together. Postprandial lipemia itself is one of the causes of insulin resistance, and since postprandial lipemia is exaggerated in these syndromes, postprandial studies may be the best situation to investigate insulin resistance. Furthermore, postprandial hyperlipidemia has been closely linked to atherosclerosis making this the major risk factor in insulin resistance. One of the less well studied aspects of postprandial lipemia, which is closely related to insulin resistance, is peripheral fatty acid trapping and adipose tissue distribution. In HIV lipodystrophy, aberrant adipose tissue deposition and impaired peripheral fatty acid trapping are

important determinants of many of the metabolic disturbances seen in this disorder. Therefore, HIV lipodystrophy is an interesting model to elucidate the molecular mechanisms regulating fatty acid trapping and adipose tissue distribution. Recent studies have clearly shown that components of the complement system play an important role in lipoprotein metabolism and fatty acid regulation. Especial attention has been drawn to the complement component 3 (C3) which is also a strong predictor of the metabolic syndrome. C3 is synthesized by adipose tissue during lipolysis of triglyceride rich lipoproteins. This process seems to be disturbed in different situations of insulin resistance like the metabolic syndrome, type 2 diabetes and familial combined hyperlipidemia. C3 may be a potential link between inflammation and lipoprotein dysmetabolism. Surprisingly, C3 concentrations are also modulated by several interventions designed to treat the metabolic syndrome, like statins. Other inflammatory markers like leukocyte count and activation behave similar to C3 concentrations, especially during the postprandial phase. In this overview we will discuss several metabolic aspects of two examples of the insulin resistance syndrome, type 2 diabetes and HIV lipodystrophy.

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*Acrónimos:* Apo, Apolipoprotein; ASP, acylation stimulating protein; AUC, area under the curve; C3, Complement component 3; CRP, C-reactive protein; CVD, cardiovascular disease; FFA, free fatty acids; HAART, highly active antiretroviral therapy; HSL, hormone sensitive lipase; IMT, intima-media thickness; LPL, lipoprotein lipase; HBA, hydroxybutyric acid; RXR, retinoid X receptor; IL, interleukin; NCEP, National Cholesterol Education Program; NRTIs, nucleoside analogue reverse-transcriptase inhibitors; NNRTI, non-nucleoside analogue reverse-transcriptase inhibitor; PI, protease inhibitor; PPAR, peroxisome proliferator-activated receptor; SREBP-1, sterol regulatory element-binding protein-1; TZDs, thiazolidinediones; TG, triglyceride; RLP-C, remnant-like particle cholesterol; TRPs, triglyceride-rich particles.

**Key Words:** Chylomicrons; Fatty acids; Triglycerides; Cardiovascular disease.

## TWO FACES OF METABOLIC DYSREGULATION

Atherosclerosis is the main cause of mortality in patients with type 2 diabetes<sup>1</sup>. Important cardiovascular risk factors in type 2 diabetes are unfavorable body fat distribution, insulin resistance, dyslipidemia, hypertension, and a prothrombotic and proinflammatory state<sup>2</sup>. Most of these risk factors are strongly interrelated and are part of the "insulin resistance syndrome" or the "metabolic syndrome" as was elegantly described by Reaven in 1988<sup>3</sup>. In 1998, the National Cholesterol Education Program (NCEP) has endorsed the importance of the metabolic syndrome in cardiovascular risk assessment by introducing a case definition of the metabolic syndrome based on clinically easily obtainable anthropometric and laboratory parameters<sup>4</sup>. Using this definition, the metabolic syndrome is present when at least three out of five risk determinants (increased waist circumference, increased blood pressure, increased fasting plasma triglycerides, low HDL-cholesterol and elevated fasting plasma glucose) are present<sup>4</sup>.

Recently, it was shown that the NCEP definition of the metabolic syndrome is associated with an increased risk for cardiovascular disease (CVD)<sup>5</sup>. The incidence of the metabolic syndrome is rapidly increasing in Western societies and therefore a dramatic rise in CVD has to be expected<sup>6</sup>. Most likely these effects are a result of a changing Western lifestyle that is increasingly sedentary and characterized by a hypercaloric diet, a reduction in physical activity and an increasing prevalence of obesity. These changes are also likely to increase the incidence of type 2 diabetes. It is estimated that the prevalence of type 2 diabetes worldwide will be doubled in 2010 compared with 1999<sup>7</sup>. In the Netherlands, the prevalence of type 2 diabetes today is approximately 300.000, and is expected to increase to 500.000 by 2010<sup>8</sup>.

The metabolic syndrome is closely linked to body fat distribution. Especially abdominal obesity is associated with several metabolic risk factors and an increased risk of type 2 diabetes<sup>9</sup>. However, mounting evidence indicates that absolute or partial lack of body fat may result in a similar metabolic risk profile. Several forms of congenital and acquired lipodystrophies have been related to dyslipidemia, insulin resistance and early-onset type 2 diabetes<sup>10</sup>. During the last years, much attention has been directed to the increasing prevalence of lipodystrophy among HIV-infected patients receiving highly active antiretroviral therapy (HAART)<sup>10</sup>. The introduction of HAART in 1996 has led to a dramatic decrease in morbidity and mortality due to AIDS<sup>11</sup>. However, HAART is strongly associated with changes in body fat distribution, insulin resistance, early-onset type 2 diabetes

and dyslipidemia<sup>12-17</sup>. The clustering of these risk factors shows striking similarities with the metabolic syndrome, as described in HIV-negative individuals<sup>2,6</sup>. As survival of subjects with HIV increases, CVD may become an important complicating factor in the management of these patients.

