Metabolic dysregulation and interventions in type 2 diabetes mellitus and HIV-lipodystrophy

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Metabolic dysregulation and interventions in type 2 diabetes mellitus and HIV-lipodystrophy.
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Utrecht, Universiteit Utrecht, Faculteit Geneeskunde.
Proefschrift Universiteit Utrecht, met een samenvatting in het Nederlands.

Limited edition: 650 copies
ISBN: 90-393-40552

Lay-out: Roy Sanders, Ede
Printed by: FEBO druk B.V., Enschede

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Metabole dysregulatie en interventies bij type 2 diabetes mellitus en HIV-lipodystrophy

(met een samenvatting in het Nederlands)

Proefschrift

Ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de Rector Magnificus, Prof. Dr. W.H. Gispen ingevolge het besluit van het College voor Promoties in het openbaar te verdedigen op dinsdag 11 oktober 2005 des ochtends te 10.30 uur

Door

Jeroen Peter Hans van Wijk
Geboren op 25 januari 1977 te Utrecht

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The publication of this thesis was financially supported by the Dutch Organization for Scientific Research, the Netherlands Heart Foundation, the Dutch Diabetes Foundation, the J.E. Jurriaanse Foundation, Abbott, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Lilly, Novo Nordisk, Pfizer, Sanofi-Aventis, Servier and Sopachem.

To my parents

Contents

Published in part in:
Drugs 2004;64(Suppl.2):19-41 and
Curr Atheroscler Rep 2005; 7(5): 369-74

1

Introduction and outline of the thesis

Two faces of metabolic dysregulation

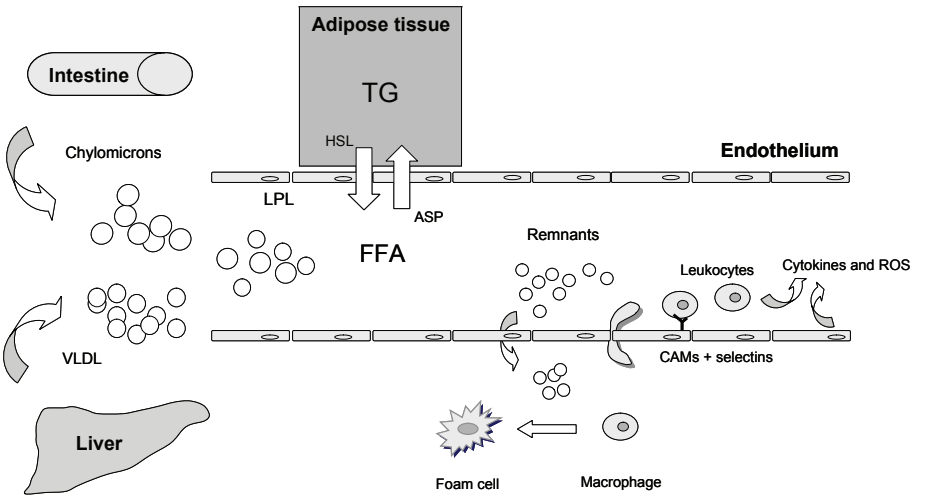
Atherosclerosis is the main cause of mortality in patients with type 2 diabetes[1]. Important cardiovascular risk factors in type 2 diabetes are insulin resistance, dyslipidemia, hypertension, unfavorable body fat distribution and a prothrombotic and proinflammatory state[2]. Most of these risk factors are strongly interrelated and are part of the "insulin resistance syndrome" or the "metabolic syndrome" as was elegantly described by Reaven in 1988[3]. 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[4]. 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[4]. Recently, it was shown that the NCEP definition of the metabolic syndrome is associated with an increased risk for cardiovascular disease (CVD)[5]. The incidence of the metabolic syndrome is rapidly increasing in Western societies and therefore a dramatic rise in CVD has to be expected[6]. 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[7]. In the Netherlands, the prevalence of type 2 diabetes today is approximately 300.000, and is expected to increase to 500.000 by 2010[8].

The metabolic syndrome is closely linked to body fat distribution. Especially abdominal obesity is closely associated with several metabolic risk factors and an increased risk of type 2 diabetes[9]. However, mounting evidence is indicating 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[10]. 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)[10]. The introduction of HAART in 1996 has led to a dramatic decrease in morbidity and mortality due to AIDS[11]. However, HAART is strongly associated with changes in body fat distribution, insulin resistance, early-onset type 2 diabetes and dyslipidemia[12-17]. The clustering of these risk factors shows striking similarities with the metabolic syndrome, as described in HIV-negative individuals[2-6]. As survival of subjects with HIV increases, CVD may become an important complicating factor in the management of these patients. The focus of this thesis is on metabolic dysregulation and pharmacological interventions in type 2 diabetes mellitus and HIV-lipodystrophy.

Postprandial lipid metabolism and atherosclerosis

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[18,19]. In addition, the ability of insulin to suppress VLDL secretion is impaired in insulin-resistant disease states[18,19]. Especially the increase of large VLDL1 particles initiates a sequence of events that generates small dense LDL and low HDL-cholesterol[18]. 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*)[20]. 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

Figure 1
Postprandial lipid metabolism and atherosclerosis



In the postprandial phase, hepatic and intestinal TRPs compete for clearance by LPL, which hydrolyzes TG into glycerol and FFA, leaving atherogenic remnant particles. Adipose tissue plays a crucial role in regulating postprandial FFA concentrations. Insulin and ASP are the principal determinants of adipocyte FFA trapping. Insulin inhibits lipolysis by suppressing HSL activity. ASP (C3adesArg) is an immunologically inactive cleavage product of C3 and stimulates FFA and glucose uptake in adipocytes. TRPs and their remnants accumulate in the subendothelial space, where they promote atherosclerosis by the formation of foam cells. Atherosclerosis is nowadays regarded as a low-grade chronic inflammatory disease, initiated by endothelial activation triggered by cardiovascular risk factors. 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. The latter is most specific for monocytes and lymphocytes, whereas neutrophils are absent in the atherosclerotic lesion until it is ruptured. However, upon activation, resident and recruited neutrophils may affect endothelial function via the production of pro-inflammatory cytokines and oxidative stress

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[18,21]. Hence, exaggerated and prolonged postprandial hyperlipidemia is an important characteristic of the diabetic dyslipidemia[18,22,23]. 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[24]. It has been shown that postprandial TG are better predictors of subclinical atherosclerosis than fasting TG concentrations[22,23,25]. 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[26]. Even in fasting normolipidemic subjects, increased postprandial lipemia has been linked to atherosclerosis[27-29]. 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[30-32].

Adipocyte fatty acid trapping

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[33]. 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[34]. Second, increased hepatic FFA delivery is a main determinant of VLDL secretion and postprandial lipemia[18,19]. 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[35]. In an animal model of CD36 deficient mice, increased hepatic FFA delivery has been linked to increased hepatic β-oxidation reflected in increased plasma levels of HBA[36]. Postprandial HBA appearance in plasma may, therefore, serve as a marker of postprandial hepatic FFA delivery. Third, FFA may also directly impair vasoreactivity[37].
Insulin and acylating-stimulating protein (ASP) are the principal determinants of adipocyte FFA trapping (*Figure 1*). Insulin inhibits the basal lipolytic rate by suppressing hormone-sensitive lipase (HSL) activity[38]. Complement component C3 (C3) is secreted by adipose tissue and is also involved in adipocyte FFA trapping. 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[39]. The effects of C3/ASP and insulin on adipocyte FFA trapping are additive and independent[40]. Chylomicrons are strong activators of adipocyte C3 production *in vitro*[41], and it has been shown that after a high-fat meal plasma C3 concentrations increase[42,43],

especially when insulin effects are blunted[44]. 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[45], leading to an exaggerated and prolonged postprandial C3 response[42], eventually resulting in abnormal diversion of FFA to the liver and VLDL overproduction.

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[46]. 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[47], but also in patients with HIV-lipodystrophy[48]. Moreover, low adiponectin levels are associated with a moderately increased CVD risk in diabetic men[49]. 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

Atherosclerosis is nowadays regarded as a low-grade chronic inflammatory disease, involving a series of highly specific cellular and molecular responses[50,51]. Atherogenesis is initiated by endothelial activation triggered by several cardiovascular risk factors[52]. 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 1*). A higher content of inflammatory cells in the atherosclerotic lesion renders the plaque vulnerable with an increased risk of rupture[53]. The importance of 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[54].

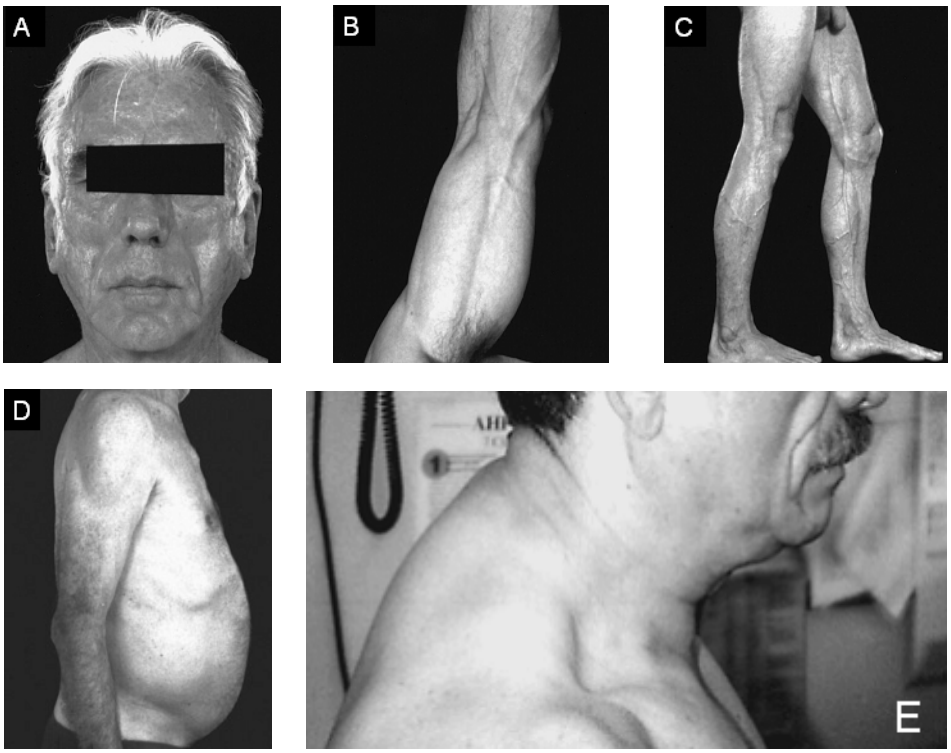
Markers of inflammation, such as the blood leukocyte count and C-reactive protein (CRP), are independent predictors of future CVD[55,56]. CRP is a sensitive acute-phase reactant produced by the liver in response to cytokines. IL-6 is the major cytokine responsible for hepatic CRP production and is itself also associated with CVD[57]. Even subjects with a low CRP concentration are at increased cardiovascular risk if they have a blood leukocyte count in the higher 25th percentile[58]. Differential leukocyte counts (monocytes and neutrophils) are also related to CVD[55]. Interestingly, the best association with CVD has been demonstrated for neutrophils[55]. Their role in the pathophysiology of atherosclerosis is not entirely clear, as these cells are absent in the atherosclerotic lesion until it is ruptured[59]. However, upon activation, resident and recruited neutrophils

may affect endothelial function via the production of pro-inflammatory cytokines and generation of oxidative stress[60]. The blood leukocyte count is increased in subjects with type 2 diabetes and impaired glucose tolerance[61,62]. In addition, type 2 diabetic patients have increased expression of leukocyte activation markers[61], 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[60,63,64]. 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[60]. 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[65]. 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[60]. In patients with type 2 diabetes, a significant rise in CRP levels was observed after ingestion of a high-fat meal[66]. 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

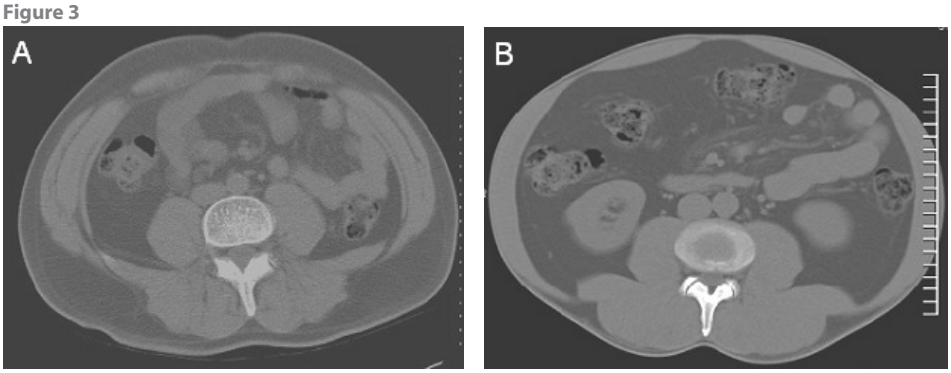
The introduction of HAART in 1996 has led to a dramatic decrease in AIDS-related mortality[11]. However, HAART is strongly associated with lipodystrophy and metabolic risk factors[12-17]. Lipodystrophy is characterized by changes in body fat distribution, including subcutaneous fat loss, intra-abdominal fat accumulation and development of a buffalo hump (*Figures 2 and 3*)[12-14]. 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[14]. 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). All three classes of antiretroviral agents may be 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[67]. The etiology of lipodystrophy appears to be multifactorial, including HIV drug inhibitory effects on adipocyte differentiation and alteration of

Figure 2



Pictures of HIV-lipodystrophy. Lipodystrophy is characterized by subcutaneous fat loss in the face (A), arms (B) and legs (C), and intra-abdominal (D) and dorsocervical (E) fat accumulation. Adapted from internet

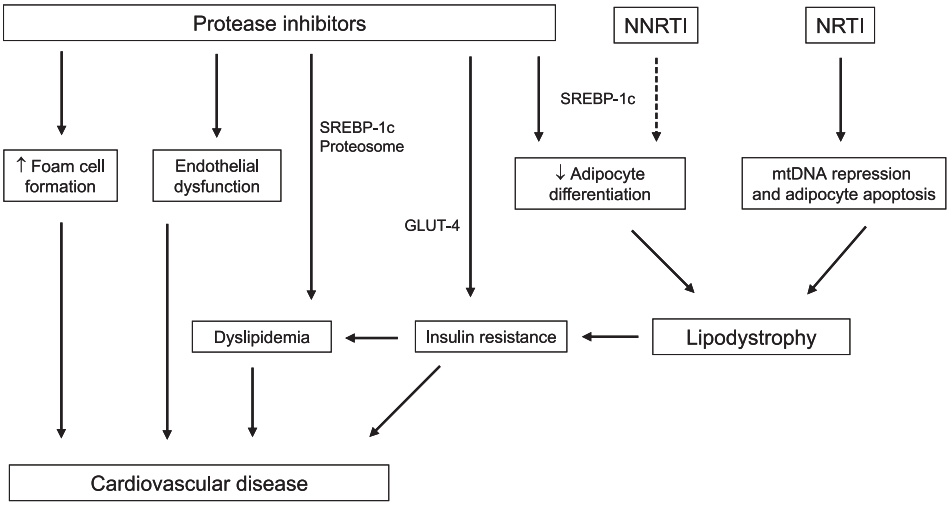
Figure 3



Body fat distribution using single-slice abdominal computer tomography (L4-L5) in a healthy subject (A) and a patient with HIV-lipodystrophy (B). Subcutaneous abdominal fat is low, while visceral abdominal fat is high in the patient with HIV-lipodystrophy

mitochondrial functions (*Figure 4*). For example, PIs impede adipocyte differentiation through altered expression and nuclear localization of sterol regulatory element-binding protein-1 (SREBP-1) and peroxisome proliferator-activated receptor-γ (PPAR-γ)[68,69], which are essential for adipogenesis. NRTIs may induce mitochondrial dysfunction and apoptosis of adipocytes by inhibition of mitochondrial DNA polymerase γ, and depletion of mitochondrial DNA[70].

Figure 4
Pathogenesis of HAART-associated lipodystrophy and cardiovascular risk in HIV-infected patients



The etiology of lipodystrophy is multifactorial, including HIV drug inhibitory effects on adipocyte differentiation (PIs and possibly also NNRTIs) and alteration of mitochondrial functions (NRTIs). Lipodystrophy is often accompanied by several metabolic risk factors, such as insulin resistance and dyslipidemia. Insulin resistance and dyslipidemia may result from direct effects of antiretrovirals (PIs), effects of HIV infection, or indirect effects, such as changes in body fat distribution. In addition, PI therapy has been associated with endothelial dysfunction and CD36-dependent cholesteryl ester uptake in macrophages

Lipodystrophy is often accompanied by several metabolic risk factors, such as insulin resistance, glucose intolerance and dyslipidemia[15-17]. Insulin resistance may result from direct effects of antiretrovirals, effects of HIV infection, or indirect effects, such as changes in body fat distribution[71]. For example, it has been shown that PIs induce insulin resistance in vitro by reducing insulin-mediated glucose uptake by glucose transporter 4[72]. In HIV-negative adults, PIs reduce insulin sensitivity as early as 4 weeks after administration, without changing body fat distribution[73,74]. 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[71,75,76]. Abnormalities in glucose tolerance have been recognized in more than one-third of the patients with lipodystrophy[15]. Basal lipolytic rates are generally increased in patients with HIV-lipodystrophy, suggesting impaired action of HSL[77]. In addition, several studies have reported elevated FFA levels following glucose

or insulin challenges[78,79], suggesting resistance to the action of insulin to suppression of lipolysis.

The natural course of HIV infection is characterized by changes in plasma triglycerides (TG), HDL-cholesterol, and LDL particle size[80]. 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[15-17]. 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[81]. 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[82]. Except for atazanavir, all PIs cause to some extent fasting hyperlipidemia[83]. PIs suppress the breakdown of the nuclear form of SREBP in the liver, resulting in increased TG and cholesterol biosynthesis[17]. In addition, PIs suppress the proteasomal breakdown of nascent apoB, leading to VLDL oversecretion[84]. 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[85,86]. 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[87,88].

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[89]. PIs may also promote atherosclerotic lesion formation independent of dyslipidemia by increasing CD36-dependent cholesteryl ester accumulation in macrophages[90]. 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[91,92]. IMT also progresses much more rapidly in HIV-infected patients than in non-HIV cohorts[93]. 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[94]. 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 been investigated to reduce atherogenic lipoproteins in HIV-infected patients[95]. 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[96]. There is thus an urgent need for agents that improve body fat distribution and cardiovascular risk in HIV-infected patients.

Insulin-sensitizing agents

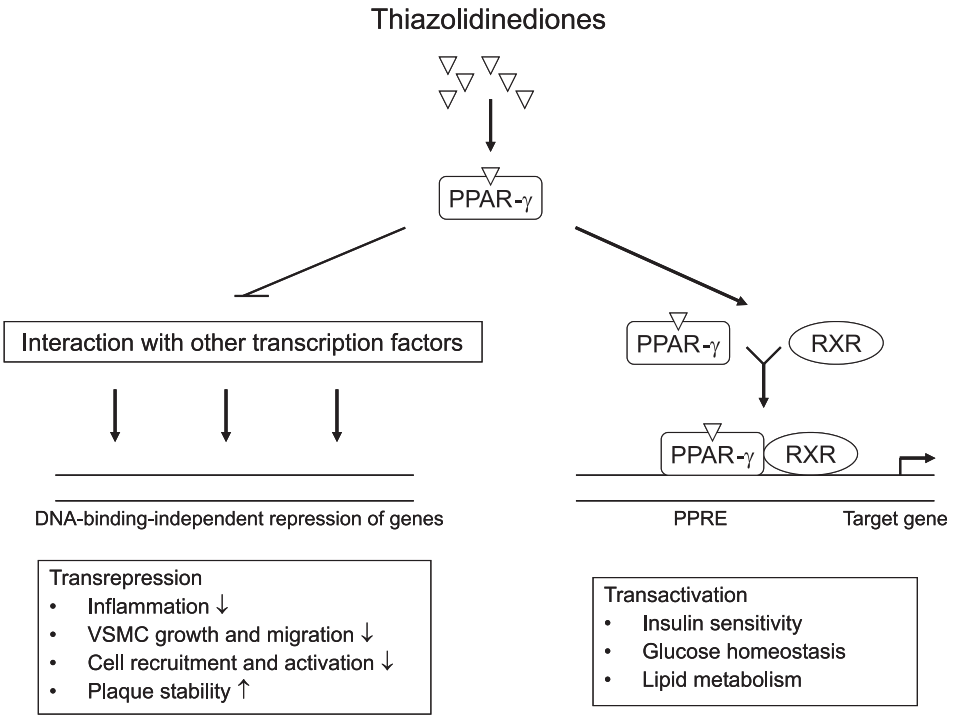
Biguanides and thiazolidinediones are used in clinical medicine to improve insulin sensitivity and glycemic control in patients with type 2 diabetes. In type 2 diabetes, both classes of drugs significantly modulate body fat distribution and several aspects of the metabolic syndrome to potentially retard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

Thiazolidinediones

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[97,98]. 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*)[97,98]. 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)[97,98].

Three PPARs (PPAR-α, PPAR-β/δ, and PPAR-γ) have been identified to date. PPAR-α is the main target for fibrates and regulates the expression of genes involved in lipid metabolism and inflammation. PPAR-β/δ stimulates FFA oxidation primarily in muscle but also in adipose tissue. PPAR-γ exhibits its regulatory effects primarily in adipocytes by interfering with insulin signaling, cytokine production, and FFA metabolism. Thiazolidinediones (TZDs) are synthetic ligands for PPAR-γ activation[97,98]. 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-γ 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-α and resistin)[98]. 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[98,99], an effect which would be desirable in patients with HIV-lipodystrophy. Interestingly, all of the major cell types in the vasculature also express PPAR-γ[100,101]. TZDs have interesting effects on these cells, which appear to be partially independent of the PPAR-γ-RXR mediated transcriptional effects[102]. There appears to be a generalized transrepression of inflammatory transcription in a DNA-binding-independent manner[102-104]. Direct beneficial vascular effects of TZDs include increased nitric oxide bio-availability[105], decreased leukocyte-endothelial cell interaction[106], reduced vascular smooth muscle cell migration and

Figure 5
Schematic overview of the metabolic and vascular actions of TZDs



The metabolic actions are mediated by receptor-dependent activation of the PPAR-γ-retinoid X receptor (RXR) complex and subsequent transcriptional activation of target genes involved in FFA metabolism and insulin signaling. In addition, TZDs can interact with other transcription factors in a DNA-binding–independent manner and exhibit anti-inflammatory properties by repressing gene expression. Eventually, both metabolic and vascular effects of TZDs may contribute to cardiovascular risk reduction

proliferation[107], and cholesterol efflux from macrophages[108]. Therefore, it is thought that TZDs, which were primarily introduced to improve glycemic control, may also have benefits on atherosclerotic disease progression.

Biguanides

Metformin, a biguanide, has been available for the treatment of type 2 diabetes for many years[109]. Over this period of time, it has become one of the most widely prescribed anti-hyperglycemic agent. Its mechanism of action involves reduction of hepatic insulin resistance and glucose output, leading to significant reductions in glucose and insulin levels[109,110]. 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[111,112]. 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[113]. Moreover, in the United Kingdom Prospective Diabetes Study, metformin decreased cardiovascular mortality in overweight type 2 diabetic patients[114].

Outline of the thesis

To compare the effects of rosiglitazone and pioglitazone on fasting lipid profile in patients with type 2 diabetes mellitus.

To study the effects of rosiglitazone on postprandial lipemia and inflammation in patients with type 2 diabetes mellitus.

To study postprandial fatty acid metabolism in relation to body fat distribution in HIV-infected patients.

To investigate functional and structural markers of atherosclerosis in relation to the presence of the metabolic syndrome in HIV-infected patients.

To compare the effects of rosiglitazone and metformin for treatment of HIV-lipodystrophy.

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Artorioscler Thromb Vasc Biol 2003; 23(10): 1744-9

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Thiazolidinediones and blood lipids in type 2 diabetes: a summary analysis

Abstract

Objective

We evaluated study population characteristics and treatment effects on blood lipids between studies in which either rosiglitazone (RSG) or pioglitazone (PIO) was investigated in patients with type 2 diabetes.

Research Design and Methods

We performed a summary analysis of all published double-blind, placebo-controlled studies with RSG (4 and 8 mg/d) and PIO (15, 30 and 45 mg/d). Data were analyzed using the random-effects model.

Results

Nineteen trials met our inclusion criteria yielding 5304 patients, 3236 in studies with RSG and 2068 in studies with PIO. Subjects treated with PIO were more obese and showed more pronounced hyperglycemia and dyslipidemia (increased triglycerides and decreased HDL-cholesterol) at baseline than subjects treated with RSG. Using weighted linear regression analysis, studies with PIO showed greater beneficial effects on triglycerides, total cholesterol and LDL-cholesterol, after adjustment for the respective lipid levels at baseline. RSG 8 mg/d showed greater increases in total cholesterol and LDL-cholesterol than RSG 4 mg/d. PIO 30 mg/d showed greater reductions in TG than PIO 15 mg/d.