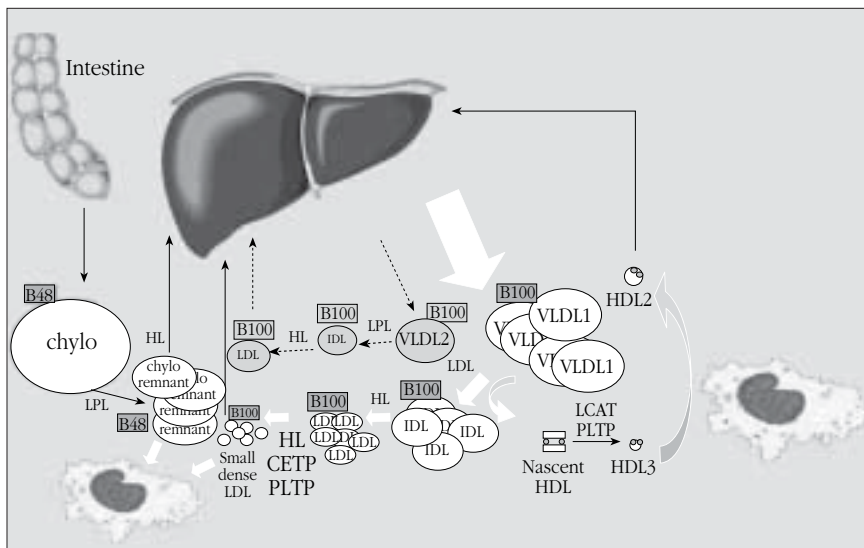
The focus of this paper is on metabolic dysregulation and pharmacological interventions in type 2 diabetes mellitus and HIV-lipodystrophy.

## POSTPRANDIAL LIPID METABOLISM AND ATHEROSCLEROSIS (FIGURE 1)

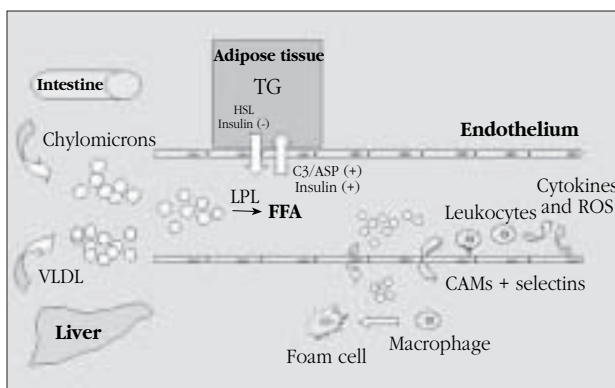
Dyslipidemia is one of the main modifiable risk factors in subjects with insulin resistance and type 2 diabetes. Increased hepatic free fatty acid (FFA) delivery has been postulated as a major contributor of dyslipidemia, because it could lead to hepatic overproduction of TG-rich VLDL particles<sup>18,19</sup>. In addition, the ability of insulin to suppress VLDL secretion is impaired in insulin-resistant disease states<sup>18,19</sup>. Especially the increase of large VLDL1 particles initiates a sequence of events that generates small dense LDL and low HDL-cholesterol<sup>18</sup> (Figure 1).

It is important to realize that TG-rich particles (TRPs) are mainly produced postprandially, and people are non-fasting most part of the day. Endogenous TRPs (VLDL, containing apoB100 as structural protein) and exogenous TRPs (chylomicrons, containing apoB48 as structural protein) compete for the same clearance mechanism, e.g. endothelium bound lipoprotein lipase (LPL), which hydrolyzes TG into glycerol and FFA, leaving atherogenic remnant particles (Figure 1)<sup>20</sup>. In the postprandial phase due to limited LPL availability, competition at the level of LPL will occur resulting in accumulation of TRPs. This competition is most likely when fasting hypertriglyceridemia is present. In addition, the lipolytic rate, as well as the clearance of remnant particles by liver receptors, is impaired in insulin resistance<sup>18,21</sup>. Hence, exaggerated and prolonged postprandial lipemia is an important characteristic of the diabetic dyslipidemia<sup>18,22,23</sup>.

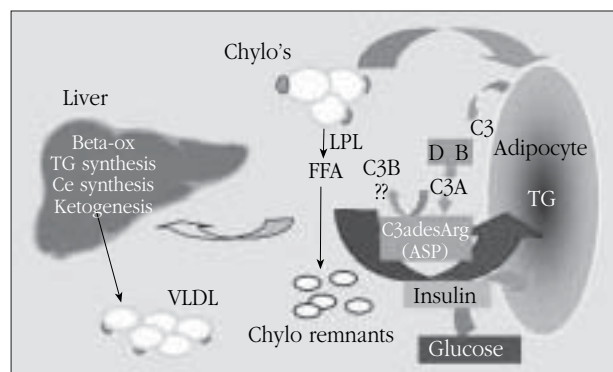
Increasing evidence suggests that postprandial hyperlipidemia contributes to atherosclerosis. Both hepatic and intestinal TRPs and their remnants accumulate in the subendothelial space, where they promote atherosclerosis by the formation of foam cells<sup>24</sup>. It has been shown that postprandial TG levels are better predictors of subclinical atherosclerosis than fasting TG concentrations<sup>22,23,25</sup>. Moreover, in the Physicians Health Study, plasma TG levels 3 to 4 hours after a meal distinguished even better between cases with future myocardial infarction and controls than fasting plasma TG levels<sup>26</sup>. Even in fasting



**Figure 1.** Lipoprotein metabolism in diabetes and the metabolic syndrome.



**Figure 2.** Postprandial lipid and fatty acid metabolism.



**Figure 3.** C3/ASP-Pathway and Postprandial Lipemia. Activation of C3 by the alternative complement pathway

normolipidemic subjects, increased postprandial lipemia has been linked to atherosclerosis<sup>27-29</sup>. Recently, Nakajima and colleagues developed a simple technique to analyze remnant-like particle cholesterol (RLP-C), and increased levels of these remnant particles have also been associated with future CVD<sup>30-32</sup>.

**PERIPHERAL FATTY ACID TRAPPING (FIGURES 2 AND 3)**

Adipose tissue plays a crucial role in regulating free fatty acid (FFA) concentrations in the postprandial period by suppressing the release of FFA in the circulation and stimulating the uptake of FFA liberated from TRPs by LPL<sup>33</sup>. This pathway is also known as the pathway of “adipocyte FFA trapping”. If adipocyte FFA trapping is disturbed, then non-adipose tissues, such as the liver, skeletal muscle and

pancreas, are exposed to excessive FFA concentrations, which may have several metabolic consequences. First, high FFA levels may aggravate insulin resistance<sup>34</sup>. Secondly, increased hepatic FFA delivery is a main determinant of VLDL secretion and postprandial lipemia<sup>18,19</sup>. Hydroxybutyric acid (HBA) is a marker of hepatic FFA oxidation. HBA is formed in liver mitochondria solely from FFA, and FFA availability is the major determinant of HBA production<sup>35</sup>. In an animal model of CD<sup>36</sup> deficient mice, increased hepatic FFA delivery has been linked to increased hepatic  $\beta$ -oxidation reflected in increased plasma levels of HBA<sup>36</sup>. Postprandial HBA appearance in plasma may, therefore, serve as a marker of postprandial hepatic FFA delivery<sup>37</sup> (36a). Thirdly, FFA may also directly impair vasoreactivity<sup>38</sup>.

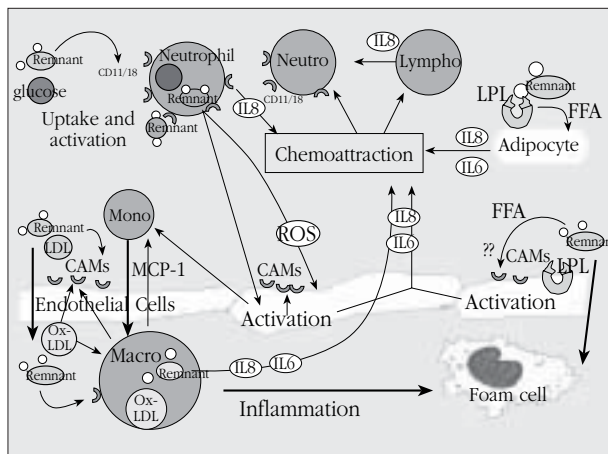
Insulin and the C3/acylating-stimulating protein (ASP)-system are the principal determinants of adipocyte FFA

trapping (Figure 2). Insulin inhibits the basal lipolytic rate by suppressing hormone-sensitive lipase (HSL) activity<sup>39</sup>. Complement component C3 (C3) is secreted by adipose tissue and is also involved in adipocyte FFA trapping (Figure 3). ASP (which is identical to C3adesArg) is an immunologically inactive cleavage product of C3 and stimulates FFA and glucose uptake in adipocytes, and inhibits HSL-mediated lipolysis<sup>40</sup>. The effects of C3/ASP and insulin on adipocyte FFA trapping are additive and independent<sup>41</sup>. Chylomicrons are strong activators of adipocyte C3 production *in vitro*<sup>42</sup>, and it has been shown that after a high-fat meal plasma C3 concentrations increase<sup>43,44</sup>, especially when insulin effects are blunted<sup>45</sup>. It is thought that effective postprandial C3-mediated diversion of FFA from the liver contributes to a healthy lipoprotein phenotype. Adipocytes from patients with familial combined hyperlipidemia are resistant to the effects of C3<sup>46</sup>, leading to an exaggerated and prolonged postprandial C3 response<sup>43</sup>, eventually resulting in abnormal diversion of FFA to the liver and VLDL overproduction<sup>43,44</sup>.

In addition to the central role of lipid storage, adipose tissue also releases a large number of cytokines and bioactive mediators that influence body weight homeostasis, inflammation, coagulation, fibrinolysis, insulin resistance, diabetes and atherosclerosis<sup>47</sup>. These various protein signals are often referred to as “adipocytokines”. Among these, adiponectin is an insulin-sensitizing and anti-inflammatory adipocytokine. Several studies report a close relationship between low adiponectin levels and insulin resistance. Adiponectin levels are not only low in patients with type 2 diabetes<sup>48</sup>, but also in patients with HIV-lipodystrophy<sup>49</sup>. Moreover, low adiponectin levels are associated with a moderately increased CVD risk in diabetic men<sup>50</sup>. Clearly, these studies emphasize the importance of adipose tissue as an active endocrine organ involved in several metabolic and inflammatory processes that are relevant for the development of atherosclerosis.