Conclusions

Studies conducted with PIO show more beneficial effects on blood lipids, but also different study population characteristics in comparison with studies conducted with RSG. Both, differences in pharmacological properties between the agents and differences in study population characteristics, are likely to have influenced the results.

Introduction

Thiazolidinediones (TZDs) are oral anti-hyperglycemic agents that reduce insulin resistance in peripheral tissues and decrease hepatic glucose production[1]. TZDs are potent synthetic ligands for peroxisome proliferator-activated receptor gamma γ (PPAR-γ) activation, which mediates the physiological response by altering transcription of genes that regulate glucose and lipid metabolism[2-4]. Currently, there are two TZDs available: rosiglitazone (RSG) and pioglitazone (PIO). Troglitazone has been retracted from the market due to a substantially increased risk of severe hepatotoxicity[5-7]. The clinical potency of TZDs correlates closely with their PPAR-γ binding affinity. RSG has a greater PPAR-γ binding affinity than PIO, which translates to a clinical dose that is approximately 1/6th that of PIO[4,8]. Accordingly, the maximum recommended dose RSG (8 mg/d) corresponds with the maximum recommended dose PIO (45 mg/d), whereas the submaximum dose RSG (4 mg/d) corresponds with the submaximum dose PIO (30 mg/d).

The anti-hyperglycemic effects of RSG and PIO are well documented. RSG and PIO both demonstrate effective glycemic control when used as monotherapy and when used in combination with other anti-hyperglycemic agents[9-12]. TZDs also have important non-glycemic effects, such as modulation of lipid metabolism. It has been suggested that RSG and PIO differ in their effects on blood lipids and lipoproteins. Several studies have shown that treatment with PIO is associated with greater beneficial effects on blood lipid levels than treatment with RSG[13-16]. Since dyslipidemia is an important risk factor for atherosclerosis, differential therapeutic modulation of lipid levels may confer a different level of protection from cardiovascular disease in patients with type 2 diabetes. Several factors need to be considered when interpreting the effects of different TZDs on blood lipids. Firstly, differences between RSG and PIO may be related to specific pharmacological properties of these agents. It has been shown that, at the same clinical dose, PIO is associated with greater PPAR-α activation than RSG[17]. PPAR-α is the main target for fibrates, a class of lipid-lowering drugs, which mainly reduce TG and increase HDL-C[18,19]. Secondly, it is well recognized that the lipid-lowering responses of fibrates and statins are enhanced in patients with more pronounced dyslipidemia at baseline[20,21]. Baseline lipid levels may therefore influence the magnitude of treatment effects by TZDs.

We performed a summary analysis of all published double-blind, placebo-controlled studies to evaluate the effects of RSG and PIO on blood lipids in patients with type 2 diabetes. In addition, we critically evaluated study population characteristics between studies conducted with RSG and PIO.

Methods

Selection criteria

We used PUBMED (http://www.ncbi.nih.gov/entrez/query.fcgi) to search the Medline database until December 2002 to identify all double-blind, randomized, placebo-controlled studies evaluating the effects of RSG or PIO on blood lipids in patients with type 2 diabetes. The Medline database was searched for the following terms: "rosiglitazone" and "pioglitazone". These searches were combined with searches for the terms "type 2 diabetes" and "placebo". The search was limited to English-language publications. No age or sex restriction was applied. Forty-six publications were identified using this search strategy. Subsequently, all full-text papers were reviewed and studies were selected on the basis of a double-blind, placebo-controlled treatment period of at least 8 weeks with either RSG (doses 4 and 8 mg/day) or PIO (doses 15, 30 and 45 mg/day). Treatment effects on blood lipids should be reported by each study. Both TZD monotherapy and combination therapy with other anti-hyperglycemic agents (e.g. sulfonylureas, metformin or insulin) were considered eligible. Studies using combination therapy of TZDs with lipid-lowering interventions (lipid-lowering agents or active lifestyle interventions) were excluded from the analysis, although a concurrent weight-maintenance diet was considered acceptable, if applied equally to all intervention arms.

Data extraction

Both, assessment of eligibility and data extraction, were performed by a single non-blinded reviewer (JvW). The following information was extracted from each study: year of publication, sample size, gender distribution, participant age, TZD monotherapy or combination therapy, concurrent weight-maintenance diet, duration of treatment with study medication, BMI and blood lipid levels (triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C)), including mean changes of each lipid parameter from baseline. Data extraction was performed for the RSG-group, the PIO-group and the accompanying placebo-groups (RSG-placebo and PIO-placebo).

Statistical analysis

Chi-squared tests were performed to test for heterogeneity of study results. Because the studies came from various geographic areas, between-study variation could be expected. Hence, all data were combined using the random-effects model of DerSimonian and Laird[22]. The random-effects model weights studies according to the sample size, the within-study variance and the between-study variance. Weights were set equal to the reciprocal of the variance. We compared study population characteristic, between different TZDs (RSG versus PIO) and between TZDs and placebo (RSG versus placebo and PIO versus placebo). In addition, we compared treatment effects on blood lipids

(mean absolute changes from baseline) between RSG and PIO. We also performed a weighted linear regression analysis for each lipid parameter (triglycerides, total cholesterol, HDL-C and LDL-C) to compare the post-treatment blood lipid levels between studies in which either RSG or PIO was used, after adjustment for the respective lipid level at baseline. In this analysis, the post-treatment blood lipid level was used as dependent variable and the baseline blood lipid level was used as independent variable. For statistical analysis, we used SPSS version 10.0 (SPSS Inc. Chicago). Statistical significance was reached if $p<0.05$ (two-sided).

Results

Study characteristics

Nineteen trials met our inclusion criteria yielding 5304 patients; 3236 patients in studies with RSG (*Table 1*)[11,12,23-31] and 2068 patients in studies with PIO (*Table 2*)[9,10,32-37]. RSG-trials and PIO-trials were comparable in gender distribution. Subjects in RSG-trials were older than subjects in PIO-trials. Sixty-six percent of the subjects in the RSG-trials received the study medication as monotherapy, whereas only 27% of the subjects in the PIO-trials received the study medication as monotherapy. A concurrent weight-maintenance diet was more prevalent in PIO-trials than in RSG-trials (52% versus 34%, respectively). Fifty-six percent of the RSG-group and 8% of the PIO-group received the maximum recommended dose (8 mg/d for RSG and 45 mg/d for PIO, respectively). Fifty-seven percent received PIO 30 mg/d and 35% PIO 15 mg/d. Mean duration of treatment was 22 weeks in the RSG-trials and 18 weeks in PIO-trials.

Baseline characteristics

The baseline characteristics of the RSG-group, PIO-group and accompanying placebo-groups (RSG-placebo and PIO-placebo) are shown in *Table 3*. Subjects in the PIO-group were significantly younger and more obese than subjects in the RSG-group. In addition, subjects in the PIO-group were characterized by a more pronounced hyperglycemia (increased fasting glucose and HbA1c) and dyslipidemia (increased TG and decreased HDL-C) than subjects in the RSG-group. There were no differences in baseline characteristics between TZDs and the accompanying placebo-groups (RSG versus RSG-placebo and PIO versus PIO-placebo, respectively).

Treatment effects of RSG and PIO

Chi-squared tests revealed no statistical evidence of heterogeneity of study results (data not shown). The treatment effects of RSG and PIO on blood lipids are shown in *Figure 1*. The treatment effects are shown as mean changes from baseline of the TZD minus placebo for each lipid parameter (delta RSG-placebo and delta PIO-placebo, respectively). PIO was associated with significantly greater beneficial effects on all blood lipid levels.

Table 1
General characteristics of studies with rosiglitazone

Study reference	Journal	Total sample size (% females)	Mono-therapy	Weight-maintenance diet	Mean age (Years)	Treatment dose (mg/d)	Duration of treatment
Miyazaki Y et al. [23]	Diabetologia, 2001	29 (45%)	Yes	Yes	55,1	8	12 weeks
Raskin P et al. [25]	Diabetes Care, 2001	313 (44%)	No	No	56,8	4 and 8	26 weeks
Lebovitz HE et al. [11]	J Clin Endocrinol Metab, 2001	493 (34%)	Yes	Yes	60,0	4 and 8	26 weeks
Phillips LS et al. [26]	Diabetes Care, 2001	908 (37%)	Yes	No	57,5	4 and 8	26 weeks
Raskin P et al. [24]	Diabetologia, 2000	208 (39%)	Yes	No	58,5	4 and 8	8 weeks
Nolan JJ et al. [27]	Diabetic medicine, 2000	278 (38%)	Yes	No	62,6	4 and 8	8 weeks
Wolffenbuttel BHR et al. [12]	Diabetic Medicine, 2000	375 (44%)	No	No	61,3	4	26 weeks
Gómez-Perez FJ et al. [29]	Diab Met Res Rev, 2002	105 (74%)	No	No	53,1	4 and 8	26 weeks
Fonseca V et al. [28]	JAMA, 2000	339 (32%)	No	Yes	58,2	4 and 8	26 weeks
Patel J et al. [30]	Diabetes Obes Metab, 1999	155 (31%)	Yes	Yes	58,3	4	12 weeks
Carey DG et al. [31]	Obesity Research, 2002	33 (18%)	Yes	Yes	56.1	8	16 weeks
Pooled characteristics		3236 (39%)	66%	34%	58.6	56% max dose	22 weeks

Table 2
General characteristics of studies with pioglitazone

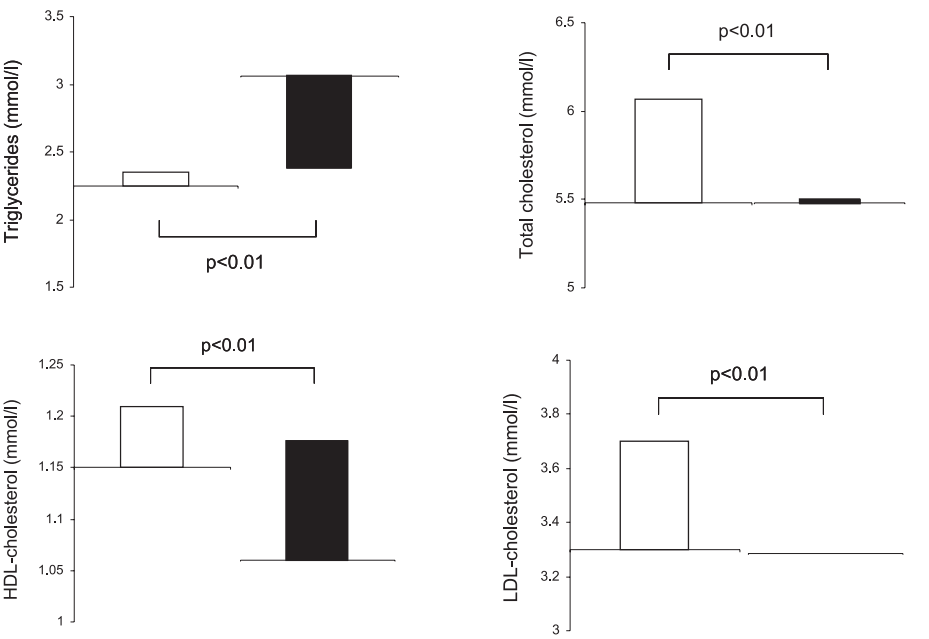
Study reference	Journal	Total sample size (% females)	Mono-therapy	Weight-maintenance diet	Mean age	Treatment dose (mg/d)	Duration of treatment
Miyazaki et al. [32]	Diabetes Care, 2001	23 (26%)	No	Yes	54,5	45	16 weeks
Kawamori et al. [33]	Diab Res Clin Pract, 1998	30 (37%)	No	No	54,8	30	12 weeks
Einhorn D et al. [34]	Clinical Therapeutics, 2000	328 (43%)	No	Yes	55,6	30	16 weeks
Aronoff S et al. [9]	Diabetes Care, 2000	319 (42%)	Yes	No	53,7	15, 30 and 45	26 weeks
Miyazaki Y et al. [35]	Diabetes Care, 2002	45 (47%)	Yes	No	54,7	15, 30 and 45	26 weeks
Rosenblatt S et al. [36]	Coron Artery Dis, 2001	197 (47%)	Yes	Yes	54,5	30	16 weeks
Kipnes MS et al. [10]	Am J Med, 2001	560 (41%)	No	Yes	56,7	15, 30	16 weeks
Rosenstock J et al. [37]	Int J Clin Pract, 2002	566 (53%)	No	No	57.1	15, 30	16 weeks
Pooled characteristics		2068 (45%)	27%	52%	55,8	8% max dose	18 weeks

Table 3
Baseline characteristics

	RSG studies		PIO studies	
	RSG-group	RSG-placebo	PIO-group	PIO-placebo
Number of patients (n)	2194	1042	1328	740
Age (years)	58.6 (0.2) *	58.9 (0.3) #	55.8 (0.27)	56.2 (0.35)
BMI (kg/m^2)	29.7 (0.09) *	29.6 (0.13) #	32.2 (0.22)	32.0 (0.20)
Glucose (mmol/l)	12.01 (0.07) *	11.63 (0.09) #	13.35 (0.10)	13.24 (0.14)
HbA1c (%)	9.0 (0.03) *	8.9 (0.05) #	9.8 (0.04)	9.8 (0.05)
Triglycerides (mmol/l)	2.25 (0.04) *	2.09 (0.05) #	2.92 (0.08)	2.94 (0.11)
Cholesterol (mmol/l)	5.48 (0.02)	5.46 (0.03)	5.49 (0.03)	5.70 (0.03)
HDL-C (mmol/l)	1.15 (0.01) *	1.15 (0.01) #	1.07 (0.01)	1.08 (0.01)
LDL-C (mmol/l)	3.30 (0.02)	3.31 (0.03)	3.25 (0.03)	3.28 (0.04)

All data were analyzed using the random-effects model. Data are mean (SE).
In this analysis, comparisons between different TZDs (RSG versus PIO) and between different placebo groups (RSG-placebo versus PIO-placebo) were performed. * p<0.001 versus PIO-group; # p<0.001 versus PIO-placebo

Figure 1



The mean treatment effects of TZDs minus the mean treatment effect of the accompanying placebo for each lipid parameter (RSG = white bars; PIO = black bars).

Influence of baseline lipid levels on treatment effects

Since subjects in studies with PIO were more dyslipidemic at baseline than subjects in studies with RSG, we performed a weighted linear regression analysis for each lipid parameter. Using this analysis, post-treatment TG (β=0.45, $p<0.01$), TC (β=0.56, $p<0.001$) and LDL-C (β=0.31, $p<0.05$) were higher in studies with RSG than in studies with PIO. However, post-treatment HDL-C was not significantly different between RSG and PIO (β=0.02, NS).

Treatment effects of RSG and PIO per treatment dose

Treatment with maximum recommended TZD dose was more prevalent in the RSG-group than in the PIO-group. Therefore, we performed a subgroup analysis in which we evaluated the effects of RSG and PIO on blood lipids per treatment dose (*Table 4*). The maximum and submaximum recommended doses RSG (8 and 4 mg/d, respectively) had similar effects on TG and HDL-C. However, RSG 8 mg/d was associated with significantly greater increases in TC and LDL-C compared with RSG 4 mg/d. PIO 30 mg/d was associated with significantly greater reductions in TG than PIO 15 mg/d. The different doses of PIO had comparable effects on TC, HDL-C and LDL-C.

Table 4
Treatment effects of RSG and PIO per treatment dose

	RSG 4 mg/d	RSG 8 mg/d
Δ Triglycerides (mmol/l)	+ 0.13 (0.06)	+ 0.05 (0.07)
Δ Cholesterol (mmol/l)	+ 0.52 (0.04) *	+ 0.70 (0.04)
Δ HDL-C (mmol/l)	+ 0.05 (0.01)	+ 0.06 (0.01)
Δ LDL-C (mmol/l)	+ 0.34 (0.03) *	+ 0.48 (0.04)

* $p<0.05$ versus RSG 8 mg/d

	PIO 15 mg/d	PIO 30 mg/d	PIO 45 mg/d
Δ Triglycerides (mmol/l)	- 0.44 (0.08) #	- 0.66 (0.07)	- 0.38 (0.18)
Δ Cholesterol (mmol/l)	- 0.01 (0.06)	+ 0.01 (0.05)	+ 0.10 (0.15)
Δ HDL-C (mmol/l)	+ 0.10 (0.02)	+ 0.09 (0.02)	+ 0.11 (0.04)
Δ LDL-C (mmol/l)	+ 0.08 (0.06)	- 0.01 (0.04)	+ 0.15 (0.12)

# $p<0.05$ versus PIO 30 mg/d

Δ Triglycerides, Δ Cholesterol, Δ HDL-C and Δ LDL-C is the difference in concentration for triglycerides, total cholesterol, HDL-C and LDL-C, respectively, between the active treatment and placebo group for each specific TZD dose. Data are mean (SE).

Subgroup analysis of monotherapy trials and combination therapy trials

Since monotherapy was more prevalent in studies with RSG, we evaluated treatment effects of RSG and PIO on blood lipids for monotherapy trials and combination therapy trials separately (*Table 5*). RSG combination therapy trials showed greater beneficial

Table 5
Treatment effects of RSG and PIO for monotherapy trials and combination therapy trials

Rosiglitazone trials

	Monotherapy	Combination therapy
Δ Triglycerides (mmol/l)	+ 0.21 (0.06) *	- 0.06 (0.07)
Δ Cholesterol (mmol/l)	+ 0.68 (0.03) *	+ 0.46 (0.05)
Δ HDL-C (mmol/l)	+ 0.03 (0.01) *	+ 0.11 (0.01)
Δ LDL-C (mmol/l)	+ 0.43 (0.03) *	+ 0.33 (0.04)

* p<0.05 versus combination therapy

Pioglitazone trials

	Monotherapy	Combination therapy
Δ Triglycerides (mmol/l)	- 0.51 (0.09) #	- 0.57 (0.06) #
Δ Cholesterol (mmol/l)	+ 0.05 (0.07) #	- 0.01 (0.05) #
Δ HDL-C (mmol/l)	+ 0.09 (0.02) #	+ 0.10 (0.01)
Δ LDL-C (mmol/l)	+ 0.07 (0.06) #	+ 0.02 (0.04) #

# p<0.05 versus Rosiglitazone-trials

Δ Triglycerides, Δ Cholesterol, Δ HDL-C and Δ LDL-C is the difference in concentration for triglycerides, total cholesterol, HDL-C and LDL-C, respectively, between the active treatment and placebo group. Data are mean (SE).

effects on all lipid levels than RSG monotherapy trials. PIO combination therapy trials showed similar effects on blood lipids compared with PIO monotherapy trials. PIO monotherapy trials showed greater beneficial effects on all lipid levels compared with RSG monotherapy trials, whereas PIO combination therapy trials showed greater beneficial effects on TG, TC and LDL-C than RSG combination therapy trials.

Discussion

In clinical practice, there is much debate concerning potential different effects of RSG and PIO on blood lipids. This may have important implications, since dyslipidemia is a major risk factor for atherosclerosis in patients with type 2 diabetes. Since no data on prospective, randomized, double-blind, PIO versus RSG studies are available, we performed a summary analysis of all published double-blind, placebo-controlled studies with either RSG or PIO. The main outcome of our summary analysis is that studies with PIO showed more beneficial treatment effects on blood lipids in comparison with studies with RSG, but important differences in baseline characteristics exist between the study populations.

During the last years, TZDs have received increasing attention for the treatment of patients with type 2 diabetes. The anti-hyperglycemic effects of RSG and PIO are well documented and appear to be equivalent between comparable doses of the two

agents[13]. In addition to glucose lowering, TZDs influence lipid metabolism, most likely by directing a PPAR-γ mediated change in adipocyte metabolism and insulin sensitivity. Hence, TZDs could potentially modulate the characteristic diabetic dyslipidemia, which is characterized by increased TG, reduced HDL-C and the predominance of atherogenic small, dense LDL particles[38].

We found that studies with PIO show greater beneficial effects on TG, TC and LDL-C than studies with RSG. Whether the magnitude of these differences is sufficient to produce clinically relevant cardiovascular benefits is an open question. The current available data support a dose-dependent effect of RSG on TC and LDL-C, whereas PIO may exert dose-related effects on TG. However, only a small number of subjects was receiving the maximum recommended dose PIO.

Studies with PIO showed greater beneficial effects on TG than studies with RSG. Several factors may explain the differential effects of RSG and PIO on TG levels. First, it has been shown that, at the same clinical dose, PIO is associated with greater PPAR-α activation than RSG[17]. PPAR-α is the main target for fibrates, a class of lipid-lowering drugs, which mainly reduce TG and increase HDL-C[18,19]. Increased PPAR-α activation by PIO may explain the observed beneficial effects of PIO on TG. Second, it is well recognized that the lipid-lowering responses are partly dependent on the baseline characteristics of the study group. The lipid-lowering responses of fibrates and statins are enhanced in patients with more pronounced dyslipidemia at baseline[20,21]. In our summary analysis, we have shown that subjects treated with PIO were characterized by a more pronounced dyslipidemia (increased TG and decreased HDL-C) at baseline than subjects treated with RSG. These differences in patient baseline characteristics between studies with RSG and PIO are likely to have influenced the magnitude of the effects on TG and HDL-C. The observation that after adjustment for baseline HDL-C, there was no longer a statistically significant difference in post-treatment HDL-C between RSG and PIO, supports this hypothesis. Moreover, in a recent study with PIO, it was shown that patients with the lowest baseline HDL-C levels responded with HDL-C increases of greater magnitude than did those who had higher HDL-C levels at baseline[36]. Studies with RSG showed greater increases in TC and LDL-C compared with studies with PIO, despite similar baseline levels. Why RSG and PIO exert different effects on TC and LDL-C is an open issue. Interestingly, TZDs improve LDL particle density, causing a shift cause a shift from small, dense LDL particle to larger, buoyant LDL particles, which are less prone to oxidative modification and are therefore thought to be less atherogenic[39-42]. These changes in LDL-C density elicited by TZDs may be more meaningful than the small changes in the overall LDL-C levels.

Besides differences in baseline lipids, subjects treated with PIO were more obese and had worse glycemic control at baseline than subjects treated with RSG. In addition, a concurrent weight-maintenance diet was more prevalent in PIO-trials than in RSG-trials, whereas more subjects in RSG-trials were on monotherapy. These factors may also have influenced the results. Interestingly, RSG combination therapy trials showed

greater beneficial effects on all blood lipids compared with RSG monotherapy trials. These differences were not observed in studies with PIO. Since monotherapy was more prevalent in RSG-trials, this could have contributed to the results. Regrettably, the number of studies was limited and we could not adjust for the other relevant parameters (e.g. BMI, glycemic control), in order to more reliably estimate differences in treatment effects between studies with RSG and PIO. Although differences in study population characteristics were a confounding factor for our analysis, it should be noted that this is also one of the most interesting findings, that is often not accounted for when discussing differential effects of TZDs in clinical practice. Apparently, studies with RSG are performed in a 'different patient population' than studies with PIO. Our results emphasize the importance of study population characteristics when examining clinical data from studies performed with different TZDs. Clearly, there is a need for a direct, double-blind comparisons of the two agents in the same population.

Our data are in line with several open-label prospective or retrospective studies on effects of RSG and PIO on blood lipids[14,15]. Khan et al. performed an open-label, randomized comparison of RSG and PIO in patients previously treated with troglitazone[14]. In that study, conversion to pioglitazone was associated with significant improvements in all lipid levels, whereas conversion to RSG led to significant increases in all lipid levels, despite similar weight increase and glycemic control in the RSG-group and PIO-group. In a recent retrospective review of randomly selected medical records, it was shown that treatment with PIO was associated with greater beneficial effects on blood lipid levels than treatment with RSG, despite similar glycemic control[13]. However, this article fails to keep into account a large body of double-blind, randomized, placebo-controlled studies, which represent the gold standard for clinical analysis.

In conclusion, studies conducted with PIO show more beneficial effects on blood lipids, but also different study population characteristics in comparison with studies conducted with RSG. Both, differences in pharmacological properties between the agents and differences in study population characteristics, are likely to have influenced the results. When examining the available clinical data from studies performed with different TZDs, it is important to interpret the results in light of the prevailing study population characteristics.

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Diabetes Care 2005; 28(4): 844-9

3

Rosiglitazone improves postprandial triglyceride and free fatty acid metabolism in type 2 diabetes

Abstract

Objective

Increased postprandial lipemia is part of the diabetic dyslipidemia and is associated with accelerated atherosclerosis. We investigated the effects of the peroxisome proliferator-activated receptor-γ agonist rosiglitazone on postprandial lipemia in patients with type 2 diabetes.

Research Design and Methods

A randomized, 8-week, cross-over, placebo-controlled, double-blind trial was performed in which rosiglitazone at 4 mg was administrated twice daily in 19 patients with type 2 diabetes. Standardized 6-h oral fat-loading tests were performed after each treatment period. Postprandial curves were calculated as the total area under the curves (AUC) and the incremental area under the curves (dAUC).

Results

Rosiglitazone did not change fasting plasma triglycerides compared with placebo (1.97±0.22 vs. 1.88±0.20 mmol/l, respectively), but decreased postprandial triglyceride levels, leading to significantly lower triglyceride dAUC (-37%, $p<0.05$), without changing total triglyceride AUC. Significant postprandial triglyceride reductions in the chylomicron fraction (Svedberg flotation rate [Sf] > 400) were achieved with rosiglitazone, which resulted in a significant lower triglyceride AUC (-22%) in this fraction. The postprandial triglyceride rise in VLDL1 (Sf 60-400) was also lower after rosiglitazone (-27%), but this did not result in a significant lower triglyceride AUC. In VLDL2 (Sf 20-60), there were no significant differences in triglyceride AUC and triglyceride dAUC between rosiglitazone and placebo. Rosiglitazone decreased free fatty acid (FFA) AUC (-12%) and FFA dAUC (-18%) compared with placebo.

Conclusions

Rosiglitazone improves the metabolism of large triglyceride-rich lipoproteins and decreases postprandial FFA concentrations in type 2 diabetes. This may have clinical implications as these effects may contribute to cardiovascular risk reduction.

Introduction

Dyslipidemia is one of the main cardiovascular risk factors in type 2 diabetes[1]. An increased hepatic free fatty acid (FFA) flux has been postulated as a major contributor of diabetic dyslipidemia, because it could lead to hepatic overproduction of triglyceride(TG)-rich lipoproteins (TRL)[1]. It is important to realize that humans are non-fasting most part of the day and non-fasting TG are also predictors of atherosclerosis[2]. Exaggerated and prolonged postprandial hyperlipidemia is an important characteristic of the diabetic dyslipidemia[1-3]. Several studies have shown that, even in fasting normolipidemic subjects, impaired clearance of TRL and their remnants is linked to atherosclerosis[4-9]. Therefore, therapeutic modulation of postprandial lipemia may convey increased protection from atherosclerosis.

Thiazolidinediones (TZDs) are oral anti-hyperglycemic agents that reduce insulin resistance in peripheral tissues and decrease hepatic glucose production[10]. TZDs are potent synthetic ligands for peroxisome proliferator-activated receptor γ (PPAR-γ) activation, thereby directly influencing the transcription of genes that regulate insulin sensitivity[11]. In addition to glucose lowering, TZDs modulate lipid metabolism most likely by directing a PPAR-γ mediated change in adipocyte metabolism. Rosiglitazone, a PPAR-γ agonist, generally increases LDL-cholesterol and HDL-cholesterol[12]. Rosiglitazone decreases fasting plasma FFA levels[13], probably due to improved peripheral fat storage, but it has only minor effects on fasting plasma TG[12]. Nevertheless, rosiglitazone may improve postprandial TG clearance by stimulating lipoprotein lipase (LPL)-mediated lipolysis[14,15]. We conducted a double-blind, placebo-controlled cross-over trial to investigate the effects of rosiglitazone on postprandial lipemia in type 2 diabetes.

Methods

Subjects

Non-smoking males and non-fertile females aged 35 to 70 years with documented type 2 diabetes were considered eligible. Patients on insulin treatment were excluded. All patients were treated with oral anti-hyperglycemic agents, which continued during the study. Exclusion criteria were current or previous treatment with TZDs, HbA1c > 9%, serum creatinin > 200 μmol/l, abnormal thyrotropin (TSH), aspartate aminotransferase (AST) or alanine aminotransferase (ALT) $\geq$ 2 times the upper limit of normal, congestive cardiac failure, blood pressure >160/>95 mmHg, total cholesterol > 8 mmol/l and/or fasting TG > 5 mmol/l and an alcohol intake > 3 units/day. The study protocol was approved by the local research ethics committee of the University Medical Center Utrecht. All participants gave written informed consent.

Study design

The study was designed as a randomized, cross-over, placebo-controlled, double-blind trial. Eligible patients were randomly assigned to receive rosiglitazone 4 mg twice daily or placebo in addition to their current oral anti-hyperglycemic agents for 8 weeks. A 6-week wash-out period was included between the 2 treatment periods. At the end of each treatment period a standardized 6-h oral fat loading test was carried out. At the beginning and at the end of each 8-week treatment period, anthropometric and fasting laboratory parameters were determined. Patients were instructed to fast at least 12-h prior to each visit. No study medication or other medication was used on the morning of the study days.

Oral fat loading test and separation of lipoproteins

After placing a cannula for venous blood sampling, subjects rested for 30 minutes before administration of the fat load. Fresh cream (a 40% weight/volume fat emulsion representing a total energy content of 3700 kcal/L) was ingested within 5 minutes at a dose of 50 g fat and 3.75 g glucose per m^2 body surface[9,16]. Participants remained supine during each test and were only allowed to drink mineral water. Peripheral blood samples were obtained in sodium EDTA (2 mg/mL), kept on ice and centrifuged immediately for 15 minutes at 800 *g* at 4 °C, then plasma was stored at -80 °C. Lipoproteins were subfractionated by ultracentrifugation as described previously in detail[16,17]. Consecutive runs were carried out to float Svedberg flotation rate (Sf) > 400 (chylomicrons), Sf 60-400 (VLDL1), Sf 20-60 (VLDL2), Sf 12-20 (IDL) and Sf 2-12 (LDL).

Analytical methods

Total cholesterol, HDL-cholesterol obtained after precipitation with phosphotungstate/$MgCl_2$, and TG were measured in duplicate by colorimetric assay with the CHOD-PAP and GPO-PAP kits, respectively (Roche diagnostics, Mannheim, Germany). FFA were measured by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). For FFA measurement, a lipase inhibitor was added to the plasma in order to block ex vivo lipolysis. Total plasma apolipoprotein B (apoB) was measured by nephelometry using apoB monoclonal antibodies. Glucose, creatinin, TSH, AST and ALT were measured by standard enzymatical laboratory methods. Insulin was measured by ELISA (Mercodia, Uppsala, Sweden). For estimation of insulin sensitivity the HOMA index (= glucose*insulin/22.5) was calculated.

Statistical analysis

All values are expressed as mean ± SEM in the text, tables and figures. The area under the curve (AUC) for TG and FFA were calculated by the trapezoidal rule using GraphPad Prism version 4.0 (LA, USA). Incremental integrated AUCs (dAUC) were also calculated after correction for baseline values. Differences between rosiglitazone and placebo

were analyzed by paired t-test. During serial measurements, time effects when compared with t=0, were tested using repeated measures ANOVA with Bonferroni correction for multiple comparisons. Bivariate correlations were calculated using Spearman's correlation coefficients. Calculations were performed using SPSS/PC + 11.5 (SPSS Inc. Chicago, IL, USA). Statistical significance was taken at the 5% level. A reduction in TG-dAUC of 20% was considered clinically relevant. Power-analysis with β=0.10 and α=0.05 revealed that 15 patients had to be included to find such a reduction. Since a small drop-out was expected, we aimed to include 20 patients.

Results

General characteristics

In total, 22 diabetic patients were screened. Two patients were excluded after the screening visit because of abnormal TSH and HbA1c > 9%. One patient withdrew informed consent during the study. General characteristics of the 19 remaining participants are listed in *Table 1*. All patients were using oral anti-hyperglycemic agents which was unchanged during the study. Eight patients were treated for dyslipidemia with statins (4 with simvastatin and 4 with atorvastatin). ACE inhibitors (n=3), a β-blocking agent (n=1), a calcium antagonist (n=1) and a diuretic (n=1) were used in six patients

Table 1
General characteristics and baseline fasting metabolic profile of the study group (n=19)

Male/Female	14/5
Age (years)	60 (1)
BMI (kg/m^2)	29.2 (1.1)
Waist (cm)	101 (2)
Systolic blood pressure (mmHg)	144 (3)
Diastolic blood pressure (mmHg)	86 (2)
Glucose (mmol/l)	7.8 (0.4)
HbA1c (%)	6.2 (0.2)
Anti-hyperglycemic therapy	
SU only	6 (32%)
Metformin only	5 (26%)
Combination metformin + SU	8 (42%)
Total cholesterol (mmol/l)	4.9 (0.2)
HDL-cholesterol (mmol/l)	0.99 (0.08)
LDL-cholesterol (mmol/l)	2.99 (0.16)
Triglycerides (mmol/l)	1.95 (0.22)

SU = sulfonylureum derivative

with hypertension. Rosiglitazone was well tolerated and no patient showed significant side effects other than headache, dizziness and gastrointestinal complaints (n=3). Rosiglitazone significantly reduced ALT compared with placebo (36±2 versus 42±4 U/l, p<0.05). Significant decreases hemoglobin and hematocrit were also observed after treatment with rosiglitazone (data not shown).

Effects of rosiglitazone on fasting metabolic parameters

Rosiglitazone significantly decreased fasting plasma glucose (6.2±0.3 versus 7.2±0.5, p<0.01) and HOMA (2.06±0.35 versus 3.76±0.50, p<0.01) compared with placebo, but did not change HBA1c (6.2±0.6 versus 6.3±0.7%). The effects of rosiglitazone and placebo on fasting lipoprotein profile are listed in *Table 2*. Rosiglitazone increased total cholesterol, due to a significant increase in LDL-cholesterol, leading to an increased non-HDL cholesterol compared with placebo.

Table 2
Effects of rosiglitazone and placebo on fasting and postprandial lipids

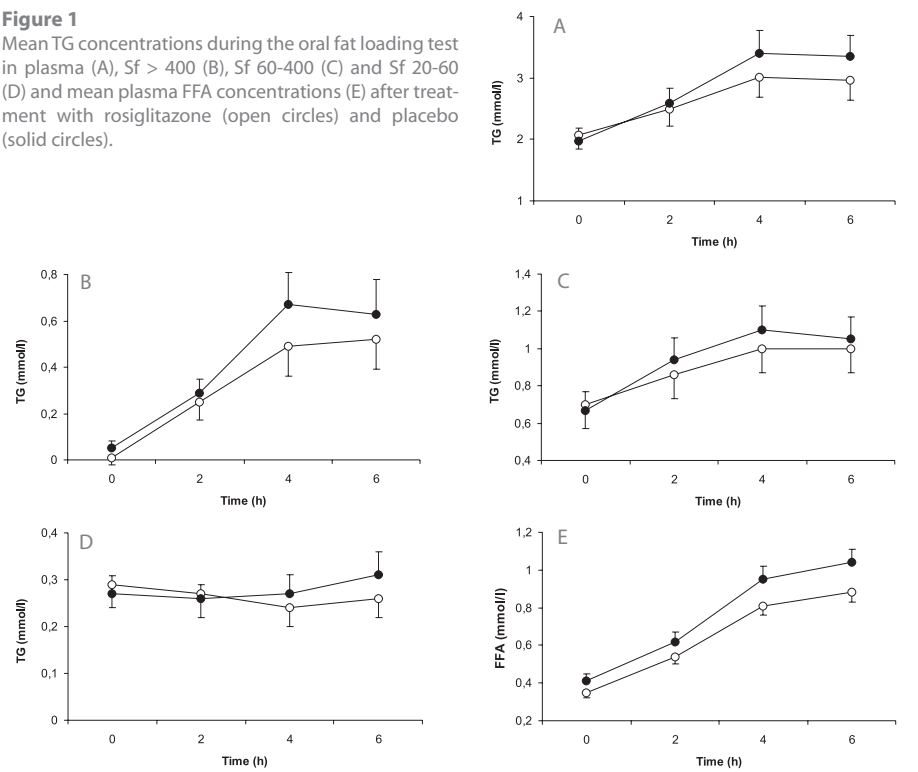
	Rosiglitazone	Placebo
Fasting cholesterol (mmol/l)		
Plasma	5.39 (0.24) *	4.96 (0.20)
Sf > 400 (chylomicron)	0.03 (0.01)	0.02 (0.01)
Sf 60-400 (VLDL1)	0.25 (0.04)	0.24 (0.04)
Sf 20-60 (VLDL2)	0.26 (0.04)	0.23 (0.03)
Sf 12-20 (IDL)	0.42 (0.06)	0.37 (0.05)
Sf 2-12 (LDL)	3.45 (0.20) *	3.14 (0.15)
HDL	1.05 (0.21)	0.98 (0.09)
Fasting plasma TG (mmol/l)	1.97 (0.22)	1.88 (0.20)
Apolipoprotein B (g/l)	0.90 (0.05)	0.86 (0.04)
Total cholesterol/HDL	5.63 (0.40)	5.54 (0.34)
Non-HDL cholesterol (mmol/l)	4.34 (0.23) *	3.98 (0.17)
Plasma TG-AUC (mmol.h/l)	14.7 (1.7)	16.0 (1.8)
Plasma TG-dAUC (mmol.h/l)	3.04 (0.65) *	4.82 (0.77)
Sf > 400 TG-AUC (mmol.h/l)	2.01 (0.50) *	2.59 (0.54)
Sf > 400 TG-dAUC (mmol.h/l)	1.83 (0.38) *	2.28 (0.44)
Sf 60-400 TG-AUC (mmol.h/l)	5.18 (0.81)	5.74 (0.70)
Sf 60-400 TG-dAUC (mmol.h/l)	1.25 (0.20) *	1.73 (0.29)
Sf 20-60 TG-AUC (mmol.h/l)	1.52 (0.25)	1.64 (0.21)
Sf 20-60 TG-dAUC (mmol.h/l)	-0.11 (0.11)	0.05 (0.08)
FFA-AUC (mmol.h/l)	3.80 (0.22) *	4.36 (0.30)
FFA-dAUC (mmol.h/l)	1.80 (0.20) *	2.24 (0.27)

Sf = Svedberg flotation rate
* p < 0.05 compared with placebo

Effects of rosiglitazone on postprandial TG and FFA

Rosiglitzone did not change fasting TG in plasma, or in the chylomicron, VLDL1 and VLDL2 fractions compared with placebo. After rosiglitazone, the postprandial TG increase in plasma was lower compared with placebo (*Figure 1*), which resulted in a significant lower TG-dAUC (*Table 2*). Total plasma TG-AUC was not different between rosiglitazone and placebo. A significant reduction in the postprandial TG content of the chylomicron fraction was achieved with rosiglitazone, which resulted in a significant lower TG-AUC (-22%, $p<0.05$) in this fraction. The postprandial TG rise in VLDL1 was also lower after rosiglitazone (-27%, $p<0.05$), but this did not result in a significant lower TG-AUC. In VLDL2, there were no significant differences in TG-AUC and TG-dAUC between rosiglitazone and placebo. Fasting FFA levels tended to be lower after treatment with rosiglitazone compared with placebo (0.35±0.03 versus 0.41±0.03 mmol/l, respectively, $p=0.06$). Rosiglitazone significantly decreased FFA-dAUC (-18%, $p<0.05$) compared with placebo, leading to significantly lower FFA-AUC (-12%, $p<0.05$). The reductions in FFA-AUC and TG-dAUC upon treatment with rosiglitazone were not related to the reduction of fasting plasma glucose ($r=0.14$ and $r=0.07$, respectively), but they were significantly related to the reduction of ALT ($r=0.53$ and $r=0.42$, respectively, $p<0.05$). A subgroup analysis of subjects on (n=8) and off (n=11) statin treatment showed similar effects of rosiglitazone on plasma TG-dAUC (-42% and-34%, respectively) and FFA-AUC (-14% and –11%, respectively).

Figure 1
Mean TG concentrations during the oral fat loading test in plasma (A), Sf > 400 (B), Sf 60-400 (C) and Sf 20-60 (D) and mean plasma FFA concentrations (E) after treatment with rosiglitazone (open circles) and placebo (solid circles).



Discussion

The main finding of the present study is that rosiglitazone improves postprandial TG metabolism in patients with type 2 diabetes. Since humans are non-fasting most part of the day and non-fasting TG are strong predictors of atherosclerosis[2-9], this may convey increased protection from cardiovascular disease in these patients. Significant effects on TG clearance were found in the chylomicron and VLDL1 fractions, suggesting a preferential action of rosiglitazone on large TRL. Finally, rosiglitazone significantly decreased postprandial FFA concentrations.

Increased postprandial lipemia is a common feature of the diabetic dyslipidemia and is associated with accelerated atherosclerosis, even in fasting normolipidemic subjects[1-9]. Especially the increase of large VLDL1 particles is associated with the generation of atherogenic remnants[1]. In one study, postprandial TG levels distinguished even better between cases with future myocardial infarction and controls than fasting plasma TG levels[2]. The data from the present study show that rosiglitazone does not change fasting TG, but decreases the postprandial TG rise in plasma (-37%), chylomicrons (-20%) and VLDL1 (-27%). Although this did not lead to a significantly lower total TG-AUC in plasma, rosiglitazone significantly reduced the total TG-AUC in the chylomicron fraction by approximately 20%. Whether these effects are sufficient to produce clinical benefit is an open issue. However, it is tempting to hypothesize that the anti-atherosclerotic effects observed with TZDs may involve improvement of postprandial lipemia[18-20]. Another interesting aspect of the study is the fact that the effects of rosiglitazone were found on top of statin treatment. In our opinion, this is of interest since statins are used by a majority of patients with type 2 diabetes, and they have been shown to improve postprandial TG metabolism. Whether further improvement of TG metabolism by rosiglitazone exerts additional vascular benefit in diabetic patients on statin treatment remains to be shown.

Data on the effects of TZDs on postprandial lipemia are scarce. In one study, there was lack of effect of pioglitazone on postprandial TG levels[21]. However, in that study, the postprandial TG level was measured only 2-h after a conventional breakfast. Our study was performed in a metabolic ward setting using an oral fat load and TG levels were measured at 2-h intervals up to 6-h postprandially. The present study is the first to show improved postprandial TG metabolism by rosiglitazone. Whether rosiglitazone also improves postprandial lipemia during insulin action, such as after a mixed meal, remains to be investigated in future studies. In most intervention studies with lipid-lowering drugs, the reduction in postprandial lipemia is more or less similar to the reduction in fasting plasma TG, which are the main determinants of postprandial lipemia[22]. To the best of our knowledge, this is the first study to describe improved postprandial TG metabolism, without lowering fasting TG levels. These results suggest other mechanisms for improved postprandial lipemia than reduced competition for the common lipolytic pathway. Firstly, TZDs are able to upregulate adipocyte LPL production through

activation of PPAR-γ[14,15], which could have contributed to the improved incremental TG-response after rosiglitazone. Unfortunately, LPL mass and activity were not measured in the present study. Secondly, it has been demonstrated that rosiglitazone directly stimulates the expression and function of lipoprotein receptor-related protein in vitro[23]. Thirdly, rosiglitazone significantly decreased postprandial FFA concentrations, probably by increasing adipocyte FFA trapping, thereby decreasing the source of hepatic VLDL production[12,24-26]. Finally, improvement of glycemic control may translate into improvement of postprandial lipemia, especially in patients with poor glycemic control. For example, in patients with poor glycemic control it has been shown that metformin[27] and glipizide[28] improve postprandial lipemia. In contrast, in diabetic patients with good glycemic control, nateglinide and glibenclamide attenuated hyperglycemia, but they did not improve postprandial lipemia[29]. Patients in our study had good glycemic control (HbA1c 6.2%). Rosiglitazone decreased fasting plasma glucose, but not HbA1c, and improved postprandial TG metabolism. Improvement of postprandial lipemia upon treatment with rosiglitazone was not related to the decrease in fasting plasma glucose. Therefore, we propose that is unlikely that the beneficial effects of rosiglitazone on postprandial lipemia are due solely to improved glycemic control, although it might have contributed.

It has been shown that in the postprandial phase, when chylomicrons and VLDL compete for clearance by LPL, the former are hydrolyzed preferentially[30]. This could partly explain the greater beneficial effects of rosiglitazone on postprandial TG clearance in large TRL (chylomicrons and VLDL1) compared with small TRL (VLDL2). Alternatively or additionally, it has been suggested that hepatic VLDL1 and VLDL2 production are independently regulated[31,32]. Acute insulin administration suppresses the rates of VLDL1-apoB production, but has no effect on VLDL2-apoB production[31]. Hence, increased insulin sensitivity by rosiglitazone may have resulted in the suppressed postprandial production of VLDL1 compared with VLDL2, as suggested by our results.

Insulin resistance is commonly associated with biochemical evidence of nonalcoholic steatohepatitis, such as increases in ALT[33]. Treatment with rosiglitazone improved insulin sensitivity and significantly reduced ALT. Interestingly, the reduction of ALT upon treatment with rosiglitazone was related to the decreases in FFA-AUC and TG-dAUC, suggesting reduced liver fat content, due to preferential adipocyte FFA storage[33,34].

In conclusion, rosiglitazone improves the metabolism of large TRL, and decreases postprandial FFA concentrations in type 2 diabetes. This may have clinical implications as these effects may contribute to cardiovascular risk reduction.

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Atherosclerosis 2005; in press

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Effects of rosiglitazone on postprandial leukocytes and cytokines in type 2 diabetes

Abstract

Objective

We postulated that in type 2 diabetes, the postprandial phase is a pro-inflammatory state that can be modulated by PPAR-γ agonists. For this purpose, we determined the effects of rosiglitazone (8 mg/d) on postprandial leukocyte counts and pro-inflammatory cytokines (IL-6 and IL-8) in patients with type 2 diabetes.

Research Design and Methods

A randomized, 8-week, cross-over, placebo-controlled, double-blind clinical trial was performed in 19 patients with type 2 diabetes. Standardized 6-h oral fat-loading tests were performed after each treatment period.

Results

During placebo treatment, blood leukocytes increased to a maximum 6-h postprandially, due to significant increases in neutrophils and lymphocytes. Concomitant postprandial increases were observed for IL-6 and IL-8, the major chemokines responsible for leukocyte recruitment. Rosiglitazone reduced the incremental area under the curves (dAUCs) for IL-6 (-63%, $p<0.01$) and IL-8 (-16%, $p<0.05$). The dAUC for leukocytes decreased with 37% ($p<0.05$), due to a specific reduction of neutrophils (-39%, $p<0.05$).

Conclusions

Rosiglitazone attenuated the postprandial increases of neutrophils, IL-6 and IL-8 in patients with type 2 diabetes. Since inflammation is a major force driving atherosclerosis, and man lives in a postprandial period most part of the day, a reduced inflammatory response after a meal may delay progression of atherosclerosis.

Introduction

Cardiovascular disease (CVD) is the main cause of mortality in patients with type 2 diabetes[1]. There is increasing awareness that chronic subclinical inflammation plays an important role in the pathogenesis of atherosclerosis[2,3]. Markers of inflammation, such as blood leukocyte counts, C-reactive protein (CRP) and monocyte chemoattractant protein-1 (MCP-1) are independent predictors of future CVD[4-10]. Even subjects with a low CRP concentration are at increased cardiovascular risk if they have a blood leukocyte count in the higher 25th percentile[11]. Differential leukocyte counts (e.g. monocytes and neutrophils) are also related to CVD[5,7]. Interestingly, the best association with CVD has been demonstrated for neutrophils[5]. Their role in the pathophysiology of atherosclerosis is not entirely clear, as these cells are absent in the atherosclerotic lesion until it is ruptured[8]. However, upon activation, resident and recruited neutrophils may affect endothelial function via the production of pro-inflammatory cytokines and oxidative stress[12]. It has been shown that type 2 diabetic patients have higher blood leukocyte counts, as well as increased expression of leukocyte activation markers compared with healthy controls[6,13,14].

Since humans are non-fasting most part of the day, this period may be of particular importance in the pathogenesis of atherosclerosis. It is known that delayed clearance of postprandial triglyceride-rich lipoproteins and their remnants is an important characteristic of the diabetic dyslipidemia and is linked to accelerated atherosclerosis[15-17]. The underlying mechanisms may involve increased generation of oxidative stress and activation of endothelial cells and leukocytes[12,18-22]. It has been shown that postprandial neutrophil recruitment, with concomitant production of pro-inflammatory cytokines and oxidative stress, may contribute to endothelial dysfunction in healthy subjects[12]. In addition, postprandial leukocyte activation has been described in healthy subjects, which may represent increased adhesive capacity of these cells to the endothelium.

Thiazolidinediones (TZDs), synthetic ligands for the peroxisome proliferator-activated receptor-γ (PPAR-γ), have been introduced in clinical medicine to improve insulin resistance in type 2 diabetes[23]. The metabolic effects of TZDs are mediated by receptor-dependent activation of the PPAR-γ-retinoid X receptor (RXR) complex and subsequent transcriptional activation of target genes[23]. Interestingly, TZDs are also potent inhibitors of inflammation, in part by receptor-independent mechanisms[24-28]. It is thought that the anti-inflammatory effects of TZDs may contribute to cardiovascular risk reduction[29].