#### INFLAMMATION AND ATHEROSCLEROSIS (FIGURE 4)

Atherosclerosis is nowadays regarded as a low-grade chronic inflammatory disease, involving a series of highly specific cellular and molecular responses<sup>51,52</sup>. Atherogenesis is initiated by endothelial activation triggered by several cardiovascular risk factors<sup>53</sup>. In the early phase of atherogenesis, resident and recruited leukocytes release various inflammatory mediators, bind to the endothelium and eventually transmigrate into the arterial wall (Figure 4). A higher content of inflammatory cells in the atherosclerotic lesion renders the plaque vulnerable with an increased risk of rupture<sup>54</sup>. The importance of



**Figure 4.** Postprandial lipemia and inflammation.

leukocytes in the atherosclerotic process is supported by animal studies that have shown reductions of plaque formation and endothelial dysfunction when adherence of leukocytes was prevented<sup>55</sup>.

Markers of inflammation, such as the blood leukocyte count and C-reactive protein (CRP), are independent predictors of future CVD<sup>56,57</sup>. CRP is a sensitive acute-phase reactant produced by the liver in response to cytokines. IL-6 is the major cytokine responsible for hepatic CRP production and is itself also associated with CVD<sup>58</sup>. Even subjects with a low CRP concentration are at increased cardiovascular risk if they have a blood leukocyte count in the higher 25<sup>th</sup> percentile<sup>59</sup>. Differential leukocyte counts (monocytes and neutrophils) are also related to CVD<sup>56</sup>. Interestingly, the best association with CVD has been demonstrated for neutrophils<sup>56</sup>. Their role in the pathophysiology of atherosclerosis is not entirely clear, as these cells are absent in the atherosclerotic lesion until it is ruptured<sup>60</sup>. However, upon activation, resident and recruited neutrophils may affect endothelial function via the production of pro-inflammatory cytokines and generation of oxidative stress<sup>61</sup>. The blood leukocyte count is increased in subjects with type 2 diabetes and impaired glucose tolerance<sup>62,63</sup>. In addition, type 2 diabetic patients have increased expression of leukocyte activation markers<sup>62</sup>, which may represent increased adhesive capacity of these cells to the endothelium.

Since humans are non-fasting most part of the day, this period may be of particular importance in the pathogenesis of atherosclerosis. The underlying mechanisms may involve increased generation of oxidative stress and activation of endothelial cells and leukocytes<sup>61,64,65</sup>. For example, it has recently been shown that postprandially, when TG and

glucose rise, leukocyte counts increase with concomitant production of pro-inflammatory cytokines and oxidative stress, and that these changes may contribute to endothelial dysfunction<sup>61</sup>. The postprandial leukocyte increase was due to a specific increase of neutrophils, whereas the lymphocyte increase also occurred after a water (control) test. In addition, postprandial leukocyte activation has been described in healthy subjects<sup>66</sup>. Upon activation, endothelial cells produce a variety of pro-inflammatory cytokines which may facilitate recruitment and activation of leukocytes. Among those, IL-6 and IL-8 are the main cytokines responsible for leukocyte recruitment, and both show postprandial increments as well<sup>60</sup>. In patients with type 2 diabetes, a significant rise in CRP levels was observed after ingestion of a high-fat meal<sup>67</sup>. Given the close relationship between inflammation and atherosclerosis, postprandial inflammatory changes may result in increased susceptibility for premature atherosclerosis.

#### LIPODYSTROPHY AND CARDIOVASCULAR RISK IN HIV-INFECTED PATIENTS (TABLE 1)

The introduction of HAART in 1996 has led to a dramatic decrease in AIDS-related mortality<sup>11</sup>. However, HAART is strongly associated with lipodystrophy and metabolic risk factors<sup>12-17</sup>. Lipodystrophy is characterized by changes in body fat distribution, including subcutaneous fat loss, intra-abdominal fat accumulation and development of a buffalo hump<sup>12-14</sup>. Subcutaneous fat loss is most noticeable in the face, limbs and buttocks and may occur independently of central fat accumulation. Approximately half of the HAART-treated HIV-infected patients will develop changes in body fat distribution after 12-18 months of therapy<sup>14</sup>. Severe forms of lipodystrophy, especially lipoatrophy, can be disfiguring and stigmatizing, and often lead to suboptimal adherence to HAART.

The type and duration of HAART are strongly associated with the onset and severity of lipodystrophy. HAART generally consists of two nucleoside analogue reverse-transcriptase inhibitors (NRTIs) and a protease inhibitor (PI) and/or a non-nucleoside analogue reverse-transcriptase inhibitor (NNRTI). Both PIs and NRTIs have been related to the development of lipodystrophy, but the prevalence and severity of lipodystrophy are increased mostly in patients treated with both NRTIs and a PI<sup>68</sup>. The etiology of lipodystrophy appears to be multifactorial, including HIV drug inhibitory effects on adipocyte differentiation and alteration of mitochondrial functions (Figure 4). For example, PIs impede adipocyte differentiation through altered expression and nuclear

**TABLE I. Metabolic and anthropometric characteristics and clinical signs in HIV lipodystrophy compared to type 2 diabetes**