We postulated that in patients with type 2 diabetes, the postprandial phase is a pro-inflammatory state that can be modulated by PPAR-γ agonists. For this purpose, we determined the effects of a high-fat meal with and without rosiglitazone on blood leukocyte counts, pro-inflammatory cytokines (IL-6 and IL-8) and circulating markers of inflammation (CRP and MCP-1) in a randomized double-blind, placebo-controlled cross-over trial in patients with type 2 diabetes.

Methods

Subjects

Non-smoking males and non-fertile females (i.e., post-menopausal, post-ovariectomy, or sterilized by tubal ligation) aged 35 to 70 years with documented type 2 diabetes were considered eligible. Patients on insulin treatment were excluded. All patients were treated with oral anti-hyperglycemic agents, which continued during the study. Exclusion criteria were glycated hemoglobin > 9%, serum creatinin > 200 μmol/l, abnormal TSH, evidence of hepatic disease defined as ASAT or ALAT ≥ 2 times the upper limit of normal, congestive cardiac failure, systolic blood pressure > 160 mmHg and/or diastolic blood pressure > 95 mmHg, total cholesterol > 8 mmol/l and/or triglycerides > 5 mmol/l and an alcohol intake > 3 units/day. The study protocol was approved by the local research ethics committee of the University Medical Center Utrecht. All participants gave written informed consent.

Study design

The study was designed as a prospective, randomized, cross-over, placebo-controlled, double-blind trial. The effects of rosiglitazone on postprandial triglyceride and fatty acid metabolism have been described in detail elsewhere[30]. Eligible patients were randomly assigned to receive rosiglitazone 4 mg twice daily or placebo in addition to their current oral anti-hyperglycemic agents for 8 weeks. A 6-week wash-out period was present between the 2 treatment periods. At the end of each treatment period a standardized 6-h oral fat loading test was carried out. At the beginning and at the end of each 8-week treatment period, anthropometric and fasting laboratory parameters were determined. Patients were instructed to fast at least 12-h prior to each visit. No study medication or other medication was used on the morning of the study days.

Oral fat loading test

After placing a cannula for venous blood sampling, subjects rested for 30 minutes before administration of the fat load. Fresh cream was used as fat source; this is a 40% (weight/volume) fat emulsion with a poly-unsaturated/saturated fat ratio of 0.10, containing 0.001% (w/v) cholesterol and 3% (w/v) carbohydrates representing a total energy content of 3700 kcal/L. Cream was ingested within 5 minutes at a dose of 50 g fat and 3.75 g glucose per m^2 body surface. The participants remained supine during each test and were only allowed to drink mineral water. Peripheral blood samples were obtained in sodium EDTA (2 mg/mL), before the fat load and at 2-h intervals up to 6-h postprandially. Samples for leukocyte count and differentiation were kept on room temperature. All other samples were kept on ice and centrifuged immediately for 15 minutes at 800 *g* at 4 °C, then plasma was stored at -80 °C.

Analytical methods

Glucose, cholesterol, triglycerides, HDL-cholesterol, creatinine, and aminotransferases were measured by standard clinical laboratory procedures. Insulin was measured by ELISA (Mercodia, Uppsala, Sweden). For estimation of insulin sensitivity the HOMA index (= glucose*insulin/22.5) was calculated. Blood cell counts and differentials were determined automatically using a Celldyn-3500® (Abbott, USA). IL-6 and IL-8 were measured by ELISA (R&D systems, Minneapolis, Minnesota, USA). MCP-1 (Prepotech, London, UK) was measured by ELISA in accordance with the instructions of the manufacturers. The serum concentration of CRP was measured using a high sensitivity method (hs-CRP) (Quantex hs-CRP kit, Biokit, S.A., Barcelona, Spain), with lower limit of detection of 0.10 mg/L.

Statistical analysis

All values are expressed as means ± SEM in the text, tables and figures. During serial measurements, time effects when compared with t=0 h, were tested using repeated measures ANOVA with Bonferroni correction for multiple comparisons. The postprandial inflammatory response was calculated as the incremental area under the curve (dAUC) by the trapezoidal rule using Graph Pad Prism version 4.0 (LA, USA). Assumptions of normality were tested by Kolmogorov-Smirnov tests and by review of plots. Differences in fasting parameters and dAUCs between rosiglitazone and placebo were analyzed with paired t-tests (for variable with symmetric distribution) or with Mann-Whitney tests (for variables with non-parametric distribution). Bivariate correlations were calculated using Spearman's correlation coefficients. Calculations were performed using SPSS/PC + 11.5 (SPSS Inc. Chicago, IL, USA). Statistical significance was taken at the 5% level.

Results

General characteristics

Nineteen type 2 diabetic patients (M/F: 14/5, mean age 60±6 years) were included in the study. During the study, all patients continued their usual medication comprising statins (n=8), anti-hypertensive agents (n=6) and anti-hyperglycemic therapy with sulfonylurea derivatives (n=6), metformin (n=5) or a combination of both (n=8). A synopsis of the effects of rosiglitazone on anthropometric, hemodynamic and metabolic variables, that have been published in detail elsewhere[30], is listed in *Table 1*. We did not observe a decrease in HbA1c after 8 weeks rosiglitazone. Despite the fact that our study population had relatively good glycemic control, only one patient experienced hypoglycemia when taking rosiglitazone 8 mg in addition to sulphonylurea therapy. Further, no patient showed significant side effects other than headache (n=1) and gastrointestinal complaints (n=1).

Table 1
Characteristics of the study group

	Baseline	After treatment	
		Rosiglitazone	Placebo
BMI (kg/m^2)	29.2 (1.8)	29.7 (1.1)	29.5 (1.0)
Waist-to-hip ratio	0.99 (0.02)	0.98 (0.02)	0.99 (0.02)
Systolic blood pressure (mmHg)	144 (5)	145 (10)	146 (11)
Diastolic blood pressure (mmHg)	86 (3)	87 (2)	89 (2)
Glucose (mmol/L)	7.6 (0.3)*	6.2 (0.3)	7.2 (0.5)*
HbA1c (%)	6.3 (0.6)	6.2 (0.6)	6.3 (0.7)
HOMA	ND	2.1 (0.4)	3.8 (0.5)*
Hematocrit	0.43 (0.06) *	0.39 (0.06)	0.41 (0.07)*
Total cholesterol (mmol/L)	4.9 (0.9)*	5.4 (1.1)	4.9 (0.9)*
LDL-cholesterol (mmol/L)	2.99 (0.71)*	3.45 (0.85)	3.14 (0.65)*
HDL-cholesterol (mmol/L)	0.99 (0.36)	1.05 (0.91)	0.98 (0.41)
Non-HDL cholesterol (mmol/L)	3.97 (0.82)*	4.34 (1.01)	3.98 (0.75)*
Apolipoprotein B (g/L)	ND	0.90 (0.23)	0.86 (0.17)
Triglycerides (mmol/L)	1.95 (0.98)	1.97 (0.96)	1.88 (0.86)
TG-AUC (mmol.h/L)	ND	14.7 (7.4)	16.0 (7.8)

Data are mean (SEM); * $p<0.05$ versus Rosiglitazone; ND = not determined

Postprandial leukocyte counts

Blood leukocytes showed a significant increase following fat ingestion during placebo treatment (*Figure 1*), reaching a maximum 6-h postprandially (+22%). The postprandial leukocyte increase was due to significant increases in neutrophils and lymphocytes (*Figure 2*), but monocytes remained unchanged. Rosiglitazone reduced the fasting blood leukocyte count, and attenuated the postprandial leukocyte rise (dAUC, *Table 2*) compared with placebo. The postprandial leukocyte reduction by rosiglitazone was due to a selective decrease of neutrophils. No significant effects of rosiglitazone on postprandial lymphocytes or monocytes were observed. A subgroup analysis of subjects on (n=8) and off (n=11) statin treatment showed similar effects of rosiglitazone on the dAUC for neutrophils (-37% and -40%, respectively).

A significant postprandial decline in erythrocyte counts was observed, but platelet counts remained unchanged (*Figure 1*). The fasting erythrocyte count was significantly lower after rosiglitazone compared with placebo (from 4.67±0.10 to 4.42±0.11 cells 10^{12}/L, respectively, $p<0.05$). No difference in platelet counts was observed between the two treatment periods.

Figure 1
Absolute postprandial changes of leukocytes (upper panel), erythrocytes (left lower panel) and platelets (right lower panel) after treatment with rosiglitazone (open circles) and placebo (solid circles).

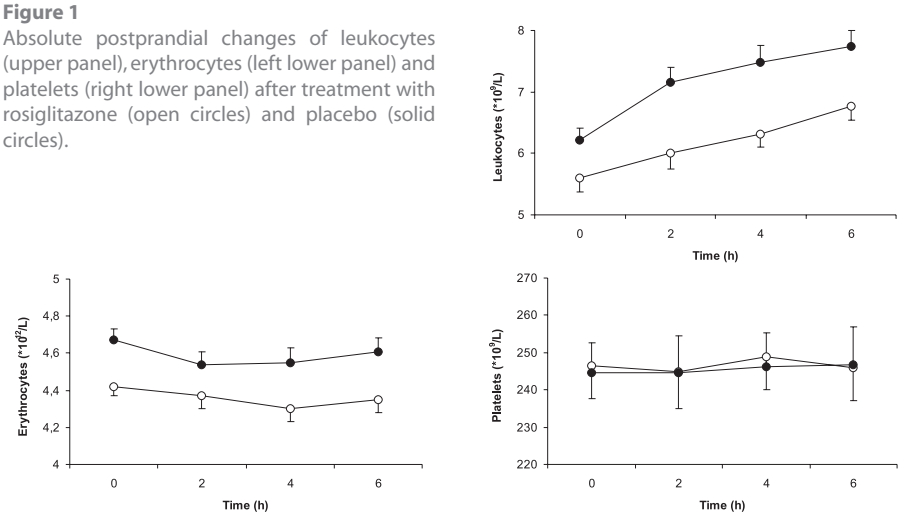


Figure 2
Relative postprandial changes of leukocytes (left upper panel), neutrophils (right upper panel) lymphocytes (left lower panel) and monocytes (right lower panel) after treatment with rosiglitazone (open circles) and placebo (solid circles).

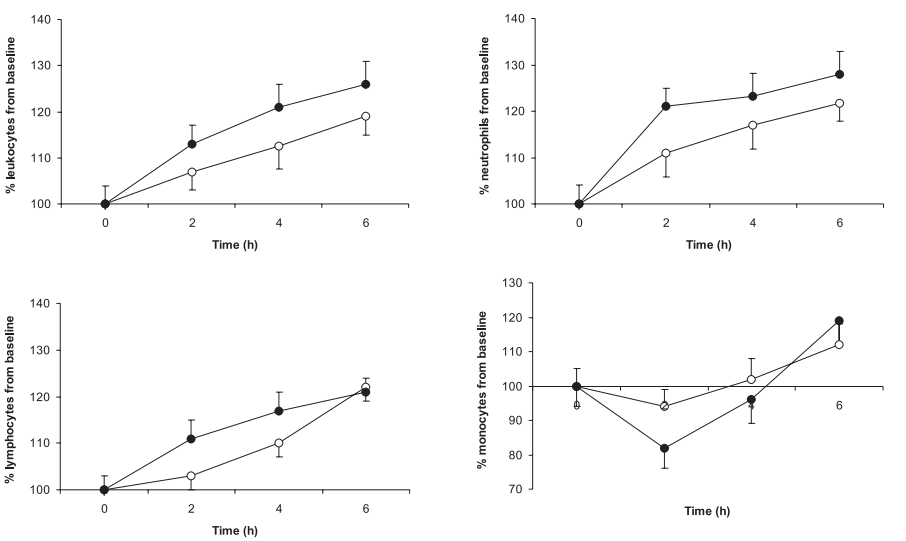


Table 2
Effects of rosiglitazone on fasting and postprandial leukocytes and cytokines

	Rosiglitazone	Placebo
Fasting leukocytes (x 10^9/L)	5.5 (0.3) *	6.2 (0.4)
dAUC for leukocytes (x 10^9.h/L)	3.3 (0.5) *	5.2 (1.2)
Fasting neutrophils (x 10^9/L)	3.1 (0.23) *	3.7 (0.3)
dAUC for neutrophils (x 10^9.h/L)	2.5 (0.4) *	4.1 (1.1)
Fasting lymphocytes (x 10^9/L)	1.8 (0.1)	1.8 (0.1)
dAUC for lymphocytes (x 10^9.h/L)	0.9 (0.3)	1.3 (0.3)
Fasting monocytes (x 10^9/L)	0.47 (0.03)	0.53 (0.08)
dAUC for monocytes (x 10^9.h/L)	0.01 (0.0.9)	-0.13 (0.31)
Fasting IL-6 (pg/ml)	1.64 (0.37)	2.06 (0.28)
dAUC for IL-6 (pg.h/ml)	0.90 (0.78) *	2.72 (1.09)
Fasting IL-8 (pg/ml)	416 (37) *	469 (56)
dAUC for IL-8 (pg.h/ml)	284 (46) *	335 (60)

Data are mean (SEM); * $p<0.05$ compared with placebo; dAUC = Incremental area under the curve

Postprandial markers of inflammation

Plasma IL-6 showed a postprandial increase after placebo treatment, reaching a maximum 4 h postprandially (*Figure 3*). Rosiglitazone did not change fasting IL-6, but reduced the dAUC for IL-6 compared with placebo. A rapid increase in IL-8 to a maximum at t=2 h followed by a subsequent return to baseline at t=4 h was observed after the fat challenge during placebo treatment. Rosiglitazone blunted this early IL-8 peak. Rosiglitazone reduced fasting IL-8 as well as the dAUC for IL-8 compared with placebo. There were no postprandial changes in plasma MCP-1 or plasma CRP (*Figure 4*) after both rosiglitazone and placebo. In comparison to placebo, rosiglitazone significantly decreased fasting CRP (from 3.8±1.1 to 1.5±0.5 mg/l, $p<0.01$), but not MCP-1 (48.9±3.9 versus 45.3±3.6 pg/ml).

Correlations

At baseline, the blood leukocyte count was significantly related to BMI ($r=0.46$, $p<0.05$), LDL-cholesterol ($r=0.57$, $p<0.01$), apolipoprotein B ($r=0.58$, $p<0.01$), systolic blood pressure ($r=0.60$, $p<0.01$) and IL-6 ($r=0.50$, $p<0.05$). In addition, the neutrophil increase after fat ingestion was related to the postprandial TG increase ($r=0.54$, $p<0.05$). The reduction in the dAUC for neutrophils by rosiglitazone was associated with the decrease in the dAUC for IL-6 ($r=0.61$, $p<0.01$) and the decrease in fasting plasma CRP ($r=0.43$, $p<0.05$), but not with the decrease in HOMA ($r=0.18$) and the postprandial TG reduction ($r=0.22$).

Figure 3
Absolute postprandial changes of IL-6 (left panel) and IL-8 (right panel) after treatment with rosiglitazone (open circles) and placebo (solid circles).

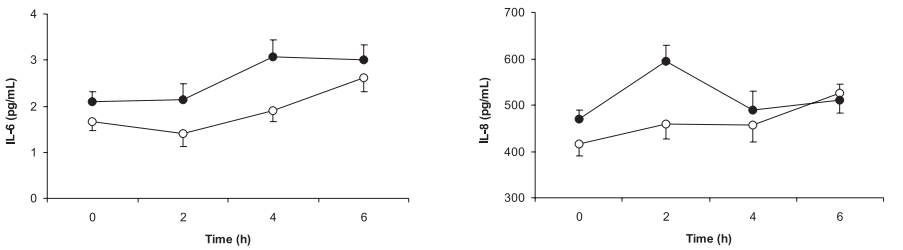
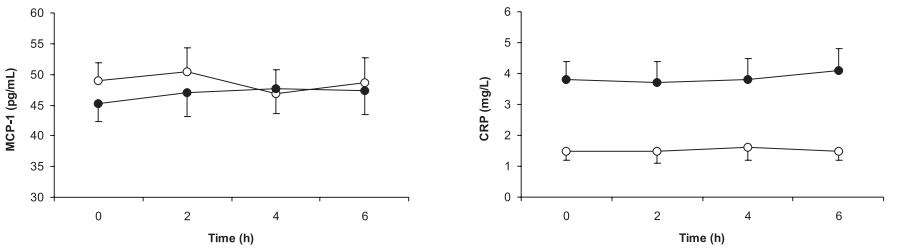


Figure 4
Absolute postprandial changes of MCP-1 (left panel) and CRP (right panel) after treatment with rosiglitazone (open circles) and placebo (solid circles).



Discussion

The postprandial period is thought to play an important role in the pathogenesis of atherosclerosis in patients with type 2 diabetes[15-20]. Atherosclerosis is an inflammatory disease, characterized by a series of highly specific cellular and molecular responses [2,3]. In the present study, the postprandial increases in blood leukocytes and the pro-inflammatory cytokines IL-6 and IL-8 were attenuated by rosiglitazone. These findings may be of clinical relevance, as humans are in a postprandial state most part of the day.

Atherosclerosis is initiated by resident and recruited leukocytes in response to endothelial injury[2,3]. The importance of 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[31]. Upon activation, endothelial cells produce a variety of cytokines, which may facilitate recruitment and activation of leukocytes[2,3]. Among those, IL-6 and IL-8 are the main cytokines responsible for leukocyte recruitment[12,32-36], and both showed postprandial increases in our group of stable regulated type 2 diabetic patients. These changes were accompanied by postprandial increases of neutrophils and lymphocytes, which may affect endothelial function via the production of pro-inflammatory cytokines and oxidative stress[12]. Given the

close relationship between inflammation and atherosclerosis, we postulate that the postprandial inflammatory changes as observed in this study may result in increased susceptibility for premature atherosclerosis.

The inflammatory response to a postprandial stimulus has been studied previously. In healthy subjects, postprandial recruitment and activation of leukocytes has been described[12,21,22]. To investigate whether the observed effects are postprandial and not simply diurnal variations, we previously studied leukocyte changes after an oral fat load and after water[12,22]. In these studies, lymphocytes increased gradually in both tests (fat and water), but neutrophils increased only after ingestion of an oral fat load. Hence, the postprandial neutrophil increase is meal-specific and may be a pro-atherogenic factor for the postprandial situation[12,22], while diurnal lymphocyte variations are meal-independent. Diurnal variations have also been described for IL-6, but in a previous study, the IL-6 increase tended to be greater following a high-fat meal in comparison to a blank test with water[12]. Postprandial leukocyte changes may occur in the population as a whole[12,22], and not only in diabetic patients. However, since leukocyte counts have been linked to future cardiovascular events[4-7], postprandial leukocyte increases may be harmful, especially in high-risk patient groups. Whether the postprandial leukocyte changes are more pronounced in type 2 diabetic patients compared with controls remains to be shown.

In other studies, it has been shown that both, high-fat and high-carbohydrate meals, produce an increase of markers of oxidative stress and endothelial activation in normal and type 2 diabetic subjects[18-20], but the increases are more substantial following the high-fat meal[20]. These findings indicate that the response to dietary fat and carbohydrates might differ with respect to the inflammatory response. We investigated the inflammatory response to a (non-physiological) high-fat meal only, which is a limitation of our study. Further studies are necessary to investigate the effects of more physiological meals (high-carbohydrate and mixed meals) on leukocyte recruitment. In our study, postprandial CRP concentrations did not change. This is in contrast with a previous study[37], in which a significant rise in postprandial CRP levels was observed in patients with type 2 diabetes. This difference, however, may be explained by differences in test meal (mixed meal in their study versus high-fat meal in our study), and by the fact that the patients in that study had worse glycemic control than the patients in our study. Despite the fact that MCP-1 expression is induced by atherogenic lipoproteins *in vitro*[38], postprandial MCP-1 levels did not change *in vivo* in our patient group.

There is increasing interest in the potential benefits of TZD therapy on several components of the metabolic syndrome. For example, several studies have shown that TZDs inhibit markers of inflammation through transcriptional mechanisms. There appears to be a generalized repression of NFkB, CCAAT/enhancer-binding protein, and activator protein-1 mediated transcription of inflammatory genes[24-26], resulting in reduced circulating markers of inflammation[27,28]. In the present report, we sought to determine

the effects of rosiglitazone on the postprandial inflammatory response, with the main focus on postprandial leukocyte recruitment, as these cells are highly involved in the initiation and progression of atherosclerosis. Our results show that rosiglitazone reduced the postprandial leukocyte recruitment, due to a selective reduction of neutrophils. There are several mechanisms which could explain this observation. The leukocyte reduction by rosiglitazone could be the result of improved insulin sensitivity. This hypothesis is in line with the positive correlation of the leukocyte count with decreased insulin sensitivity in the Insulin Resistance Atherosclerosis Study[39]. However, in our study the leukocyte decrease by rosiglitazone was not related to the decrease in HOMA. Perhaps more likely, the leukocyte reduction could be secondary to the reductions of IL-6 and IL-8, which are the major cytokines responsible for leukocyte recruitment[12,32-36]. In line with this hypothesis, the IL-6 reduction by rosiglitazone was strongly related to the decrease of the dAUC for neutrophils. The main sources for IL-6 are adipose tissue, endothelial cells and inflammatory cells[32-35,40]. Rosiglitazone has been shown to improve adipocyte function and decrease endothelial cell activation [23,41], and both could lead to lower IL-6 levels. Finally, we have recently shown that rosiglitazone improves postprandial TG metabolism[30]. Since TG-rich lipoproteins are able to activate neutrophils and induce cytokines, the attenuation of the postprandial increases of neutrophils and cytokines in the rosiglitazone-treated patients may also be due to the reduction of postprandial lipemia.

Given the close relation between inflammation and CVD, the reduced postprandial inflammatory response upon treatment with rosiglitazone raises the prospect of reduced cardiovascular risk. In vivo evidence for anti-atherosclerotic effects of rosiglitazone has been provided. It has been shown that rosiglitazone reduces common carotid intima-media thickness progression after 48 weeks of treatment compared with placebo in non-diabetic patients with coronary artery disease[42]. Interestingly, only minor effects on insulin sensitivity and lipid profile were observed in that study. These data could be interpreted as a strong argument in favor of direct anti-atherosclerotic effects of rosiglitazone, at least in part due to repression of inflammation[29]. We observed a substantial reduction in CRP, in agreement with previous studies[27,28], which is probably clinically relevant. 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[33]. One of the questions that remains is to what extent the postprandial reductions in leukocytes and pro-inflammatory cytokines, as observed in the present study (reduction of the dAUCs for neutrophils [-39%], IL-6 [-63%] and IL-8 [-16%]), contribute to the overall attenuation of the low-grade inflammatory state and improvement of cardiovascular risk. Given the close relationship between these inflammatory markers and CVD[4-9,33-36], it is tempting to hypothesize that these effects contribute to cardiovascular risk reduction as well. Finally, the postprandial leukocyte reduction by rosiglitazone was observed in subjects with and without concomitant

statin therapy. This may be of importance since statins are used by a majority of patients with type 2 diabetes, and they also have anti-inflammatory effects[43].
Rosiglitazone did not change HbA1c in our study. Previously, it has been shown that the maximal effects of TZDs on HbA1c are reached after 16 weeks of treatment[44]. In addition, the decrease in HbA1c may depend on glycemic control at baseline. Our patients had good glycemic control, and were treated for only 8 weeks, which may explain the lack of effect of rosiglitazone on HbA1c in our study.
In conclusion, rosiglitazone attenuated the postprandial increases of blood leukocytes and the pro-inflammatory cytokines IL-6 and IL-8 in patients with type 2 diabetes. Since inflammation is a major force driving atherosclerosis, and man lives in a postprandial period most part of the day, a reduced inflammatory response after a meal may delay progression of atherosclerosis.

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Arterioscler Thromb Vasc Biol 2004; 24(5): 798-800

5

PPAR-γ agonists: shifting attention from the belly to the heart?

Second generation thiazolidinediones (TZDs), synthetic ligands for the peroxisome proliferator activated receptor-γ (PPAR-γ), have recently been introduced in clinical medicine to improve insulin resistance in type 2 diabetes. The two isoforms of PPAR-γ are 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[1]. These effects are mediated by receptor-dependent activation of the PPAR-γ -retinoid X receptor (RXR) complex and subsequent transcriptional activation of target genes[1]. The PPAR-γ1 isoform is also expressed in endothelial cells, vascular smooth muscle cells (VSMC) and monocytes/macrophages in the vasculature[2,3]. PPAR-γ agonist have been shown to have interesting effects on these cells, which appear to be partially independent of the PPAR-γ-RXR mediated transcriptional effects[4]. For example, in endothelial cells, TZDs have been shown to enhance eNOS activity by phosphorylation and to inhibit leukocyte-endothelial cell interaction[5,6]. TZDs inhibit growth factor-induced proliferation and migration of VSMC[7]. Also in vivo, in a model of angiotensin II induced hypertension, TZDs could normalize endothelial function and correct structural vascular abnormalities[8]. In monocytes/macrophages, TZDs upregulate the scavenger receptor CD36[9], and induce the cholesterol efflux pomp adenosine triphosphate-binding cassette, subfamily A, member 1 (ABCA1), suggesting altered lipid handling by macrophages whereby pro-atherogenic lipoproteins are taken up and anti-atherogenic lipoproteins are generated[10]. Finally, and perhaps most importantly, TZDs are very potent inhibitors of inflammation. There appears to be a generalized repression of NFkB, CCAAT/enhancer-binding protein, and activator protein-1 mediated transcription of inflammatory genes[11,12]. The exact mechanism is still unknown, but probably involves increased levels of co-repressor molecules or transcriptional superregulation, for example by chromatin remodeling, as has been described for other nuclear hormone receptors[13]. As a result, a broad spectum of pro-inflammatory cytokines (e.g. IL-6, TNF-α, G-CSF, CD40, MCP-1, MMP) as well as adhesion molecules (e.g. ICAM-1, VCAM-1), iNOS and C-reactive protein are suppressed[6,11-16]. The potent anti-inflammatory actions of TZDs are also illustrated by the fact that TZDs have been used to treat primary inflammatory conditions, such as colitis[17]. These modes of actions suggest that TZDs may have important anti-atherosclerotic actions. These effects can be indirect by improving insulin resistance-related metabolic risk factors. However, TZDs may also have important direct anti-atherosclerotic effects, due to repression of inflammatory transcription, resulting in restoration of endothelial function and reduced vascular (micro)inflammation.

In *Arteriosclerosis, Thrombosis, and Vascular Biology,* Sidhu et al. describe that the TZD rosiglitazone reduces common carotid intima media thickness (IMT) progression, a well established intermediate endpoint of atherosclerotic disease progression, after 48 weeks of treatment compared with placebo in non-diabetic patients with coronary

artery disease[18]. Previous studies have demonstrated that TZDs have beneficial effects on intermediate endpoints of atherosclerosis, such as endothelial function and IMT, in type 2 diabetes[19-21]. The interesting point of the study of Sidhu et al. is that these effects are not confined to diabetic patients, but can be extrapolated to patients with documented coronary artery disease without manifest diabetes. Another important point of the current study is that in these high-risk patients rosiglitazone retarded carotid IMT progression on top of statins and anti-hypertensive agents. These observations could be interpreted as a strong argument in favour of direct vascular effects of TZDs on atherosclerosis.

It should be noted that in patients with clinical cardiovascular disease the prevalence of the metabolic symdrome is very high, despite the absence of diabetes. We have recently reported that in such a cohort, almost half of the patients fulfilled the criteria of the metabolic syndrome (defined as 3 or more of the following: low HDL-cholesterol, increased triglycerides, high blood pressure, glucose intolerance and high waist circumference)[22]. Regrettably, there was no information about the prevalence of the metabolic syndrome in the Sidhu study, although a similar percentage would not be unlikely. For example, almost a quarter of the patients had impaired fasting glucose. This would indicate that the study group indeed could have had benefit from improved metabolic control by TZD treatment. However, only minor effects on metabolic parameters were observed. First, during rosiglitazone treatment, there as a small but significant reduction in HOMA, as a marker of insulin sensitivity, compared with placebo. HOMA is an independent predictor of cardiovascular events in both diabetic and non-diabetic patients[23,24]. However, this is particulary the case for high HOMA values[23].The study group in the Sidhu study had relatively low HOMA values and rosiglitazone caused quantitatively only a minimal reduction in HOMA, which makes an important role on retarded IMT progression less likely. Second, rosiglitazone-treated patients showed a small transient increase in LDL-cholesterol and triglycerides. This phenomenon is frequently observed during TZD treatment as TZDs generally cause a shift towards larger, more buoyant LDL particles, which are less prone to oxidative modification and are therefore thought to be less atherogenic[25]. Unfortunately, LDL density can not be estimated in the current study as there is no information available on apolipoprotein B. Nevertheless, on top of statin treatment, there were overall no sustained effects on lipid parameters by rosiglitazone, which makes a lipid-based explanation for the observed effect on atherosclerotic disease progression also less likely.

The fact that TZDs modulate atherosclerosis progression potentially independent of metabolic changes offers additional opportunities to improve cardiovascular risk in a broader group of high-risk patients. However, one then also has to consider potential side-effects. Edema formation and expansion of the extracellular volume is found in

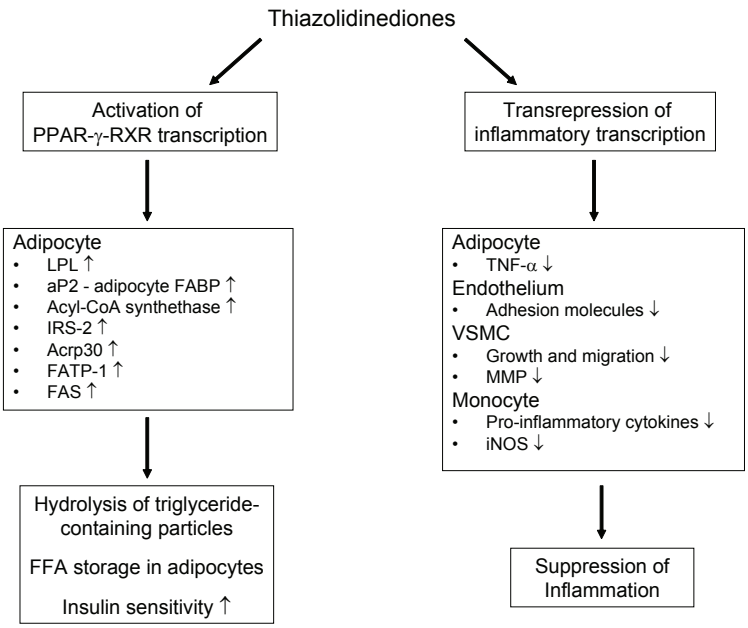
3-5% of the patients for each of the TZDs, and the incidence increases in combination with insulin[26]. Obviously, this may give rise to concern when considering treatment with TZDs in patients with cardiovascular disease that also have congestive heart failure (CHF). So far, TZDs have not been studied in patients with NYHA class III or IV CHF and therefore are not recommend for use in these patients. In the study of Sidhu et al., patients with CHF (NYHA class I to IV) were also excluded.

As we start to understand the vascular pathobiology of atherosclerosis better and better, drugs that interfere with key processes in atherosclerosis biology, such as endothelial function and vascular (micro)inflammation, become important as they potentially allow cardiovascular risk reduction beyond treatment of a risk factor. The study of Sidhu provides us with clues that TZDs, drugs that were primarily introduced to treat such a risk factor (i.e. insulin resistance), may have clinical relevant effects on the pathobiology of atherosclerosis.

Putative mechanisms by which TZDs reduce atherosclerosis

TZDs can improve FFA trapping in adipocytes by altering the transcription of genes involved in FFA storage (aP2-adipocyte FABP, FATP-1, FAS and Acyl-CoA synthase). Improved adipocyte differentiation, in combination with increased insulin receptor-mediated signaling (IRS-2) and increased secretion of a fat-specific secreted protein (Acrp30) leads to increased insulin sensitivity. In monocytes/macrophages, TZDs promote the uptake of lipids by the scavenger receptor CD36, but also induce the cholesterol efflux pomp (ABCA1), leading to reversed cholesterol transport. These effects are all mediated by receptor-dependent activation of the PPAR-γ-RXR complex and subsequent transcriptional activation of target genes.

TZDs have also been shown to have interesting effects on vascular cells, which appear to be partially independent of the PPAR-γ-RXR mediated transcriptional effects. There appears to be a generalized repression of NFkB, CCAAT/enhancer-binding protein, and activator protein-1 mediated transcription of inflammatory genes, which may result in restoration of endothelial function and reduced vascular (micro)inflammation



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J Clin Endocrinol Metab 2005; 90(6): 3575-82

6

In vivo evidence of impaired peripheral fatty acid trapping in patients with HIV-associated lipodystrophy

Abstract

Objective

The use of antiretroviral combination therapy in HIV has been associated with lipodystrophy and several metabolic risk factors. We postulated that patients with HIV-lipodystrophy have impaired adipose tissue free fatty acid (FFA) trapping and, consequently, increased hepatic FFA delivery.

Research Design and Methods

We investigated FFA, hydroxybutyric acid (HBA; reflecting hepatic FFA oxidation) and triglyceride (TG) changes after a high-fat meal in HIV-infected males with (LIPO, n=26) and without (NONLIPO, n=12) lipodystrophy and in healthy males (n=35). Because defective peripheral FFA trapping has been associated with impaired action of complement component 3 (C3), we also determined postprandial C3 concentrations.

Results

The LIPO group had higher HOMA compared with the other groups. Area under the curves (AUCs) for FFA, HBA and TG were higher in the LIPO group compared with the NONLIPO group and controls. No differences in TG-AUC, FFA-AUC and HBA-AUC were observed between the NONLIPO group and controls. In HIV-infected patients, FFA-AUC and HBA-AUC were inversely related to subcutaneous adipose tissue area. Plasma C3 showed a postprandial increase in healthy controls, but not in the HIV-infected groups. C3 was not related to body fat distribution, neither with postprandial FFA and HBA.

Conclusions

The present data suggest disturbed postprandial FFA metabolism in patients with HIV-lipodystrophy, most likely due to inadequate incorporation of FFA into TG in subcutaneous adipose tissue, but do not support a major role for C3 in these patients. The higher postprandial HBA levels reflect increased hepatic FFA delivery, and may aggravate insulin resistance and dyslipidemia, leading to an increased cardiovascular risk.

Introduction

The use of highly active antiretroviral therapy (HAART) in HIV-infected patients has been associated with peripheral lipoatrophy, intra-abdominal fat accumulation and development of a buffalo hump[1-3]. Approximately half of the HAART-treated HIV-infected patients will develop at least one of these symptoms after 12-18 months of therapy[3]. Severe forms of HIV-lipodystrophy can be disfiguring and stigmatizing, and often lead to suboptimal adherence to HAART. Moreover, the associated insulin resistance and dyslipidemia increase the risk of cardiovascular disease[4].

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 triglyceride(TG)-rich lipoproteins by lipoprotein lipase (LPL)[5]. This pathway is also known as the pathway of "adipocyte FFA trapping". In patients with HIV-lipodystrophy, this process may be impaired, because there is not sufficient subcutaneous adipose tissue to capture FFA. The results could be postprandial accumulation of FFA, which are exposed to extra-adipose tissues, such as the liver, skeletal muscle and pancreas, aggravating insulin resistance and overproduction of VLDL particles[5,6].

Increased hepatic FFA delivery is a main determinant of VLDL secretion and postprandial lipemia in subjects with insulin resistance[6]. 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[7]. Therefore, postprandial HBA appearance in plasma may serve as a marker of postprandial hepatic FFA delivery. The postprandial HBA increase is higher in patients with familial combined hyperlipidemia (FCHL) compared with controls, and this is paralleled by increased postprandial FFA levels[8]. In an animal model of CD36 deficient mice, increased hepatic FFA delivery has been linked to increased hepatic β-oxidation reflected in increased plasma levels of HBA[9].

It has been recognized that complement component C3 (C3) is involved in peripheral FFA trapping[10,11]. C3adesArg (which is identical to acylating-stimulating protein, ASP) is an immunologically inactive cleavage product of C3 and stimulates FFA and glucose uptake in adipocytes[10,11]. Chylomicrons are strong activators of adipocyte C3 production[12,13], and it has been shown that after a high-fat meal plasma C3 concentrations increase[13-15], especially when insulin effects are blunted[16]. It is thought that effective postprandial C3-mediated diversion of FFA from the liver contributes to a healthy lipoprotein phenotype. Several groups have shown that adipocytes from patients with familial combined hyperlipidemia (FCHL) are resistant to the effects of C3[17-19], leading to an exaggerated and prolonged postprandial C3 response[15], eventually resulting in abnormal diversion of FFA to the liver and VLDL overproduction[8,15].

We postulated that patients with HIV-lipodystrophy have impaired peripheral FFA trapping, leading to postprandial FFA accumulation and increased hepatic FFA

delivery. For this purpose, we investigated FFA, HBA and TG changes after an acute oral fat challenge (10 h, 50 g/m^2) in HAART-treated HIV-infected male patients with and without lipodystrophy and in healthy normolipidemic controls. Because impaired peripheral FFA trapping has been associated with impaired action of C3 in patients with FCHL, we also determined postprandial C3 concentrations.

Methods

Subjects

Males aged between 18 and 65 years with a documented HIV infection were recruited from the Department of Infectious Disease of the University Medical Center Utrecht. Inclusion criteria were HIV-RNA < 10.000 copies/ml and HAART for at least 18 months with no changes in the treatment regimen during 6 months prior to inclusion. Exclusion criteria were the presence of HIV-related symptoms, renal- and/or liver disease, diabetes mellitus, use of lipid-lowering medication and an alcohol intake > 3 U/day. The presence of HIV-lipodystrophy was defined as self-reported symptoms of loss of subcutaneous fat (face, arms, legs and buttocks) with or without increased abdominal girth or development of a buffalo hump. These findings were confirmed by the investigator (JPHvW) before enrolment. This definition may be considered a limitation, but we did not perform whole-body dual-energy X-ray absorptiometry (DEXA) and were, hence, unable to use the objective case definition of lipodystrophy as published by Carr et al[20]. Healthy controls were recruited by advertisement and met the same inclusion as the HIV-infected patients. Participants visited our department after a 12 h fast for blood sampling and anthropometric measurements. Body fat mass was estimated using bioimpedance analysis (RJL systems, Detroit, USA). The study protocol was approved by the local research ethics committee of the University Medical Center Utrecht. All participants gave written informed consent.

Oral fat loading test and separation of lipoproteins

After placing a cannula for venous blood sampling, subjects rested for 30 minutes before administration of the fat load. Fresh cream (a 40% weight/volume fat emulsion representing a total energy content of 3700 kcal/L) was ingested within 5 minutes at a dose of 50 g fat and 3.75 g glucose per m^2 body surface[13-16]. Participants remained supine during the test and were only allowed to drink mineral water. Peripheral blood samples were obtained in sodium EDTA (2 mg/mL) and lithium-heparin tubes, kept on ice and centrifuged immediately for 15 minutes at 800 *g* at 4 °C, then plasma was stored at -80 °C. Lipoproteins were subfractionated by ultracentrifugation as described previously in detail[21]. Consecutive runs were carried out to float Svedberg flotation rate (Sf) > 400 (chylomicrons), Sf 60-400 (VLDL1), Sf 20-60 (VLDL2), Sf 12-20 (IDL) and Sf 2-12 (LDL). Cholesterol was measured in each fraction. TG were measured only in Sf > 400, Sf 60-400 and Sf 20-60.

Analytical methods

Total cholesterol and TG were measured in duplicate by colorimetric assay with the CHOD-PAP and GPO-PAP kits, respectively (Roche diagnostics, Mannheim, Germany). FFA were measured by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). For FFA measurements, a lipase inhibitor (Orlistat, Roche, Basel, Switzerland) was added to the plasma in order to block ex vivo lipolysis. Total serum C3 concentrations were measured by nephelometry (Dade Behring Nephelometry type II). Total plasma C3 measured in our study represented C3, C3b and C3c production. Since C3a is the least immunogenic part of C3 and much smaller than the complete C3 molecule, the contribution of C3a or ASP to total C3 measured in our assay is neglectable[15]. Hydroxybutyric acid (HBA) was measured spectrophotometrically by the principle of NADH to NAD+ conversion after adding 3-hydroxybutyrate dehydrogenase. For this purpose, 0.5mL blood from the lithium-heparin tubes was denutriated by adding 1mL of 0.7M $HClO_4$, immediately after collection. ApolipoproteinB (apoB) was measured by nephelometry using apoB monoclonal antibodies (Behring Diagnostics NV, OSAN 14/15). Glucose was measured by standard enzymatical laboratory methods (Vitros 250; Johnson/Johnson, Rochester, NY, USA). Insulin was measured by ELISA (Mercodia, Uppsala, Sweden). For estimation of insulin sensitivity the HOMA index (= glucose*insulin/22.5) was calculated. CD4 cell counts were determined by flow cytometry, and HIV-RNA was determined by ultrasensitive assay

Cross-sectional computer tomography

A single-slice cross-sectional CT scan at the L_4-L_5 level was performed as described previously[22], in order to assess distribution of subcutaneous and visceral abdominal fat (SAT and VAT, respectively). Briefly, a lateral scout image was obtained to identify the level of the L_4 pedicle, which served as the landmark for the 1-cm single-slice image. The border of the intra-abdominal cavity was outlined on the CT image and total fat and VAT areas were quantified by selecting an attenuation range of –250 to –50 Hounsfield Units. SAT was calculated as the difference between total fat area and VAT.

Statistical analysis

Data are expressed as mean ± SD in the text, tables and figures. Total integrated area's under the curves (AUC) were calculated by the trapezoidal rule using GraphPad Prism version 4.0. Incremental integrated AUCs (dAUC) were also calculated after correction for the baseline value. Differences between two groups were analyzed by independent t-test. Comparisons between the three groups were performed with repeated measures ANOVA with least significance difference test as post hoc analysis test, with Bonferroni correction to the P value. During serial measurements, time effects when compared with T = 0 h, were tested using repeated measures ANOVA with Bonferroni correction for multiple comparisons. In HIV-infected patients, bivariate correlations were calculated using Pearson's correlation coefficients. All significantly correlated

variables were used as independent variables in stepwise multiple regression analysis with FFA-AUC and HBA-AUC as dependent variables. TG, insulin and HOMA values were log transformed before analysis due to non-parametric distribution. Calculations were performed using SPSS/PC + 11.5 (SPSS Inc. Chicago, IL, USA). Statistical significance was taken at the 5% level.

Results

General characteristics and fasting lipoprotein profile

Thirty-eight HIV-infected patients were included in the study. Twenty-six of them were characterized as having clinical evident HIV-lipodystrophy according to both patient and investigator. All HIV-infected patients in both groups were currently receiving HAART with nucleoside reverse transcriptase inhibitors and a protease inhibitor and/or a non-nucleoside reverse transcriptase inhibitor (*Table 1*).

Table 1
Baseline characteristics of HIV-infected patients with and without lipodystrophy and healthy controls

	LIPO group (n=26)	NONLIPO group (n=12)	Healthy controls (n=35)	P value from ANOVA
Age (years)	48 (9)	46 (6)	48 (7)	0.92
BMI (kg/m^2)	23.3 (2.4) *	25.1 (1.2)	24.1 (2.5)	0.03
Waist-to-hip ratio	0.99 (0.05) *	0.92 (0.05)	0.91 (0.06)	0.01
Total body fat mass (kg)	10.5 (5.1) #	15.5 (4.7)	ND	
SAT (cm^2)	80 (44) #	168 (145)	ND	
VAT (cm^2)	175 (75)	161 (48)	ND	
Systolic BP (mmHg)	135 (14) *	127 (11)	122 (13)	0.01
Diastolic BP (mmHg)	83 (9)	77 (8)	82 (10)	0.17
Glucose (mmol/l)	5.51 (0.87)	5.10 (0.40)	5.19 (0.61)	0.30
Insulin (mU/l)	8.0 (4.5) *#	3.9 (1.8)	2.9 (2.8)	0.003
HOMA	1.94 (1.73) *#	0.96 (1.41)	0.68 (0.64)	0.001
Time since diagnosis of HIV (yrs)	8.3 (4.1)	7.9 (3.5)	NA	
Time on HAART (yrs)	5.4 (3.2)	4.9 (2.6)	NA	
Current PI use (%)	73%	75%	NA	
Current NNRTI use (%)	35%	33%	NA	
Current NRTI use (%)	100%	100%	NA	
Current stavudine use (%)	15%	16%	NA	
HIV-RNA (copies/ml)	257 (58)	150 (26)	NA	
CD4 cell count (x 10^6 cells/ml)	724 (335)	584 (258)	NA	

* p<0.05 versus healthy controls # p<0.05 versus the NONLIPO group ND = not determined
NA = not applicable SAT = subcutaneous adipose tissue VAT = visceral adipose tissue
HOMA = homeostasis model assessment index (glucose*insulin/22.5) PI = protease inhibitor
NNRTI = non-nucleoside reverse transcriptase inhibitor NRTI = nucleoside reverse transcriptase inhibitor

General characteristics and the fasting metabolic profile of HIV-infected patients with (LIPO group, n=26) and without (NONLIPO, n=12) lipodystrophy and healthy controls (n=35) are shown in *Table 1*. The time since diagnosis of HIV infection, the time on HAART and immunological parameters were similar between the LIPO and NONLIPO group. Total body fat mass and SAT, which were measured only in the HIV-infected patients, were significantly lower in the LIPO group than in the NONLIPO group. The LIPO group had higher HOMA than the NONLIPO group and healthy controls. Fasting lipoprotein profile of the study group is listed in *Table 2*. Fasting plasma TG were increased in the LIPO group compared with the other groups, due to higher TG levels in the Sf > 400, Sf 60-400 and Sf 20-60 fractions. Apolipoprotein B and total cholesterol were similar between the LIPO and NONLIPO groups, but they were increased in both compared with healthy controls. LDL-cholesterol was lower in the LIPO group compared with the NONLIPO group.

Table 2
Fasting lipoprotein profile of HIV-infected patients with and without lipodystrophy and healthy controls

	LIPO group (n=26)	NONLIPO group (n=12)	Healthy controls (n=35)	P value from ANOVA
Cholesterol (mmol/l)				
Plasma	5.67 (0.96) *	5.60 (0.94) *	4.83 (0.79)	0.01
Sf > 400 (chylomicrons)	0.12 (0.25) *	0.019 (0.026)	0.019 (0.016)	0.03
Sf 60-400 (VLDL1)	0.43 (0.18) *	0.32 (0.18) *	0.14 (0.12)	0.04
Sf 20-60 (VLDL2)	0.38 (0.21) *#	0.23 (0.09) *	0.15 (0.09)	0.02
Sf 12-20 (IDL)	0.39 (0.25) *	0.29 (0.16)	0.19 (0.14)	0.03
Sf 2-12 (LDL)	3.00 (0.90) #	3.68 (0.76) *	3.14 (0.68)	0.04
HDL	1.12 (0.33) *	1.18 (0.23)	1.21 (0.25)	0.009
Triglycerides (mmol/l)				
Plasma	3.55 (2.07) *#	1.78 (0.59)	1.34 (0.42)	0.01
Sf > 400 (chylomicrons)	0.33 (0.10) *#	0.01 (0.02)	0.02 (0.03)	0.001
Sf 60-400 (VLDL1)	1.35 (1.05) *#	0.68 (0.40) *	0.36 (0.31)	0.01
Sf 20-60 (VLDL2)	0.50 (0.36) *#	0.19 (0.14)	0.15 (0.10)	0.04
Cholesterol/HDL-C ratio	5.46 (1.71) *	4.92 (1.17)	4.56 (1.52)	0.02
Apolipoprotein B (g/l)	1.13 (0.30) *	1.08 (0.20) *	0.90 (0.18)	0.01
LDL-C/apoB ratio	2.64 (0.68) *	3.43 (0.42)	3.52 (0.39)	0.02

Sf = Svedberg flotation rate; * $p<0.05$ versus healthy controls; # $p<0.05$ versus the NONLIPO group

Postprandial triglyceride and free fatty acid changes

In addition to higher fasting plasma TG, the postprandial TG increase (*Figure 1*) was greater in the LIPO group than in the NONLIPO group and healthy controls, which resulted in a higher TG-dAUC and total TG-AUC (*Table 3*). Although fasting FFA values were not significantly different between the groups, FFA levels increased postprandially more in the LIPO group than in the other groups.