	HIV lipodystrophy	Type 2 DM
<i>Cardiovascular:</i>		
Elevated plasma cholesterol	+/-	+/-
Elevated plasma TG	++	+
Decreased HDL-C	+/-	+
Large TG-rich VLDL	+	+
Postprandial Hyperlipidemia	+	+
Impaired peripheral fatty acid trapping	++	+
Enhanced hepatic fatty acid flux	++	+
Elevated plasma apoB	+	+
Insulin Resistance	+	+
<i>Liver:</i>		
Increased liver fat	+	+
<i>Fat:</i>		
Decreased subcutaneous fat	++	-
Increased intra-abdominal fat	++	++
<i>Muscle:</i>		
Impaired glucose uptake	+	++
Decreased glucose phosphorylation	+	+
<i>Pancreas:</i>		
Hyperinsulinemia	+	+

Note: +: present; -: absent; +/-: may be present or absent.

localization of sterol regulatory element-binding protein-1 (SREBP-1) and peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ )<sup>69,70</sup>, which are essential for adipogenesis. NRTIs may induce mitochondrial dysfunction and apoptosis of adipocytes by inhibition of mitochondrial DNA polymerase  $\gamma$ , and depletion of mitochondrial DNA<sup>71</sup>.

Lipodystrophy is often accompanied by several metabolic risk factors, such as insulin resistance, glucose intolerance and dyslipidemia<sup>15-17</sup>, thereby sharing many aspects with diabetes (Table 1). Insulin resistance may result from direct effects of antiretrovirals, effects of HIV infection, or indirect effects, such as changes in body fat distribution<sup>72</sup>. For example, it has been shown that PIs induce insulin resistance

in vitro by reducing insulin-mediated glucose uptake by glucose transporter 4<sup>73</sup>. In HIV-negative adults, PIs reduce insulin sensitivity as early as 4 weeks after administration, without changing body fat distribution<sup>74,75</sup>. Direct effects of NRTIs and NNRTIs on insulin sensitivity have not been demonstrated, but these classes may contribute to insulin resistance indirectly through changes in body fat distribution. Insulin resistance in this population has been related to visceral fat accumulation and subcutaneous fat loss<sup>72,76,77</sup>. Abnormalities in glucose tolerance have been recognized in more than one-third of the patients with lipodystrophy<sup>15</sup>. Basal lipolytic rates are generally increased in patients with HIV-lipodystrophy, suggesting impaired action of HSL<sup>78</sup>. In addition, several studies have reported elevated FFA levels following glucose or insulin challenges<sup>79,80</sup>, suggesting resistance to the action of insulin to suppression of lipolysis.

The question whether HIV-lipodystrophy is associated to impaired adipocyte FFA trapping has also been investigated. For this purpose, postprandial studies were carried out where FFA, hydroxybutyric acid (HBA), as a measure of hepatic FFA oxidation, and TG changes were recorded in HIV-infected males with and without lipodystrophy and in healthy controls. We found that the area under the curves (AUCs) for FFA, HBA and TG were higher in the patients with lipodystrophy compared with the other groups, suggesting impaired adipocyte FFA trapping that contributes to postprandial lipemia in these patients<sup>81</sup>. Postprandial FFA and HBA levels were both negatively associated with subcutaneous abdominal fat. These data are suggestive for impaired ability to store FFA as TG in subcutaneous adipocytes in patients with HIV-lipodystrophy, but additional defects in lipases (e.g. HSL and LPL) may also contribute. The higher postprandial HBA levels in the lipodystrophic patients indicated increased hepatic FFA delivery, which may aggravate insulin resistance and dyslipidemia, ultimately leading to an increased cardiovascular risk in these patients.

The natural course of HIV infection is characterized by changes in plasma triglycerides (TG), HDL-cholesterol, and LDL particle size<sup>82</sup>. Following the introduction of PI-containing HAART, multiple studies have demonstrated more pronounced atherogenic changes in lipid profile, including increases in plasma TG and LDL-cholesterol, and decreases in HDL-cholesterol<sup>15-17</sup>. In addition, increases in apolipoprotein B (apoB) have been described, often associated with the predominance of atherogenic small, dense LDL particles. Of specific concern is the fact that use of NRTIs and PIs

in combination, particularly among older subjects with normalized CD4 cell counts and suppressed HIV replication, is associated with a lipid profile known to increase the risk of CVD<sup>83</sup>.

The most pronounced changes in lipid profile have been observed with the PI ritonavir. In HIV-negative volunteers, ritonavir increased TG, apoB and VLDL-cholesterol as early as 2 weeks after administration<sup>84</sup>. Except for atazanavir, all PIs cause to some extent fasting hyperlipidemia<sup>85</sup>. PIs suppress the breakdown of the nuclear form of SREBP in the liver, resulting in increased TG and cholesterol biosynthesis<sup>17</sup>. In addition, PIs suppress the proteasomal breakdown of nascent apoB, leading to VLDL oversecretion<sup>86</sup>. The severity and prevalence of dyslipidemia in HIV-infected patients may also depend on HIV disease stage and the concomitant presence of lipodystrophy and insulin resistance. Of the NNRTIs, efavirenz is associated with higher levels of cholesterol and TG than is nevirapine, whereas both increase HDL-cholesterol<sup>87,88</sup>. A potential interesting observation is the fact that switching of a PI-based regimen to a NNRTI-based regimen may partly reverse atherogenic lipoprotein changes<sup>89,90</sup>.