Figure 1
Mean plasma triglyceride (upper panel), free fatty acid (left lower panel) and hydroxybutyric acid (right lower panel) concentrations during the oral fat loading test in HIV-infected patients with (closed squares) and without (open squares) lipodystrophy and in healthy controls (closed circles)

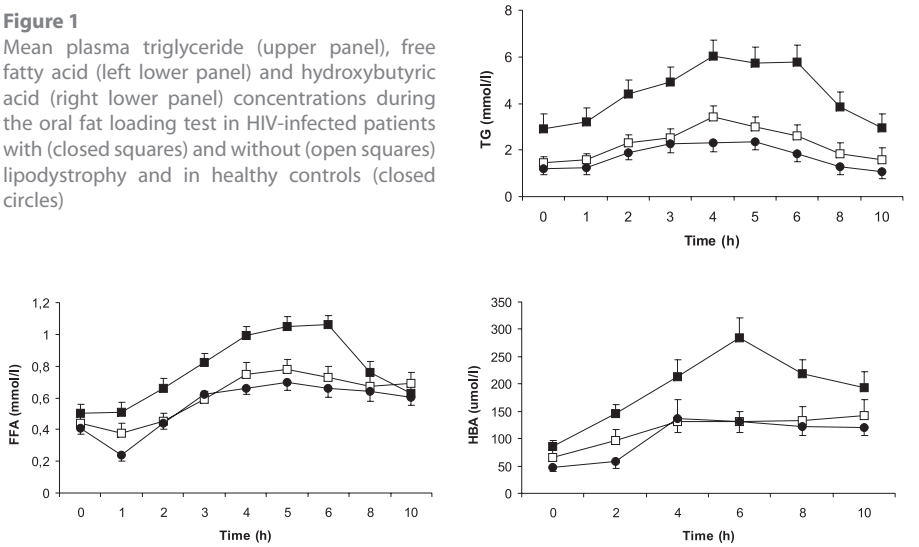


Table 3
Fasting levels and total and incremental area's under the curves for triglycerides, free fatty acids, hydroxybutyric acid and complement component 3 during an oral fat loading test

	LIPO group (n=26)	NONLIPO group (n=12)	Healthy controls (n=35)	P value from ANOVA
Fasting TG (mmol/l)	3.55 (2.07) *#	1.78 (0.59)	1.41 (0.42)	0.01
TG-AUC (mmol.h/l)	45.0 (22.8) *#	22.7 (10.7)	17.4 (6.1)	0.001
TG-dAUC (mmol.h/l)	15.8 (10.7) *#	8.0 (4.7) *	3.9 (3.4)	0.001
Fasting FFA (mmol/l)	0.51 (0.22)	0.43 (0.20)	0.42 (0.25)	0.70
FFA-AUC (mmol.h/l)	8.0 (2.0) *#	6.3 (1.0)	5.9 (1.2)	0.002
FFA-dAUC (mmol.h/l)	3.0 (1.7) *#	1.9 (1.7)	1.0 (1.6)	0.007
Fasting HBA (μmol.h/l)	87 (56)	66 (34)	47 (53)	0.003
HBA-AUC (μmol.h/l)	1954 (1123) *#	1188 (567)	1067 (501)	0.008
HBA-dAUC (μmol.h/l)	1135 (1060) *#	527 (398)	598 (459)	0.009
Fasting C3 (g/l)	1.00 (0.23) *	0.89 (0.17)	0.85 (0.11)	0.03
C3-AUC (g.h/l)	9.9 (2.5) *	8.9 (1.9)	8.95 (1.6)	0.04
C3-dAUC (g.h/l)	0.024 (0.67) *	0.029 (0.24) *	0.45 (0.32)	0.01

AUC = total area under the curve; dAUC = incremental area under the curve; *p<0.05 versus healthy controls; # p<0.05 versus the NONLIPO group

The incremental FFA response (FFA-dAUC) was higher in the LIPO group than in the NONLIPO group and healthy controls, leading to a higher absolute FFA response (FFA-AUC) in the former compared with the other groups. There were no differences in FFA-AUC and FFA-dAUC between the NONLIPO group and healthy controls.

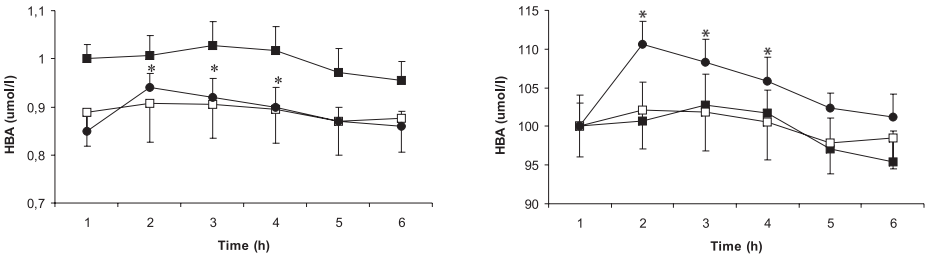
Hydroxybutyric acid changes in response to an oral fat loading test

Fasting HBA values were higher in the LIPO group compared with healthy controls, with intermediate concentrations in the NONLIPO group. The postprandial HBA increase (HBA-dAUC) was two times higher in the LIPO group compared with the NONLIPO group and healthy controls (*Figure 1*), which resulted in a significantly higher HBA-AUC. HBA-AUC and HBA-dAUC were similar between the NONLIPO group and healthy controls.

Complement component 3 changes in response to an oral fat loading test

Fasting C3 was higher in the LIPO group compared with healthy controls, with intermediate concentrations in the NONLIPO group. C3 showed a significant increase in healthy controls (*Figure 2*), reaching maximum concentrations 2-h postprandially. In contrast, in the LIPO and NONLIPO group, there were no significant changes in C3 levels after the oral fat load. Hence, the incremental C3 response (C3-dAUC) was higher in healthy controls compared with both, the LIPO and NONLIPO group. However, the total C3-AUC was higher in the LIPO group compared with healthy controls, due to the higher fasting levels.

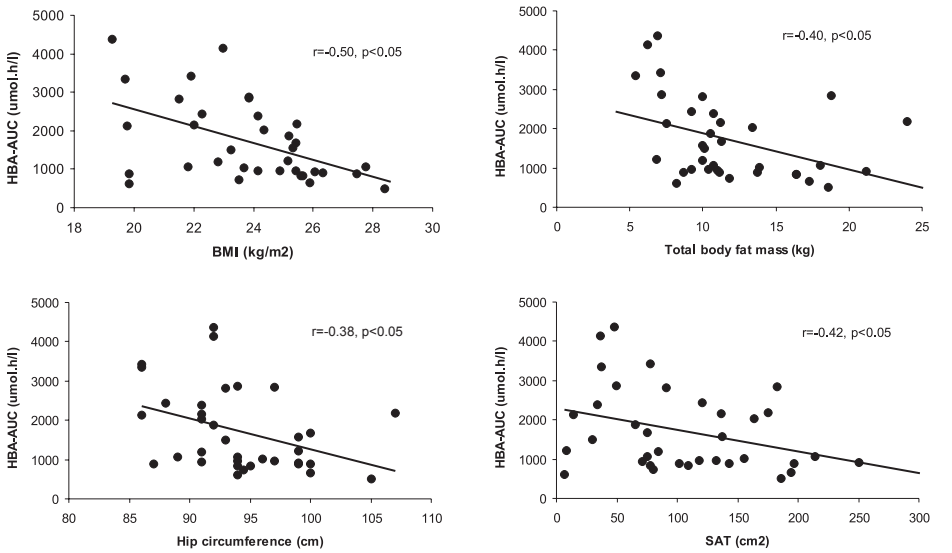
Figure 2
Mean absolute (left panel) and relative (right panel) changes of complement component 3 (C3) during the oral fat loading test in HIV-infected patients with (closed squares) and without (open squares) lipodystrophy and in healthy controls (closed circles). * p<0.05 versus baseline



Correlations between postprandial lipemia and other parameters

HBA-AUC was positively associated with FFA-AUC and FFA-dAUC (r=0.38 and r=0.32, respectively, p<0.05 for each), and inversely related to BMI (r=-0.50, p<0.01, *Figure 3*), total body fat mass (r=-0.40, p<0.05), hip circumference (r=-0.38, p<0.05) and SAT (r=-0.42, p<0.05), but not to waist circumference and VAT. Using stepwise multiple regression

Figure 3
Correlations between HBA-AUC and BMI (left upper panel), total body fat mass (right upper panel), hip circumference (left lower panel) and subcutaneous adipose tissue area (right lower panel) in HIV-infected patients. From 5 subjects, the anthropometric data were incomplete. Hence, correlations are given for the remaining 33 subjects



analysis, HBA-AUC was best predicted by fasting HBA (standardized β=0.35, p<0.05) and BMI (standardized β=-0.46, p<0.005), explaining 35% of the variation. FFA-AUC was significantly related to total body fat mass (r=-0.37, p<0.05), SAT (r=-0.35, p<0.05) systolic and diastolic blood pressure (r=0.39 for both, p<0.05), HOMA (r=0.40, p<0.05), apoB (r=0.48, p<0.01) and TG-AUC (r=0.71, p<0.001). Fasting FFA (standardized β=0.34, p<0.001), HBA-AUC (standardized β=0.27, p<0.005) and TG-AUC (standardized β=0.62, p<0.001) were the best predictors of FFA-AUC, explaining 77% of the variation. TG-AUC was related to fasting TG (r=0.94, p<0.05), cholesterol (r=0.36), apoB (r=0.56), HDL-cholesterol (r=-0.42), systolic blood pressure (r=0.37, p<0.05) and HOMA (r=0.68, p<0.05), but not with body fat distribution. Fasting plasma C3 was related to fasting plasma glucose (r=0.62, p<0.01), HOMA (r=0.54, p<001), apoB (r=0.61, p<0.01), cholesterol (r=0.48, p<0.05), plasma TG (r=0.71, p<0.01), HDL-cholesterol (r=-0.37, p<0.05), systolic blood pressure (r=0.42, p<0.05), but not with body fat distribution or HBA-AUC.

Discussion

The data of the present study suggest impaired adipocyte FFA trapping that contributes to postprandial lipemia in patients with HIV-lipodystrophy. The negative relationships between postprandial concentrations of FFA and HBA and subcutaneous adipose tissue area indicate that this fat compartment contributes most to the trapping defect. Our data do not support the concept that impaired FFA trapping in HIV involves

malfunctioning of the C3-system, since there was no difference in the postprandial C3 response between both HIV-infected groups.

Adipose tissue FFA trapping plays a crucial role in regulating FFA concentrations in the postprandial period[5]. Despite similar fasting FFA levels, HIV-infected patients with lipodystrophy showed a much greater postprandial FFA increase than the patients without lipodystrophy and the controls. Postprandial FFA and HBA levels were both negatively associated with SAT, but not to VAT despite its direct portal drainage. These data are suggestive for impaired ability to store FFA as TG in subcutaneous adipose tissue in patients with HIV-lipodystrophy. In vivo evidence supporting this concept has been provided in a small study using oral labeled TG, showing markedly diminished TG clearance and increased flux of labeled FFA in patients with HIV-lipodystrophy, indicating defective LPL function[23]. In addition, defects in hormone-sensitive lipase(HSL)-mediated inhibition of lipolysis have been described in the same population[24-26]. Hypertriglyceridemia in HIV has been associated with increases in lipolysis, FFA oxidation and hepatic re-esterification[25,26]. Although increased FFA release from adipose tissue contributes to hypertriglyceridemia, insulin-induced suppression of lipolysis did not normalize the VLDL-TG secretion rate[25], suggesting additional defects in TG clearance[23].

The definition of lipodystrophy in patients with HIV infection is often arbitrary. Our subgroup allocation was based on self-reported symptoms and physician clinical judgment. The results of the CT scans showing a significant lower SAT in the patients with lipodystrophy justifies this subgroup allocation approach.

If adipocyte FFA trapping is disturbed, then non-adipose tissues are exposed to excessive FFA concentrations, which may have several metabolic consequences. First, elevated postprandial FFA may aggravate insulin resistance. Indeed, the patients with lipodystrophy had higher HOMA compared with the patients without lipodystrophy. Also supportive is the close relationship between HOMA and FFA-AUC observed in the HIV-infected patients. Second, our data demonstrate marked increased postprandial HBA levels in patients with HIV-lipodystrophy. As ketogenesis (HBA production) occurs predominantly in hepatocytes, and FFA availability is a major determinant of rates of ketone body production in man[7], postprandial ketone body appearance may reflect hepatic FFA delivery. Previously, the severity of insulin resistance in patients with HAART-associated lipodystrophy has been related to the extent of fat accumulation in the liver[27]. Hepatic FFA accumulation may therefore play a causative role in the development of insulin resistance in these patients. Third, FFA reaching the liver may upregulate the production of apoB-containing TG-rich particles by the liver[25,26]. Although fasting apoB levels were similar between HIV-infected patients with and without lipodystrophy, TG levels were almost two-fold increased in the patients with lipodystrophy. A possible explanation may be that protease inhibitor-containing regimens increased the secretion of VLDL particles in both groups, regardless of the presence of lipodystrophy, by inhibiting proteosomal degradation of apoB in the liver[28,29]. Hence, the two-fold increased TG levels in HIV-infected patients with lipodystrophy

caused relatively TG-enriched VLDL. Besides elevated hepatic production, defects in LPL-mediated TG clearance may contribute to hypertriglyceridemia in this population[23]. In agreement, our data show an exaggerated and prolonged postprandial TG response in the patients with lipodystrophy. Finally, it should be noted that, despite similar apoB, the patients with lipodystrophy had lower LDL-cholesterol than the patients without lipodystrophy, suggesting the presence of atherogenic small dense LDL particles in the former. Taken together, disturbed postprandial FFA metabolism may induce a vicious circle of metabolic risk factors that increase cardiovascular risk in patients with HIV-lipodystrophy[4].

Insulin and ASP are principal determinants of FFA trapping by adipose tissue. The ability of insulin to suppress FFA release and to up-regulate LPL-mediated TG clearance is impaired in subjects with insulin resistance[30-32]. Our data suggest similar impairments in patients with HIV-lipodystrophy. The pathway of FFA trapping is also regulated by the C3-system. However, our data do not support the concept that impaired FFA trapping in HIV involves malfunctioning of the C3-system, since there was no difference in the postprandial C3 response between both HIV-infected groups. Moreover, C3 was not associated with body fat distribution, neither with FFA and HBA levels. In a previous study, the absolute production of ASP as well as the percentage conversion of C3 to ASP were significantly lower in HIV-infected subjects with lipodystrophy than in subjects without lipodystrophy or control subjects[33]. We observed a strong relationship between C3 and several parameters of the insulin resistance syndrome, in agreement with literature[13-17,34,35].

Several studies have investigated FFA metabolism in relation to body composition and insulin resistance in patients with HIV-lipodystrophy[24-26,36-39]. However, most of these studies have been performed in the fasting state or under hyperinsulinemic conditions. Basal lipolytic rates are generally increased in patients with HIV-lipodystrophy[24-26], suggesting impaired action of HSL. In addition, several studies have reported elevated FFA levels following glucose or insulin challenges[37-39], suggesting resistance to the action of insulin to suppression of lipolysis. High FFA levels have been related to markers of insulin resistance and body composition in HIV-infected patients[24,37]. For example, FFA levels after standard glucose challenge were positively associated with VAT[24]. In contrast, fasting FFA levels were inversely associated with SAT in the same study. Our study using a high-fat meal (under low-insulin conditions) showed that increased postprandial FFA levels were related to markers insulin resistance (HOMA) and lipoatrophy (low SAT) in HIV-infected patients.

In conclusion, the results of the present study suggest disturbed postprandial FFA metabolism in patients with HIV-lipodystrophy, most likely due to inadequate incorporation of FFA into TG in subcutaneous adipose tissue. The higher postprandial HBA levels reflect increased hepatic FFA delivery, and may aggravate several metabolic risk factors, ultimately leading to an increased risk for cardiovascular disease in these patients.

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Submitted

7

Functional and structural markers of atherosclerosis in HIV-infected patients

Abstract

Objective

Antiretroviral combination therapy in HIV has been associated with cardiovascular risk factors that cluster in the metabolic syndrome (MS). We investigated functional and structural markers of atherosclerosis in HIV-infected patients in relation to the presence of the MS.

Research Design and Methods

Thirty-seven HIV-infected patients underwent assessment of flow-mediated vasodilation (FMD), aortic pulse-wave velocity (PWV) and carotid intima-media thickness (IMT). Age-matched type 2 diabetic patients (n=15) and healthy controls (n=15) served as reference groups for FMD and PWV measurements.

Results

Fifteen HIV-infected patients (41%) fulfilled the National Cholesterol Education Program criteria of the MS. FMD was similarly impaired in HIV-infected patients without the MS (MS- group) and the diabetic patients (5.1±0.4% and 4.9±0.6%, respectively) compared with controls (8.8±0.7%). HIV-infected patients with the MS had even more impaired FMD (2.5±0.3%), as well as increased IMT compared with the MS- group. Aortic PWV was increased in the diabetic patients only. In HIV-infected patients, FMD was related to metabolic parameters, while aortic PWV and IMT were related to parameters of HIV infection, time on antiretroviral combination therapy, inflammatory parameters (C-reactive protein and leukocytes) and metabolic parameters.

Conclusions

Endothelial function is similarly disturbed in HIV-infected patients without the MS and diabetic patients, suggesting increased cardiovascular risk in HIV-infected patients, even in the absence of metabolic risk variable clustering. The presence of the MS in HIV is associated with even more advanced atherosclerotic changes. Presumably, both HIV infection and antiretroviral therapy may promote atherosclerosis through mechanisms involving endothelial cells, either directly or indirectly via metabolic risk factors.

Introduction

The use of highly active antiretroviral therapy (HAART) in HIV has been associated with insulin resistance, glucose intolerance, unfavorable fat distribution and dyslipidemia [1-4]. The clustering of these risk factors shows striking similarities with the metabolic syndrome (MS), as described in HIV-negative individuals[5]. The increasing prevalence of the MS in HIV is cause for concern, because the MS is associated with an increased risk for cardiovascular disease (CVD)[5]. As survival of patients with HIV increases, CVD may become an important complication in the management of these patients. Despite the close relationship between HAART and metabolic risk factors, controversy surrounding HIV infection and CVD still exists. For example, in a large retrospective study, use of HAART was associated with a large benefit in terms of AIDS-related mortality that was not diminished by any increase in the rate of cardiovascular events or related mortality [6]. However, 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[7].

Endothelial dysfunction is an early marker of atherosclerosis and can be assessed clinically by ultrasound assessment of brachial artery flow-mediated vasodilation (FMD). Flow-mediated vasodiltaion is correlated with the severity and extend of coronary sclerosis[8], and predicts future cardiovascular events[9]. Ultrasound measurement of carotid intima-media thickness (IMT) is a well-accepted, noninvasive method of assessing early changes in vascular structure, and is widely used as a surrogate marker for atherosclerotic disease[10]. Aortic pulse-wave velocity (PWV) is a non-invasive measurement of arterial stiffness, and is associated with end-organ alterations, such as increased ventricular stress and arterial intima–media thickening[11]. Aortic PWV is also an independent predictor of cardiovascular mortality[12]. Assessment of all three preclinical atherosclerotic markers may provide important information on both functional and structural stages of atherosclerosis.

In a cross-sectional study of HIV-infected adults it was shown that those on a protease inhibitor(PI)-containing regimen had marked impaired FMD compared with those not taking PIs[13]. However, in that study, FMD was not compared with HIV-negative reference groups and the relative contributions of antiretroviral agents, chronic inflammation due to viral infection and metabolic risk factors and their interactions are difficult to identify. Structural vascular abnormalities are also present in patients with HIV infection. Carotid IMT is higher in HIV patients than in age-matched control subjects[14,15], and progresses much more rapidly than previously reported rates in non-HIV cohorts[16]. Arterial stiffness has not been investigated in HIV-infected adults. Clearly, most studies using intermediate CVD endpoints suggest an increased risk for premature atherosclerosis in HIV-infected patients.

It is becoming increasingly important to identify those HIV-infected patients that have the highest risk for atherosclerosis. However, there is still a lack of studies on

cardiovascular risk assessment using established intermediate endpoints in HIV-infected patients. In the present study, we investigated FMD, aortic PWV and IMT in HIV-infected males on stable HAART and subdivided the group in those with and without the MS as defined by the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII) guidelines[17]. In addition, we related these well-established parameters of the vasculature to clinically easily obtainable anthropometric, haemodynamic and laboratory parameters. The data on FMD and aortic PWV were compared with data from age-matched type 2 diabetic patients, who are known to have a marked increased cardiovascular risk[18], and healthy males as controls.

Methods

Subjects

Males aged between 18 and 70 years with a documented HIV infection were recruited from the Department of Infectious Disease of the University Medical Center Utrecht. Inclusion criteria were HIV-RNA <10.000 copies/ml and HAART for at least 12 months. Exclusion criteria were the presence of opportunistic infectious disease and/or malignancies, renal- and/or liver disease, diabetes mellitus and the use of lipid-lowering and/or anti-hypertensive agents. The study protocol was approved by the local research ethics committee of the University Medical Center Utrecht. All participants gave written informed consent. The data on FMD and aortic PWV were compared with data from 15 age-matched type 2 diabetic and 15 healthy males. The diabetic patients were all treated with oral anti-hyperglycemic agents (5 with sulfonylureum derivatives, 4 with metformin and 6 with a combination of both) and had glycated hemoglobin <9%. None of the diabetic subjects were taking statins and/or anti-hypertensive agents. Further, the diabetic patients and the healthy controls met the same inclusion criteria as the HIV-infected patients. At inclusion, length, weight, blood pressure and waist and hip circumference were measured.

Oral glucose tolerance test

A standard oral glucose tolerance tests (OGTT) was performed in the HIV-infected patients only. The patients visited our department after a 10 h fast. After placing a cannula for venous blood sampling, subjects rested for 15 minutes before administration of the glucose load (75 g). Peripheral blood samples were obtained in sodium EDTA (2 mg/ml) before and at regular 30-minute intervals up to 2-h following glucose ingestion. All samples were kept on ice and centrifuged immediately for 15 minutes at 800g at 4°C and stored at -80°C until assayed.

Endothelial function

Nitric oxide (NO)-dependent flow mediated vasodilation (FMD), as percentage diameter change in the brachial artery after reactive hyperemia, was measured non-invasively

by ultrasonography[19]. Measurements were performed at the elbow of the right arm using a vessel wall-movement system (Wall Track System, Pie Medical), which consists of an ultrasound imager with a 10 MHz linear array transducer connected to a data acquisition system and a personal computer. Three measurements were averaged to calculate a baseline diameter of the brachial artery. By inflation of a blood pressure cuff for 5 min at a pressure of 200 mmHg, ischaemia was applied to the forearm distal to the location of the transducer. Ultrasonography continued for 5 min after cuff release, with measurements at 30-second intervals. The widest lumen diameter was taken as a measure for maximal vasodilation. Nitroglycerin (400 μg) was used to determine endothelium-independent vasodilation. All measurements were performed by the same technician with subjects supine in a quiet, temperature-controlled (20°C to 22°C) environment after at least 15 minutes of rest. All subjects were requested to refrain from smoking on the morning of the vascular measurements

Pulse-wave velocity

Arterial stiffness was assessed non-invasively by aortic pulse-wave velocity (PWV)[20]. Aortic PWV was calculated as distance/transit time (in centimeters per second) of the pulse wave from the base of the neck for the common carotid to the right femoral artery. The pulse waves at each of these sites were obtained sequentially with a tonometric sensor (Sphygmocor, Atcor Medical, Australia). Pulse transit time was determined as the average of 10 consecutive beats. The distance traveled by the pulse waveform was measured over the participant's torso. The validation of this automatic method and its reproducibility have been published previously[20]. The measurements were performed twice in each patient and then averaged to obtain the mean aortic PWV, which was used for the statistical analysis.

Carotid intima-media thickness

To measure carotid intima-media thickness (IMT), ultrasonography of the left and right common carotid artery (CCA) was performed with a 10 MHz linear-array transducer (ATL UltraMark IV). In accordance with the Rotterdam Study ultrasound protocol[21], a careful search was performed for all interfaces of the anterior (near) and posterior (far) walls of the distal CCA. The optimal longitudinal image was frozen on the R wave of the ECG and stored on videotape. This procedure was repeated four times for both sides. From the videotape, the frozen images were digitized and displayed on the screen of a personal computer using additional dedicated software[22]. With a cursor, the interfaces of the distal CCA were marked across a length of 10 mm. The beginning of the dilatation of the distal CCA served as a reference point for the start of the measurement. The average of the IMT of each of the four frozen images was calculated. For each individual, the IMT was determined as the average of near- and far-wall measurements of both the left and right arteries. The reproducibility of IMT measurements has been previously described [22]. Measurement of IMT was performed in the HIV-infected patients only.

Analytical methods

Total and HDL-cholesterol, triglycerides, apolipoprotein B, glucose and glycated hemoglobin were measured using standard laboratory procedures. Ultracentrifugation was used to isolate LDL. Insulin was measured by ELISA (Mercodia, Uppsala, Sweden). Blood leukocyte counts were determined automatically using a Celldyn-3500® (Abbott, USA). Plasma CRP was measured using a high sensitivity method (hs-CRP) (Quantex hs-CRP kit, Biokit, S.A., Barcelona, Spain), with lower limit of detection of 0.10 mg/L. HIV viral load was determined by ultrasensitive assay (Roche Diagnostics Amplicor HIV-1 Monitor assay, Pleasanton, CA, USA) and CD4 cell counts were determined by flow cytometry.

Statistical analysis

Data are expressed as mean (SEM) in the text, tables and figures. The NCEP-ATPIII guidelines were used to identify subjects with the MS[17]. The MS was present when at least three out of five risk determinants (increased waist circumference, increased blood pressure, elevated fasting TG, low HDL-cholesterol and elevated fasting glucose) were present. Differences between two groups were tested with Mann-Whitney tests. Comparisons between three groups or more were performed with one-way ANOVA, with least significance difference test as post hoc analysis test. Bivariate correlations for the total group of HIV-infected patients were calculated using Spearson's correlation coefficients. All significantly correlated variables were used as independent variables in stepwise multiple regression analysis with FMD, aortic PWV and IMT as dependent variables. Calculations were performed using SPSS/PC + 11.5 (SPSS Inc. Chicago, IL, USA). Statistical significance was taken at the 5% level.

Results

Characteristics of the study group

Of the thirty-seven HIV-infected patients included in the study, fifteen (41%) fulfilled the NCEP-ATPIII criteria of the MS. Elevated fasting plasma TG (68%), increased blood pressure (54%) and low HDL-cholesterol (41%) were the most prevalent components of the MS, whereas high fasting glucose (3%) and high waist circumference (5%) were less prevalent. None of the healthy controls fulfilled the criteria of the MS, whereas all type 2 diabetic patients had the MS. All HIV-infected patients were currently receiving HAART with nucleoside reverse transcriptase inhibitors and a PI and/or a non-nucleoside reverse transcriptase inhibitor (*Table 1*). Baseline characteristics and metabolic profile of the HIV-infected groups, the diabetic patients (n=15) and healthy males (n=15) are shown in *Table 2*. Apolipoprotein B was similarly increased in the diabetic patients and the MS- group as compared with the healthy controls, and it was highest in the MS+ group. Nine HIV-infected patients (all in the MS+ group) had impaired glucose tolerance (IGT).

Table 1
Virological and immunological characteristics and HAART of the HIV-infected patients

	MS+ group (n=15)	MS- group (n=22)
Time since diagnosis of HIV (yrs)	8.5 (0.8)	7.5 (5.2)
Time on HAART (yrs)	4.8 (0.5)	4.2 (0.6)
CD4 cell count (10^6 cells/L)	604 (105)	719 (58)
HIV viral load (copies/ml)	1114 (824)	813 (201)
HIV viral load < 50, n (%)	12 (80%)	17 (77%)
Protease inhibitors, n (%)	10 (67%)	15 (68%)
NNRTI, n (%)	5 (33%)	7 (32%)
NRTI, n (%)	15 (100%)	22 (100%)

Data are means (SEM), unless other indicated
NNRTI = non-nucleoside reverse transcriptase inhibitor
NRTI = nucleoside reverse transcriptase inhibitor
There were no differences in virological and immunological parameters and HAART between the groups

Functional and structural vascular assessment

Hemodynamic, inflammatory and vascular parameters of the study group are shown in *Table 3*. Baseline brachial artery lumen diameter measurements were not different between the groups (*Table 3*). Flow-mediated vasodilation was similarly impaired in the MS- group and the diabetic patients compared with the controls (*Figure 1*, panel A). The MS+ group had even more impaired FMD than the MS- group and the diabetic patients. Endothelium-independent vasodilation was higher in the controls than in the other groups. Carotid IMT was markedly increased in the MS+ group compared with the MS- group (*Table 3*). The diabetic patients had increased aortic PWV compared with the other groups (*Figure 1*, panel B), but no difference in aortic PWV was observed between the MS+ group and the MS- group. The HIV-infected patients with IGT (n=9) had increased aortic PWV compared with those with normal glucose tolerance (9.2±0.7 versus 7.7±0.6 cm/s, p=0.03). The patients on a PI-containing regimen (n=25) had a tendency towards more impaired FMD compared with those not taking PIs (n=12, 4.1±0.4 versus 5.3±0.6%, respectively, p=0.09), but similar aortic PWV (8.1±1.1 versus 7.9±0.9 cm/sec, respectively).

Determinants of vascular measurements in HIV-infected patients

Correlations between the vascular measurements and anthropometric, haemodynamic and laboratory parameters for the total group of HIV-infected patients (n=37) are shown in *Table 4*. Using stepwise multiple regression analysis, FMD was best predicted by apolipoprotein B (standardized β=0.30, p=0.01), explaining 40% of the variation. Aortic PWV was best predicted by the mean arterial pressure (standardized β=0.14,

Table 2
Characteristics of HIV-infected patients with (MS+ group) and without (MS- group) the metabolic syndrome, type 2 diabetic patients and healthy controls

	MS+ group (n=15)	MS-group (n=22)	Type 2 diabetic males (n=15)	Healthy males (n=15)	P value from ANOVA
Age (years)	50 (3)	47 (2)	53 (2) ‡	51 (3)	0.75
BMI (kg/m^2)	24.4 (0.5) †	23.6 (0.4)	28.4 (1.1) *‡	24.1 (0.7)	0.03
Waist circumference (cm)	94 (2) †	92 (2)	101 (3) *‡	94 (3)	0.02
Waist-to-hip ratio	1.00 (0.02) *	0.98 (0.01) *	0.98 (0.02) *	0.92 (0.02)	0.01
Smoking, n (%)	2 (13%)	3 (14%)	2 (13%)	2 (13%)	0.88
Total cholesterol (mmol/l)	6.2 (0.2) *†‡	5.4 (0.2)	5.3 (0.2)	5.0 (0.3)	0.02
LDL-cholesterol (mmol/l)	2.99 (0.2)	3.30 (0.2)	3.32 (0.25)	3.00 (0.19)	0.21
HDL-cholesterol (mmol/l)	1.05 (0.05) *	1.18 (0.05)	1.06 (0.13) *‡	1.22 (0.11)	0.04
Triglycerides (mmol/l)	3.82 (0.74) *†	2.01 (0.43)	2.67 (0.41) *	1.48 (0.10)	0.02
Apolipoprotein B (g/l)	1.29 (0.05) †‡	1.03 (0.04)	0.94 (0.07)	ND	0.02
Glycated hemoglobin (%)	5.2 (0.1) †	5.2 (0.07) †	6.2 (0.2)	ND	0.03
Fasting glucose (mmol/l)	5.4 (0.3) †	5.1 (0.2)	7.6 (0.4) *‡	5.0 (0.3)	0.01
Fasting insulin (mU/l)	9.0 (1.4) *‡	5.8 (1.4)	11.8 (1.9) *‡	4.2 (1.2)	0.01
2-h glucose (mmol/l)	8.0 (0.7) ‡	6.5 (0.3)	ND	ND	
2-h insulin (mU/l)	74.6 (18.9) ‡	29.9 (6.8)	ND	ND	

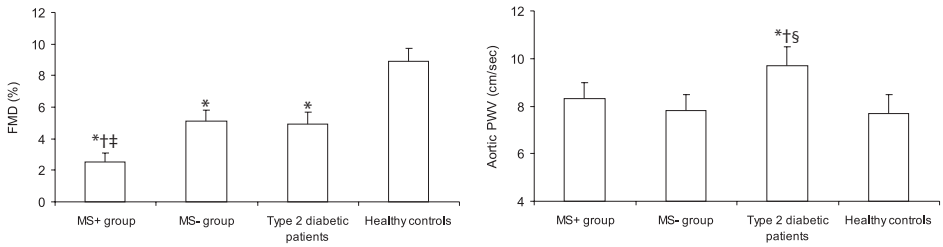
Data are means (SEM). ND = not determined. * $p<0.05$ versus healthy males. † $p<0.05$ versus diabetic patients. ‡ $p<0.05$ versus MS- group.
The criteria of the metabolic syndrome (waist, TG and HDL-cholesterol) were not statistically analyzed between the MS+ and MS- groups

Table 3
Hemodynamic, inflammatory and vascular parameters in HIV-infected patients with (MS+ group) and without (MS- group) the metabolic syndrome, type 2 diabetic patients and healthy controls

	MS+ group (n=15)	MS-group (n=22)	Type 2 diabetic males (n=15)	Healthy males (n=15)	P value from ANOVA
Systolic BP (mmHg)	140 (4)	127 (2)	144 (3) *‡	126 (2)	0.03
Diastolic BP (mmHg)	84 (2)	80 (2)	87 (2) *‡	78 (2)	0.04
Mean arterial pressure (mmHg)	104 (2) *	96 (2)	108 (3) *‡	96 (2)	0.03
CRP (mg/l)	5.8 (1.7) *	3.9 (1.0) *	3.1 (1.6) *	0.87 (0.66)	0.007
Leukocyte count (cells x10^9/l)	7.3 (2.4) *	6.7 (2.0) *	6.2 (1.8) *	5.6 (1.4)	0.02
Neutrophils (cells x10^9/l)	4.1 (0.5) *	3.6 (0.3) *	3.7 (0.3) *	3.1 (0.3)	0.04
Lymphocytes (cells x10^9/l)	2.4 (0.2) *†	2.4 (0.2) *†	1.8 (0.1)	1.8 (0.1)	0.03
Monocytes (cells x10^9/l)	0.64 (0.05)	0.59 (0.05)	0.53 (0.08)	0.40 (0.06)	0.12
Brachial artery diameter (mm)	4.56 (0.17)	4.45 (0.13)	4.51 (0.14)	4.47 (0.09)	0.67
NTG (%)	12.2 (1.6) *	13.0 (1.5) *	13.1 (1.8) *	18.5 (2.0)	0.02
Carotid IMT (mm)	0.85 (0.05) ‡	0.65 (0.03)	ND	ND	

Data are means (SEM); ND = not determined; * p<0.05 versus healthy males; † p<0.05 versus diabetic patients; ‡ p<0.05 versus MS- group. The criteria of the metabolic syndrome (SBP and DBP) were not statistically analyzed between the MS+ and MS- groups

Figure 1
Mean FMD (left panel) and aortic PWV (right panel) in HIV-infected patients with (MS+ group) and without (MS- group) the metabolic syndrome, type 2 diabetic patients and healthy controls. * p<0.05 versus healthy controls. † p<0.05 versus MS- group. ‡ p<0.05 versus type 2 diabetic patients. § p<0.05 versus MS+ group



p=0.03) and the 2-h glucose concentration (standardized β=0.24, p=0.02), explaining 73% of the variation. Age (standardized β=0.54, p=0.02), apolipoprotein B (standardized β=0.44, p=0.03) and HIV viral load (standardized β=0.33, p=0.04) were the best predictors of IMT, explaining 76% of the variation. In both, the HIV-infected patients and the diabetic patients, glycated hemoglobin levels were not associated with the vascular measurements (data not shown).

Table 4
Spearman's correlation coefficients (r) between vascular measurements and other parameters for the total group of HIV-infected patients (n=37)

	FMD	Aortic PWV	Carotid IMT
Age			0.39 *
Time since diagnosis of HIV		0.40 *	
Time on HAART		0.38 *	0.38 *
HIV viral load			0.47 **
CRP			0.33 *
Blood leukocyte count			0.38 *
Systolic blood pressure	-0.32 *	0.40 *	0.35 *
Diastolic blood pressure		0.43 *	
Mean arterial pressure		0.45 **	
Triglycerides	-0.33 *		
Cholesterol	-0.42 *		0.38 *
Apolipoprotein B	-0.46 **		0.43 *
Fasting glucose	-0.36 *		
2-h glucose		0.40 *	0.36 *
Fasting insulin		0.37 *	
2-h insulin	-0.35 *		

Only significant correlation coefficients are given; * $p<0.05$; ** $p<0.01$

Discussion

As survival of patients with HIV increases, it is becoming increasingly important to identify those patients that are at increased risk for accelerated atherosclerosis. In the present study, we determined the relationship between intermediate endpoints of CVD and clinically easily obtainable anthropometric, haemodynamic and laboratory parameters in HIV-infected patients. Our main findings are that HIV-infected patients without the MS have endothelial dysfunction similar to that of type 2 diabetic patients. The presence of the MS in HIV was associated with even more advanced functional and structural vascular abnormalities.

In the present study, 41% of the patients fulfilled the definition of the MS according to the NCEP-ATPIII guidelines. In the U.S., approximately 24% of the general population fulfills this definition[23]. Elevated TG were the most prevalent component of the MS, whereas an increased waist circumference and high fasting plasma glucose were less prevalent. Despite normal fasting glucose levels, 24% of the HIV-infected patients demonstrated IGT (24%). On average, both HIV-infected groups study group had normal waist circumference (<102 cm), but a relatively high waist-to-hip ratio. Hence, the presentation of the MS in HIV-infected patients may differ from that in the general

population, due to differences in body composition and direct effects of antiretrovirals on glucose and lipid homeostasis. We have chosen to use the NCEP definition for the MS, because these criteria do not include OGTT and urinary albumin, making it more easily applicable for routine screening of HIV-infected patients than the WHO definition[24]. Atherosclerosis is characterized and preceded by endothelial dysfunction[8,9]. In the present study, HIV-infected patients without the MS showed endothelial dysfunction comparable to that of age-matched type 2 diabetic patients, who are known to have a marked increased risk for CVD[18]. These data suggest increased CVD risk in HIV-infected patients, even in the absence of metabolic risk variable clustering. Several factors may explain this observation. Firstly, in HIV-infected patients, the endothelium could be activated either directly by HIV or by a leukocyte-mediated inflammatory cascade triggered by HIV infection. Several studies have shown that HIV-associated proteins (gp120 and Tat) interact with chemokine receptors and induce endothelial cell apoptosis[25,26]. Endothelial activation may also occur by cytokines secreted in response to leukocyte activation by HIV[27-29]. We observed elevated blood leukocyte counts and elevated CRP levels in the HIV-infected patients, most likely due to chronic immune activation associated with HIV infection[27-29]. However, besides chronic immune activation, CRP levels have also been related to body composition in HIV-infected patients[30]. In HIV-negative subjects, both leukocyte counts and CRP have been linked to endothelial dysfunction and future cardiovascular events[31-34]. Secondly, it should be noted that the MS- group was characterized by high apolipoprotein B, despite the absence of other metabolic risk factors, and apolipoprotein B was also closely related to endothelial dysfunction in our study. This may be due to direct inhibitory effects of PIs on proteosomal apolipoprotein B degradation in the liver[35]. Thirdly, antiretroviral agents may also directly induce endothelial dysfunction. For example, when healthy volunteers were given indinavir for 4 weeks, significant endothelial dysfunction was observed, independent of the lipid profile[36]. Whether this effect also occurs in HIV-infected patients is not known.

In our study, the patients on a PI-containing regimen showed a tendency towards more pronounced endothelial dysfunction compared with those on a NNRTI-containing regimen, which may be significant if repeated in a larger group. In our study, we excluded patients with HIV-RNA > 10.000 copies/ml and patients with AIDS-related diseases. Lymphocyte counts were normal in our group of HIV-infected patients, while these are generally low in untreated HIV-infected patients, and in patients with AIDS-related diseases. Almost 80% of the patients had HIV-RNA < 50 copies/ml. However, some patients demonstrated discordant responses in virologic and immunological parameters. Virologic failure, immunologic failure, and clinical progression have distinct time courses and may occur independently or simultaneously. In patients with a history of extensive prior treatment and drug resistance complete viral suppression is often difficult to achieve. Thus, the goal is to preserve immunologic function and prevent clinical progression, even with ongoing viremia. Even partial virologic suppression of

HIV-RNA correlates with clinical benefits probably due to diminished viral fitness.

As expected, the presence of the MS was associated with more severe endothelial dysfunction, as well as a marked increased IMT, in our cohort of HIV-infected males, suggesting more advanced functional and structural atherosclerotic changes. Hence, the NCEP-ATPIII criteria of the MS may also be used in HIV-infected patients in order to identify subjects at risk for accelerated atherosclerosis. Other observational studies have shown that IMT in HIV-infected patients is related to several traditional risk factors[14-16], but when a control group was added to the analysis, HIV infection was also an independent predictor of IMT[16]. Furthermore, progression of IMT has been related to nadir CD4 counts (≤ 200)[16], which were unfortunately not available in most of our patients. We did not find a relationships between vascular parameters and the actual CD4 cell count, but the results of our study suggest that metabolic, virologic and inflammatory parameters, time since diagnosis of HIV infection and time on HAART may contribute to structural atherosclerotic changes in HIV-infected patients. It needs to be noted that we only measured intermediate markers of CVD, and not CVD endpoints. Nevertheless, given the observed metabolic profile and vascular abnormalities, at least a number of HIV-infected patients in our study group would be eligible for lipid-lowering therapy.

A limitation of the study is the absence of IMT measurements in the diabetic and healthy volunteer groups which precludes a comparison in vascular structure between these groups and the HIV-infected subjects. In addition, the nitroglycerin response was lower in both the HIV-infected and the diabetic patients compared with the controls. Impaired smooth muscle responsiveness has been observed previously in patients with type 2 diabetes[37]. No difference in nitroglycerin responsiveness was observed between both HIV-infected groups and the diabetic patients, allowing comparisons in endothelial function assessed by FMD between these groups.

The presence of the MS per se was not associated with increased arterial stiffness, despite impaired endothelial function and increased IMT in these patients. In contrast, the HIV-infected patients with IGT (n=9) showed increased aortic PWV, comparable to that of type 2 diabetic patients. This may suggest that increased arterial stiffness is confined to HIV-infected subjects with deteriorating glucose tolerance status. Previous studies support a close relation between IGT, type 2 diabetes and arterial stiffness[38,39]. The close associations between aortic PWV and the 2-h glucose concentration and insulin sensitivity support this concept. Aortic PWV was also associated with the time since diagnosis of HIV infection and the time on HAART.

In summary, endothelial function is disturbed in HIV-infected patients, even in the absence of metabolic risk variable clustering. The presence of the MS is associated with even more advanced atherosclerotic changes in HIV-infected patients. Presumably, both HIV infection and antiretroviral therapy may promote atherosclerosis through mechanisms involving endothelial cells, either directly or indirectly via metabolic risk

factors. Future studies are necessary to investigate whether treatment of metabolic risk factors could lead to reduced cardiovascular risk in these patients.

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Ann Intern Med 2005; in press

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Comparison of rosiglitazone and metformin for treating HIV-lipodystrophy: a randomized trial

Abstract

Objective

The use of antiretroviral combinationtherapy in HIV has been associated with lipodystrophy and cardiovascular risk factors. We compared the effects of the peroxisome proliferator-activated receptor-γ agonist rosiglitazone (8 mg/d) and metformin (2 g/d) for the treatment of HIV-lipodystrophy.

Research Design and Methods

An open, randomized 6-month clinical trial was performed in thirty-nine HIV-infected male patients with lipodystrophy. Main outcome measures were insulin sensitivity estimated by oral glucose tolerance test, subcutaneous and visceral abdominal fat measured by single-slice computer tomography, endothelial function measured by flow-mediated vasodilation, and fasting plasma measurements. Two patients in the metformin-group discontinued the study. Complete case analysis was performed.

Results

Compared with metformin, rosiglitazone increased subcutaneous abdominal fat (between treatment change from baseline 27 cm^2 [95% CI, 7 to 46 cm^2]) and visceral abdominal fat (between treatment change from baseline 24 cm^2 [95% CI, 6 to 51 cm^2]). The area under the curve for insulin after the oral glucose tolerance test decreased similarly with both agents, but only rosiglitazone increased adiponectin levels. Metformin showed greater benefits on fasting lipid profile than rosiglitazone. Flow-mediated vasodilation increased statistically significantly with metformin (mean change 1.5% [95% CI, 0.4 to 3.3%]), and not with rosiglitazone (mean change 0.7% [95% CI, -1.1 to 2.7%]); the metformin versus rosiglitazone increases were not statistically different. Rosiglitazone and metformin did not change C-reactive protein.

Conclusions

The findings of the present study emphasize the importance of individualized care in HIV-infected patients. Although rosiglitazone may partly correct lipoatrophy, metformin improves visceral fat accumulation, fasting lipid profile and endothelial function.

Introduction

Highly active antiretroviral therapy (HAART) in HIV has greatly reduced AIDS-related mortality[1], but is associated with changes in bodyfat distribution (e.g. lipodystrophy), including peripheral fat loss and central fat accumulation[2-4]. Severe forms of lipodystrophy are a major cosmetic concern and can lead to suboptimal adherence to HAART. In addition, lipodystrophy is associated with metabolic risk factors, including insulin resistance and dyslipidemia[2-6], which have been correlated to surrogate markers of cardiovascular disease[7-9]. Clearly, as survival of patients with HIV increases, cardiovascular disease could become an important complication in the management of these patients[10].

Metformin and rosiglitazone are used in clinical medicine to improve glycemic control in patients with type 2 diabetes[11,12]. However, these agents may also be useful in nondiabetic patients with insulin resistance. Metformin is thought to act mainly by decreasing hepatic insulin resistance and glucose output[11]. Rosiglitazone is an agonist for peroxisome proliferator-activated receptor-γ (PPAR-γ) activation, thereby influencing directly the transcription of genes that regulate glucose and lipid metabolism[12]. PPAR-γ is preferentially expressed in adipose tissue, and the improvement of insulin resistance is partly secondary to enhanced fatty acid storage in subcutaneous adipocytes and improved adipocyte function, as reflected by the altered secretion of adiponectin[12]. *In vitro*, rosiglitazone promotes adipogenesis, even in the presence of a protease inhibitor [13]. Although metformin and rosiglitazone have been investigated separately[14-19], there are no studies available comparing directly the effects of rosiglitazone and metformin for the treatment of HIV-lipodystrophy.

Endothelial dysfunction is an early marker of atherosclerosis and can be assessed clinically by ultrasound assessment of brachial artery flow-mediated vasodilation. Flow-mediated vasodilation is correlated with degree of atherosclerosis[20], and impaired flow-mediated vasodilation is an independent predictor of future cardiovascular events[21-23]. In HIV-infected patients, the use of protease inhibitors has been linked to endothelial dysfunction[7]. However, pravastatin did not improve flow-mediated vasodilation in HIV-infected patients, despite reductions in atherogenic lipoproteins[24]. In HIV-negative individuals, rosiglitazone and metformin improve endothelial function[25-27], but the effects of either of these agents in HIV-infected patients are not known.

We conducted a randomized study to compare the effects of rosiglitazone and metformin on insulin sensitivity, body fat distribution and endothelial function in patients with HIV-lipodystrophy.

Methods

Patients

Males aged between 18 and 70 years with a documented HIV infection were recruited from the Department of Infectious Disease of the University Medical Center Utrecht between March 2003 and January 2004. At the HIV clinic, approximately 600 patients are currently treated for HIV infection, of which an estimated 25% has clinical signs of lipodystrophy. Inclusion criteria were HIV-RNA values < 10.000 copies/ml, the presence of lipodystrophy, and treatment with HAART for at least 18 months with no changes in the treatment regimen during 6 months prior to inclusion. Exclusion criteria were the presence of opportunistic infectious disease or malignancies, renal, thyroid- and/or liver disease, BMI > 30 kg/m^2, fasting plasma glucose > 7 mmol/L, triglycerides > 10 mmol/L and/or total cholesterol > 8 mmol/L and an alcohol intake > 3 units (36 grams) per day or history of alcohol abuse within the last 5 years, current TZD therapy or known sensitivity to TZDs, clinical evidence of congestive heart failure, use of any other investigational agent within the last 4 weeks, concurrent therapy with glucocorticoids, testosteron, anabolic steroids or growth hormones, the use any agent that could potentially interfere or interact with rosiglitazone, history of non-compliance to medical regimens and/or patients who are considered potentially unreliable and the presence of any concomitant condition which, in the opinion of the investigator, could interfere with the interpretation of efficacy and safety data gathered in this trial. The presence of HIV-lipodystrophy was defined as self-reported symptoms of loss of subcutaneous fat (face, arms, legs and buttocks) with or without increased abdominal girth or development of a buffalo hump. These findings were confirmed by the investigator (JPHvW) before enrollment. Clinical criteria for HIV-lipodystrophy are controversial, but at the start of the study, there were no uniformly approved objective criteria for diagnosing lipodystrophy[28]. We used subjective criteria for lipodystrophy that have been used previously[17,19].

Study design

Most of the recruited patients were consecutively seen patients with suspected lipodystrophy. At inclusion, fasting blood was obtained and anthropometric measurement were performed. Total body fat mass was estimated using bio-impedance analysis (RJL systems, Detroit, USA). Concomitant medication and known cardiovascular risk factors, such as the smoking habit, dietary pattern, physical exercise and hypertension, were recorded. In addition, a thorough physical examination was performed. Eligible patients underwent an oral glucose tolerance test, single-slice abdominal computer tomography and assessment of flow-mediated vasodilation. Subsequently, participants were randomly assigned in blocks of four to receive rosiglitazone (8 mg/day) or metformin (2 g/day) for 26 weeks by using a computer-

generated list. Neither the investigators nor the patients were blinded to drug assignment. Allocation concealment was ensured by an independent pharmacist. Participants were requested not to change their regular diet, physical exercise and smoking habit during the study. At the end of the period the same measurements were performed. Patients' self-reported and physicians' impressions of body fat distribution were also evaluated by open-ended questionnaire. Patients visited the hospital after 2 and 4 months of treatment for safety evaluation, which included an open-ended questionnaire, physical examination and blood sampling. During these visits, adherence to study medication was also evaluated by open-ended questionnaire, although pill counting was not performed. The participants also underwent a standardized oral fat loading test before and after treatment with the study drugs. The effects of rosiglitazone and metformin on postprandial lipid and fatty acid metabolism will be published separately. The study protocol was approved by the local research ethics committee of the University Medical Center Utrecht. All participants gave written informed consent.