Clearly, the presence of metabolic risk factors in HIV-infected patients may predispose to accelerated atherosclerosis. In a cross-sectional study, the use of PIs was associated with endothelial dysfunction, which is an early marker of atherosclerosis<sup>91</sup>. PIs may also promote atherosclerotic lesion formation independent of dyslipidemia by increasing CD36-dependent cholesteryl ester accumulation in macrophages<sup>92</sup>. Carotid intima-media thickness (IMT), which is considered to be a strong predictor for cardiovascular events, is increased in HIV-infected patients as compared with age-matched control subjects<sup>93,94</sup>. IMT also progresses much more rapidly in HIV-infected patients than in non-HIV cohorts<sup>95</sup>. Moreover, in a multicenter prospective study, HAART was independently associated with a 26 percent relative increase in the rate of myocardial infarction per year of antiretroviral drug exposure during the first four to six years of use<sup>96</sup>. During the last years, increasing attention has been directed to the management of lipodystrophy and cardiovascular risk in HIV-infected patients. Statins and fibrates have shown to reduce atherogenic lipoproteins in HIV-infected patients<sup>97</sup>. However, these agents are unlikely to improve body fat distribution. The results of switching antiretroviral therapy on lipodystrophy and metabolic risk factors have been rather disappointing<sup>98</sup>. There is thus an urgent need for agents that improve body fat distribution and cardiovascular risk in HIV-infected patients.

## TREATMENT OF DYSLIPIDEMIA IN DIABETES AND LIPODYSTROPHY

There is no doubt that besides lifestyle intervention aimed to reduce body weight and improve glucose regulation, the most solid cornerstone for the reduction of cardiovascular risk in patients with type 2 diabetes is the use of statins<sup>99,22</sup>. These agents not only improve the fasting lipid profile<sup>100,101</sup>, but they also improve postprandial lipemia, reduce small dense LDL concentrations, increase HDL-C concentrations, enhance endothelial vessel function and unexpectedly, may sometimes improve peripheral fatty acid trapping<sup>102,103</sup>. Furthermore, statins have anti-inflammatory effects that seem to be independent from their lipid lowering properties<sup>101,104</sup>.

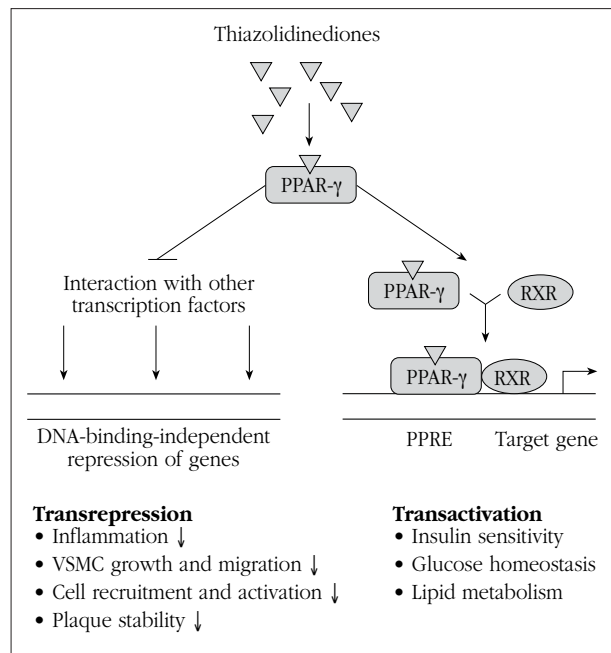
Insulin sensitizing agents have also been attributed positive effects on diabetic dyslipidemia, especially metformin and thiazolidinediones may be useful in this context.

### INSULIN-SENSITIZING AGENTS

Biguanides and thiazolidinediones are used in clinical medicine to improve insulin sensitivity and glycemic control in patients with type 2 diabetes. In type 2 diabetes, both classes of drugs significantly modulate body fat distribution and several aspects of the metabolic syndrome to potentially retard atherosclerotic disease progression. Both classes may also have a role in treating patients with other insulin-resistant conditions, and may be a valuable asset for the treatment of HIV-lipodystrophy.

### Thiazolidinediones (Figure 5)

The peroxisome-proliferator-activated receptors (PPARs) are a subfamily of the 48-member nuclear-receptor superfamily and regulate gene expression in response to ligand binding<sup>105,106</sup>. Upon activation by their ligands, PPARs form heterodimers with the nuclear retinoid X receptor (RXR) and bind to specific PPAR response elements in the promoter region of their target genes (transactivation, Figure 5)<sup>105,106</sup>. In addition, PPARs can interact with other transcription factors in a DNA-binding-independent manner and exhibit anti-inflammatory properties by repressing gene expression (transrepression)<sup>105,106</sup>. Three PPARs (PPAR- $\alpha$ , PPAR- $\beta/\delta$ , and PPAR- $\gamma$ ) have been identified to date. PPAR- $\alpha$  is the main target for fibrates and regulates the expression of genes involved in lipid metabolism and inflammation. PPAR- $\beta/\delta$  stimulates FFA oxidation primarily in muscle but also in adipose tissue. PPAR- $\gamma$  exhibits its regulatory effects primarily in adipocytes by interfering with insulin signaling, cytokine production, and FFA metabolism.



**Figure 5.** Thiazolidinediones: mode of action.

Thiazolidinediones (TZDs) are synthetic ligands for PPAR- $\gamma$  activation<sup>105,106</sup>. Currently, there are two TZDs available: rosiglitazone and pioglitazone. A third TZD, troglitazone, has been retracted from the market in 2000 due to a substantially increased risk of severe hepatotoxicity. PPAR- $\gamma$  is preferentially expressed in adipose tissue and the improvement of insulin resistance in skeletal muscle and liver tissue is probably secondary to enhanced lipid storage in subcutaneous adipocytes and improved adipocyte function, as reflected by the altered secretion of adipocytokines (e.g. adiponectin, IL-6, TNF- $\alpha$  and resistin)<sup>106</sup>. In patients with type 2 diabetes, TZDs improve insulin sensitivity despite an increase in body fat mass during treatment. The 2-4 kg increase in fat mass occurs almost exclusively in the subcutaneous fat compartment<sup>106,107</sup>, an effect which would be desirable in patients with HIV-lipodystrophy. Interestingly, all of the major cell types in the vasculature also express PPAR- $\gamma$ <sup>108,109</sup>. TZDs have interesting effects on these cells, which appear to be partially independent of the PPAR- $\gamma$ -RXR mediated transcriptional effects<sup>110</sup>. There appears to be a generalized transrepression of inflammatory transcription in a DNA-binding-independent manner<sup>110-112</sup>. Direct beneficial vascular effects of TZDs include increased nitric oxide bio-availability<sup>113</sup>, decreased leukocyte-endothelial cell interaction<sup>114</sup>, reduced vascular smooth muscle cell migration and proliferation<sup>115</sup>, and cholesterol efflux from macrophages<sup>116</sup>. Therefore, it is thought that TZDs, which were primarily

introduced to improve glycemic control, may also have benefits on atherosclerotic disease progression.

We determined the effects of rosiglitazone on postprandial leukocytes, pro-inflammatory cytokines (IL-6 and IL-8), CRP and MCP-1 in a randomized double-blind, placebo-controlled cross-over trial in patients with type 2 diabetes<sup>117</sup>. We observed that a high-fat meal increased neutrophils and pro-inflammatory cytokines in these patients. These postprandial inflammatory changes may result in increased susceptibility for premature atherosclerosis. Compared with placebo, we demonstrated that rosiglitazone attenuated the postprandial rise of neutrophils (-39%), IL-6 (-63%) and IL-8 (-18%). We also observed a substantial reduction in fasting CRP, in agreement with previous studies<sup>15,16</sup>, which is most likely clinically relevant. IL-6 is the major cytokine responsible for hepatic CRP production and is also independently associated with CVD<sup>17</sup>. One of the questions that remain is to what extent the postprandial reductions in neutrophils and pro-inflammatory cytokines contribute to the overall attenuation of the low-grade inflammatory state and improvement of cardiovascular risk. Since inflammation is a major force driving atherosclerosis, and type 2 diabetes is characterized by postprandial hyperlipidemia, a reduced inflammatory response after a meal may contribute to cardiovascular risk reduction in diabetes.

So far, only one large randomized clinical trial has been published on hard end-points in type 2 diabetics treated with pioglitazone<sup>118</sup>. In this PROactive trial, a slight but significant reduction was observed in mortality and major cardiovascular events, which was a secondary endpoint of the trial<sup>118</sup>. The composite primary endpoint did not reach clinical significance. Further studies using other TZDs, like rosiglitazone in the RECORD trial, will be needed to decide whether TZDs can improve the cardiovascular risk profile of these patients above the classical interventions available (statins, aspirin, ACE inhibitors and AII inhibitors). Finally, whereas PPAR- $\gamma$  agonists can markedly improve insulin resistance and glycemic control, PPAR- $\alpha$  agonists (fibrates) can be used in the treatment of diabetic dyslipidemia<sup>18</sup>. Hence, the combined actions of the dual PPAR- $\alpha/\gamma$  agonists appear ideally suited to decrease the risk for CVD in patients with type 2 diabetes<sup>19</sup>. This class of agents may thus be a valuable asset for the prevention of CVD in patients with type 2 diabetes in the near future.

### Biguanides

Metformin, a biguanide, has been available for the treatment of type 2 diabetes for many years<sup>119</sup>. Over this period of time,

it has become one of the most widely prescribed anti-hyperglycemic agents. Its mechanism of action involves reduction of hepatic insulin resistance and glucose output, leading to significant reductions in glucose and insulin levels<sup>119,120</sup>. Whether metformin also has an insulin-sensitizing effect in peripheral tissues remains controversial. Metformin may also have beneficial effects on abdominal obesity, dyslipidemia and plasminogen activator inhibitor-1 levels<sup>121,122</sup>. In patients with type 2 diabetes, metformin improves endothelial function by mechanisms involving glucose-lowering, reduction of insulin resistance, antioxidant effects, lipid-lowering and direct vasodilative effects<sup>119</sup>. Moreover, in the United Kingdom Prospective Diabetes Study, metformin decreased cardiovascular mortality in overweight type 2 diabetic patients<sup>122</sup>.

In order to determine more clearly which risk factors should be targeted to reduce cardiovascular risk in HIV-infected patients we investigated whether agents that improve insulin resistance could improve endothelial function. For this purpose, the effects of rosiglitazone and metformin on insulin sensitivity, body fat distribution and flow-mediated vasodilation were directly compared in a randomized clinical trial. Rosiglitazone increased subcutaneous abdominal fat in patients with HIV-lipodystrophy, despite ongoing HAART, most likely by increasing FFA storage in subcutaneous adipocytes. Since quality of life, including body image, have become crucial in HIV-infected patients, this may be an important beneficial effect in patients with HIV-lipodystrophy. Whether the increase in subcutaneous abdominal fat by rosiglitazone is accompanied by changes in adipocyte differentiation and altered cytokine expression in adipose tissue remains to be elucidated.

Our results are similar to those observed by Hadigan and colleagues<sup>123</sup>, but in contrast to those observed by others<sup>117bc</sup>. It is therefore clinically relevant to identify those patients that are most likely to benefit from treatment with rosiglitazone. Our data suggest that especially those patients with marked lipoatrophy and insulin resistance may benefit from treatment with rosiglitazone. In addition, the patients on stavudine treatment did not experience an increase in subcutaneous abdominal fat with rosiglitazone in our study, which may explain the lack of efficacy in prior studies by other groups that had a baseline imbalance and overuse of stavudine in the rosiglitazone-arm<sup>117bc124,125</sup>. Whether TZDs are able to prevent or delay the onset of lipodystrophy remains to be shown.

Metformin reduced subcutaneous and visceral abdominal fat, in agreement with previous studies in HIV-infected

patients, suggesting benefits in patients with predominant visceral adiposity<sup>126-128</sup>. Metformin and rosiglitazone showed similar benefits on insulin sensitivity, but only rosiglitazone increased adiponectin levels, most likely due to improved adipocyte function. Despite increased insulin sensitivity, a detrimental effect on fasting lipid profile was seen in some rosiglitazone-treated patients. Rosiglitazone should, therefore, be used with caution in patients with HIV-lipodystrophy, in particular in hyperlipidemic patients, or prescribed in conjunction with lipid-lowering agents. So far, prior studies have not examined whether in HIV, modulation of insulin resistance translates into vascular benefit. We found that metformin, but not rosiglitazone, improved endothelial function in patients with HIV-lipodystrophy. Whether the improvement of endothelial function is sufficient to produce clinical benefit is not known, but it may be relevant. Previously, statin treatment was not able to improve endothelial function in HIV-infected patients, despite reductions in atherogenic lipoproteins<sup>129</sup>.

Many questions remain to be answered in relation to HIV patients and cardiovascular risk reduction. For example, it is still unclear whether oral glucose tolerance tests and the NCEP-ATPIII definition of the metabolic syndrome are useful for routine screening of HIV-infected patients. Despite normal fasting glucose levels, many HIV-infected patients have IGT, and IGT is closely linked to vascular abnormalities<sup>130,131</sup>. The presence of the metabolic syndrome according to the NCEP-ATPIII criteria in HIV-infected patients is also associated with functional and structural atherosclerotic changes<sup>131</sup>. However, so far it is not known whether in HIV the clustering of risk factors within the metabolic syndrome has more predictive power regarding cardiovascular risk than individual risk factors. Also, data on progression from the metabolic syndrome and IGT to type 2 diabetes in HIV-infected patients would be interesting in order to determine whether a glucose tolerance test would be a useful clinical tool in the management of HIV-infected patients on HAART. Until these issues have been investigated, evaluation and management of individual cardiovascular risk factors using the currently available guidelines is recommended.

Metformin may be best for the viscerally obese, overweight, dyslipidemic patient, whereas it may not be appropriate for

the patient with predominantly lipodystrophy, as they may suffer a further loss of subcutaneous fat. In patients with HIV-lipodystrophy, metformin has greater benefits on vascular function than rosiglitazone<sup>130,131</sup>. Rosiglitazone may be best for lipodystrophic patients. However, rosiglitazone should be used with caution in hyperlipidemic patients. If used, this must be accompanied by careful monitoring of the lipid profile. Treatment with metformin is not recommended in patients with renal- or liver disease or elevated lactic acid levels. Regarding TZDs, it is important to note that rosiglitazone is a substrate for CYP2C8 and is unlikely to affect CYP3A4 metabolism on concomitantly administered drugs, such as PIs. Future studies are necessary to investigate the effects of combination therapy with rosiglitazone and metformin in HIV-infected patients. In addition, dual PPAR- $\alpha/\gamma$  agonists may be useful to treat both dyslipidemia and lipodystrophy in HIV-infected patients in the future.

Regarding pathophysiology of the dyslipidemia, HIV infected patients with lipodystrophy share many metabolic characteristics with type 2 diabetes<sup>132</sup>. Regional fat distribution is of major importance and interventions aimed to decrease intra-abdominal fat deposition even if there is an increase in the subcutaneous depots, may lead to improved cardiometabolic risk profiles in both situations.

#### CONSIDERACIONES PRÁCTICAS

- La hiperlipemia postprandial puede inducir resistencia a la insulina y se halla relacionada con el proceso de aterosclerosis.
- La hiperlipemia postprandial se halla aumentada en diversas situaciones clínicas que cursan con resistencia a la insulina como la diabetes tipo 2, la hiperlipemia familiar combinada y la lipodistrofia asociada a la infección por el VIH.
- En la lipodistrofia del VIH se han descrito diversas alteraciones del sistema de complemento asociadas a trastornos del metabolismo de las lipoproteínas y de los ácidos grasos.

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