Oral glucose tolerance test (OGTT)

Patients visited the hospital after an overnight fast. After placing a cannula for venous blood sampling in the forearm, patients rested for 30-minutes before administration of the glucose load (75 g). Peripheral blood samples were obtained in sodium EDTA (2 mg/ml) before (t=0) and at regular 15-minute intervals up to 2-h following glucose ingestion. All EDTA blood samples were kept on ice and centrifuged immediately for 15 minutes at 800g at 4°C and stored at -80°C until assayed.

Cross-sectional computer tomography

Single-slice cross-sectional computer tomography at the L_4-L_5 level was performed as described previously[29], in order to assess distribution of subcutaneous and visceral abdominal fat. Briefly, a lateral scout image was obtained to identify the level of the L_4 pedicle, which served as the landmark for the 1-cm single-slice image. Scan variables were 144-cm table height, 80 kV, 70 mA, 2 seconds, 1-cm slice thickness, and a 48-cm field of view. On the computer tomography image, the border of the intra-abdominal cavity was outlined and total and visceral abdominal fat areas were quantified by selecting an attenuation range of –250 to –50 Hounsfield Units. Subcutaneous abdominal fat was calculated as the difference between total and visceral abdominal fat. The computer tomography images were read and analyzed by an independent radiologist, who was unaware of the assignment status of the patients.

Endothelial function

Nitric oxide-dependent flow mediated vasodilation, as percentage diameter change in the brachial artery after reactive hyperemia, was measured by ultrasonography[30]. Measurements were performed at the elbow of the right arm using a Wall Track System

(Scanner 200, Pie Medical, Maastricht, The Netherlands), which consists of an ultrasound imager with a 10 MHz linear array transducer connected to a data acquisition system and a personal computer. Three measurements were averaged to calculate a baseline diameter. By inflation of a blood pressure cuff for 5-minutes at a pressure of 200 mmHg, ischaemia was applied to the forearm distal to the location of the transducer. Ultrasonography continued for 5-minutes after cuff release, with measurements at 30-second intervals. The widest lumen diameter was taken as a measure for maximal vasodilation. Nitroglycerin (400 μg) was used to determine endothelium-independent vasodilation. All measurements were performed by the same technician with patients supine in a quiet, temperature-controlled (21°C) environment after at least 15-minutes of rest. The operator was unaware of the assignment status of the patients. Endothelial function was measured before and 2-h after glucose ingestion.

Analytical methods

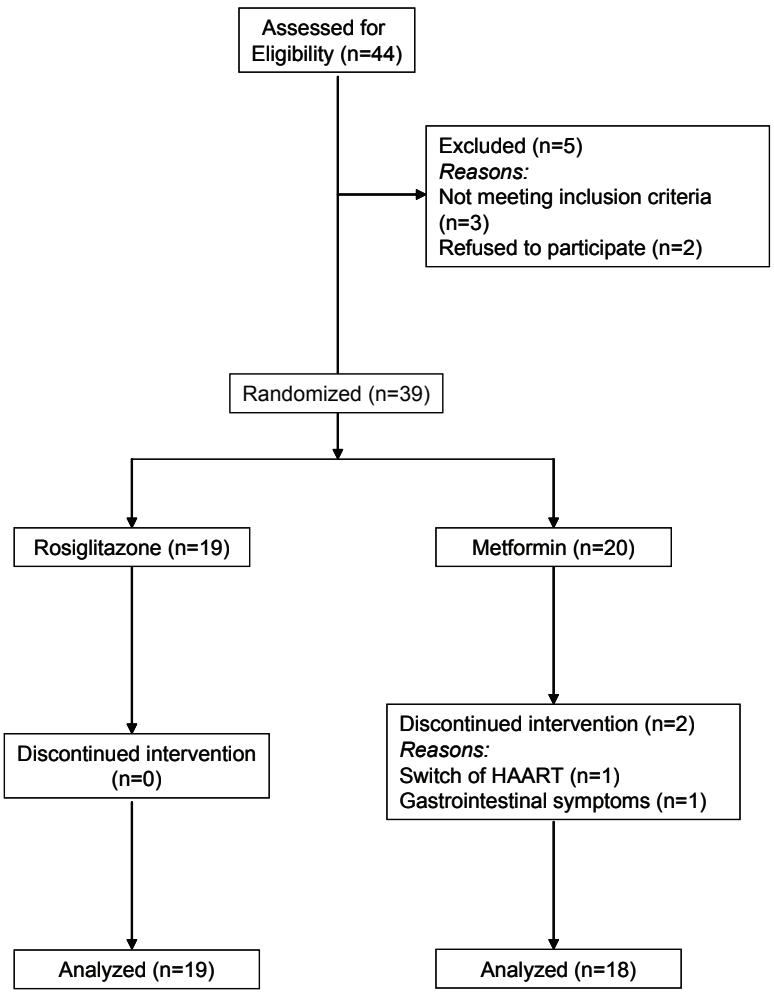
Total and high-density lipoprotein cholesterol, triglycerides, glucose, apolipoprotein B, creatinine, and serum asparate and alanine aminotransferases (AST and ALT, respectively) were measured using standard laboratory procedures, as described previously in detail[31]. LDL was isolated by ultracentrifugation[31]. Free fatty acids (FFA) were measured by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany)[31]. For FFA measurement, a lipase inhibitor was added to the plasma in order to block ex vivo lipolysis. Insulin was measured by ELISA (Mercodia, Uppsala, Sweden). C-reactive protein (CRP) was measured using a high-sensitivity method (Quantex hs-CRP kit, Biokit, S.A., Barcelona, Spain)[32]. Adiponectin was measured by ELISA (R&D systems, Minneapolis, Minnesota, USA). CD4 cell counts were determined by flow cytometry, and HIV viral load was determined by ultrasensitive assay

Statistical analysis

Assumptions of normality were tested by Kolmogorov-Smirnov tests and by review of plots. Data are presented as means and standard deviations for variables with symmetric distribution, and as medians and interquartile ranges for variables with skewed distributions. Exact ninety-five percent confidence intervals for median differences were obtained by considering the full distribution of between treatment differences[33]. During the oral glucose tolerance test, total integrated area's under the curves (AUC) were calculated by the trapezoidal rule using GraphPad Prism version 4.0 (GraphPad Software, San Diego, USA). Primary outcome measures were insulin AUC and subcutaneous and visceral abdominal fat. The secondary outcome measure was flow-mediated vasodilation. The primary analysis was to compare treatment effects between groups. For variables with symmetric distribution, we used t-tests on changes from baseline. For variables with skewed distributions, between treatment changes from baseline were compared with Mann-Withney tests. The secondary analysis was

to compare changes within each group with paired t-tests or Mann-Withney tests, as appropriate. Bivariate correlations between the changes in body fat distribution upon treatment and other parameters were calculated using Spearman's correlation coefficients. A sample size of 15 patients per arm was determined to be necessary to detect a 25% reduction in insulin AUC with 80% power and $\alpha = 0.05$. Since a small drop-out may be expected, we aimed to include 20 patients in each arm. Calculations were performed using SPSS/PC + 11.5 (SPSS Inc. Chicago, IL, USA). Statistical significance was taken at the 5% level.

Figure 1
Flow diagram of patients in the study



Results

Baseline characteristics

In total, 44 patients were screened for the study, of which 39 received the allocated study drug (*Figure 1*). Baseline characteristics of the study group are listed in *Tables 1 and 2*. We found no clinically important differences between groups in virological,

Table 1
Baseline characteristics of the study group

	Rosiglitazone group (n=19)	Metformin group (n=20)
Age (years)	47 (9)	48 (9)
Time since diagnosis of HIV (years)	8.4 (4.4)	7.5 (4.0)
Time on antiretroviral therapy (years)	5.6 (3.5)	5.4 (3.6)
Antiretroviral use, n (%)	19 (100%)	20 (100%)
Protease inhibitor, n (%)	13 (68%)	12 (60%)
NNRTI, n (%)	6 (32%)	8 (40%)
NRTI, n (%)	19 (100%)	20 (100%)
Stavudine, n (%)	4 (21%)	4 (20%)
Lipid-lowering agents, n (%)	3 (16%)	3 (15%)
Statins, n (%)	2 (11%)	2 (10%)
Fibrates, n (%)	1 (5%)	1 (5%)
Anti-hypertensive agents, n (%)	2 (11%)	1 (5%)
Anti-diarrhoeic agents, n (%)	2 (11%)	2 (10%)
Smoking, n (%)	5 (26%)	5 (25%)
Systolic blood pressure (mmHg)	135 (17)	133 (18)
Diastolic blood pressure (mmHg)	84 (13)	82 (9)

Data are mean (SD). NNRTI = non-nucleoside reverse transcriptase inhibitor.
NRTI = nucleoside reverse transcriptase inhibitor

immunological and metabolic parameters, antiretroviral therapy and body composition. Two patients in the metformin group discontinued the study; one due to a switch of antiretroviral therapy and one due to gastrointestinal symptoms (diarrhea and abdominal cramps). The remaining 37 patients completed the 6-month protocol. Complete case analysis was performed. None of the patients reported changes in dietary pattern and physical exercise during the study, while all participants reported adequate treatment compliance.

Metabolic parameters

The effects of rosiglitazone and metformin on postchallenge glucose, insulin and free fatty acid concentrations are shown in *Figure 2*. Rosiglitazone and metformin similarly decreased the AUCs for glucose (mean change -1.9, SD 1.8 and –1.1, SD 0.7 mmol/L

Table 2
Effects of rosiglitazone and metformin on metabolic and safety parameters

	Baseline		Mean change after 26 weeks		Rosiglitazone – Metformin difference	
	Rosiglitazone (n=19)	Metformin (n=18)	Rosiglitazone (n=19)	Metformin (n=18)	Mean/Median †	95% CI
Total cholesterol, mmol/L (mg/dL)	5.6 (0.9); 216 (31)	5.7 (0.9); 220 (36)	0.4 (1.0); 16 (39)	-0.4 (0.6); 15 (23) *	0.8; 31	0.3, 1.3; 12, 50
LDL-cholesterol, mmol/L (mg/dL)	3.1 (0.8); 120 (30)	3.5 (0.9); 135 (31)	0.2 (1.0); 8 (39)	-0.4 (0.8); 15 (30) *	0.6; 23	0.2, 1.1; 7, 43
HDL-cholesterol, mmol/L (mg/dL)	1.23 (0.35); 47 (13)	1.03 (0.07); 40 (3)	-0.15 (0.30); -6 (12) *	0.01 (0.21); 0.4 (8)	-0.16; -6.4	-0.35, -0.02; -14, -1
Apolipoprotein B, g/L #	1.08 (0.92, 1.21)	1.16 (0.99, 1.28)	0.09 (-0.06, 0.17)	-0.10 (-0.23, 0.03) *	0.19	0.07, 0.31
Triglycerides, mmol/L (mg/dL) #	2.3 (1.6, 3.5)	3.0 (1.6, 5.3)	0.5 (0.1, 1.1) *	-0.6 (-1.0, -0.1) *	1.1; 97	0.4, 2.6; 35, 230
	204 (142, 310)	266 (143, 470)	44 (9, 97)	-53 (-89, -9)		
Adiponectin, µg/mL #	3.8 (1.0, 7.1)	3.0 (1.4, 4.1)	3.2 (1.4, 6.8) *	0.2 (-0.8, 0.6)	3.1	1.9, 8.5
C-reactive protein, mg/L #	2.2 (1.0, 5.5)	2.9 (1.4, 7.1)	-0.05 (-1.4, 0.6)	-1.0 (-4.5, 0.1)	0.89	-1.4, 2.3
HIV viral load (copies/ml) #	50 (50, 105)	50 (50, 50)	0 (0, 0)	0 (0, 0)	0	-150, 342
CD4 cell count (x 10^9 cells/l)	0.697 (0.366)	0.574 (0.259)	0.008 (0.191)	0.043 (0.155)	-.0.035	-0.147, 0.096
Hematocrit	0.41 (0.31)	0.41 (0.31)	-0.03 (0.02) *	-0.01 (0.02)	-0.02	-0.04, -0.008
AST, U/L	41 (17)	42 (22)	-6 (26) *	-8 (16) *	2	-9, 13
ALT, U/L	37 (13)	43 (21)	-7 (13) *	-9 (20) *	3	-11, 16
Lactic acid, mmol/L	2.1 (0.4)	2.1 (0.5)	-0.2 (0.4)	0.1 (0.6)	-0.3	-0.8, 0.6
Gastrointestinal symptoms, n (%)			1 (5%)	6 (32%)		
Dizziness, n (%)			1 (5%)	0 (0%)		

Data are mean (SD); # Median (interquartile range); LDL = low-density lipoprotein; HDL = high-density lipoprotein; AST = aspartate aminotransferase; ALT = alanine aminotransferase; The adverse events that are presented are counts by patient; † Medians were obtained by considering the full distribution of between treatment differences[33]; * Significant change from baseline ($p<0.05$)

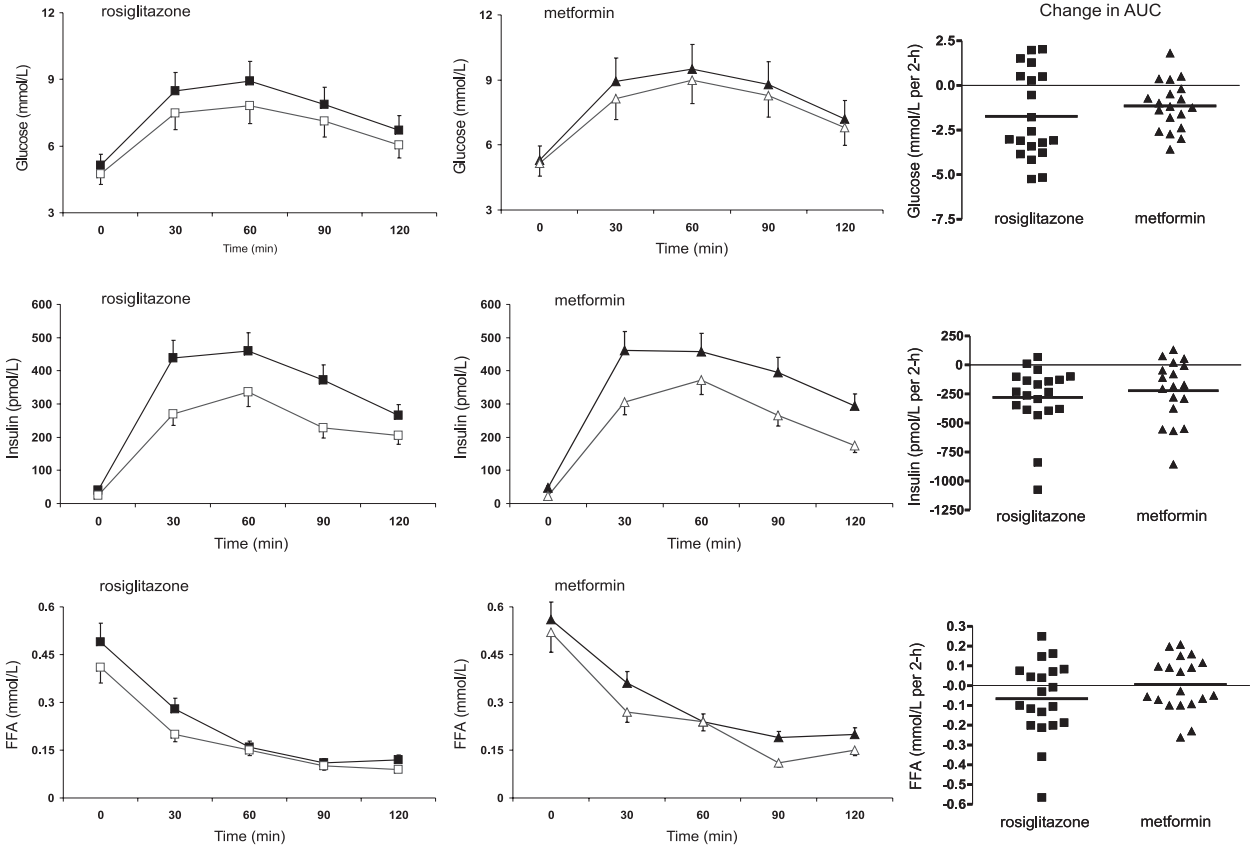


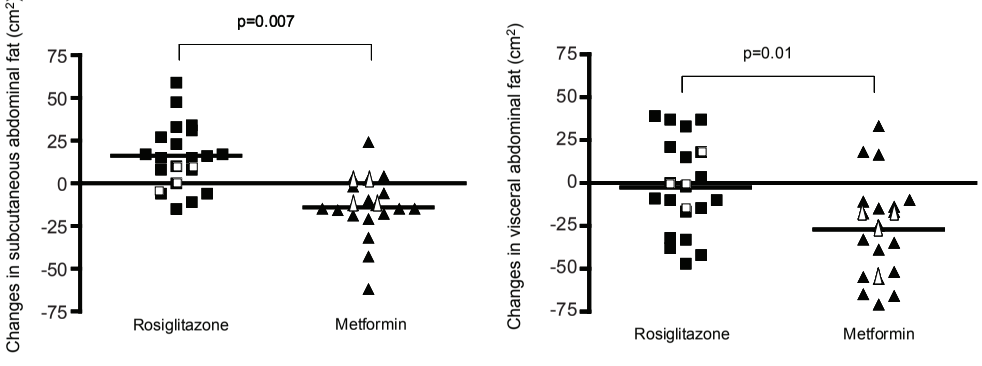
Figure 2
Effects of rosiglitazone and metformin on insulin sensitivity. Plasma glucose (upper panel), insulin (middle panel) and free fatty acid (lower panel) levels after an oral glucose tolerance test are shown. Squares indicate rosiglitazone, triangles indicate metformin. Closed symbols indicate before treatment, open symbols indicate after treatment. In the right panels, individual changes in AUC are shown. In these panels, the horizontal lines represent the mean change in AUC

Table 3
Effects of rosiglitazone and metformin on body fat distribution

	Baseline		Mean change after 26 weeks		Rosiglitazone – Metformin difference	
	Rosiglitazone (n=19)	Metformin (n=18)	Rosiglitazone (n=19)	Metformin (n=18)	Mean/Median †	95% CI
Body weight, kg #	75.7 (65, 81)	78.9 (72.1, 94.1)	2.3 (0.8, 3.2) *	-1.2 (-2.7, -0.7) *	3.2	1.8, 5.1
BMI, kg/m^2 #	23.6 (19.8, 24.9)	24.3 (23.6, 26.6)	0.4 (0.2, 0.9) *	-0.4 (-0.8, -0.1) *	0.7	0.5, 1.6
Waist circumference, cm #	92 (84, 96)	94 (91, 100)	0 (-1, 1)	-3.5 (-6.8, -1) *	3.3	1.8, 5.8
Waist-to-hip ratio	0.98 (0.05)	1.00 (0.05)	0.00 (0.03)	-0.03 (0.04) *	0.03	0.003, 0.04
Total body fat mass, kg #	10.3 (7.1, 12.2)	12.8 (10.7, 16.8)	2.9 (1.1, 4.3) *	-1.4 (-2.3, -0.2) *	4.0	1.0, 5.1
Subcutaneous abdominal fat, cm^2	98 (47) range 3, 300	103 (38) range 6, 380	16 (31) *	-11 (17) *	27	7, 46
Visceral abdominal fat, cm^2	158 (65) range 40, 400	189 (63) range 30, 400	-1 (36)	-25 (35) *	24	6, 51

Data are mean (SD); # Median (interquartile range); † Medians were obtained by considering the full distribution of between treatment differences[33]; * Significant change from baseline ($p<0.05$)

Figure 3
Effects of rosiglitazone and metformin on body fat distribution.
Individual changes in subcutaneous and visceral abdominal fat are shown. Squares indicate rosiglitazone, triangles indicate metformin. Open symbols represent the patients on stavudine treatment. Mean changes from baseline are shown as an insert (horizontal lines).

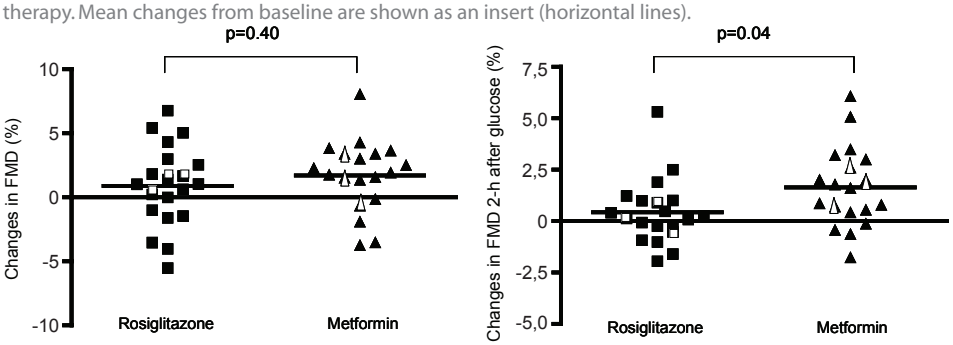


per 2-h, respectively, p=0.04 and p=0.05) and insulin (mean change –258, SD 294 and –231, SD 264 pmol/L per 2-h, respectively, p=0.01 for each). Fasting FFA levels tended to be lower after treatment with rosiglitazone (p=0.08). However, the course of FFA after the glucose load remained similar after treatment with either rosiglitazone and metformin (*Figure 2*). Compared with metformin, rosiglitazone markedly increased adiponectin levels (*Table 2*). Metformin showed greater benefits on fasting lipid profile than rosiglitazone. Serum aminotransferase levels, which when elevated may indicate the presence of steatosis[34], decreased in both treatment groups. No effects on plasma CRP were observed with either rosiglitazone and metformin.

Body fat distribution

Effects of rosiglitazone and metformin on body fat distribution are shown in *Table 3*. Compared with metformin, rosiglitazone significantly increased subcutaneous and visceral abdominal fat. Individual changes in subcutaneous and visceral abdominal fat with rosiglitazone and metformin are shown in *Figure 3*. Compared with baseline, rosiglitazone increased subcutaneous abdominal fat, while metformin decreased subcutaneous and visceral abdominal fat (*Figure 3*). The increase in subcutaneous abdominal fat by rosiglitazone was negatively related to body weight at baseline (r=-0.50, p=0.003) and positively related to the insulin AUC at baseline (r=0.43, p=0.01). In the patients on stavudine treatment (n=4) rosiglitazone did not increase subcutaneous abdominal fat (*Figure 3*). Nine patients in the rosiglitazone-group (47%), and four patients in the metformin-group (22%) reported subjective improvement of lipodystrophy, which was corroborated by clinical examination in most of these patients.

Figure 4
Effects of rosiglitazone and metformin on endothelial function.
Individual changes in fasting and postprandial flow-mediated vasodilation (FMD) are shown. Squares indicate rosiglitazone, triangles indicate metformin. Open symbols represent the patients on lipid-lowering therapy. Mean changes from baseline are shown as an insert (horizontal lines).



Vascular measurements

At baseline and at the end of the study, there were no differences in lumen diameter of the brachial artery between the groups (data not shown). Before treatment, flow-mediated vasodilation was similar in the rosiglitazone-group (mean ± SD, 4.5 ± 2.2%) and the metformin-group (mean ± SD, 4.0 ± 2.6%). Compared with baseline, flow-mediated vasodilation increased significantly with metformin (mean change ± SD, 1.5 ± 0.8%, [95% CI, 0.4 to 3.3%]), but not with rosiglitazone (mean change ± SD, 0.7 ± 1.2%, [95% CI, -1.1 to 2.7%], *Figure 4*). When directly compared with rosiglitazone, metformin did not increase flow-mediated vasodilation in the fasting state (between treatment change from baseline ± SD, 0.8 ± 1.0% [95% CI, -1.1 to 2.8%]), but increased flow-mediated vasodilation 2-h after glucose (between treatment change from baseline ± SD, 1.2 ± 0.6% [95% CI, 0.05 to 2.4%]). Metformin and rosiglitazone did not change endothelium-independent vasodilation (data not shown).

Safety evaluation

Treatment with rosiglitazone and metformin were well tolerated. In total, 8 potential adverse events were queried, which are listed in *Table 2*. All the reported side effects are known potential side effects of the study drugs. There were no increases in resting lactic acid and aminotransferase levels associated with either metformin or rosiglitazone. None of the patients developed clinically detectable edema, evidence for hepatocellular disease or congestive heart failure during the study. There was no evidence of any drug interactions between the study drugs and antiretrovirals and/or lipid-lowering agents.

Discussion

Lipodystrophy is a major clinical health problem in HIV-infected patients[2-4]. In the present study, we compared the effects of rosiglitazone and metformin for the treatment of HIV-lipodystrophy. Our findings emphasize the importance of individualized care in HIV-infected patients. Although rosiglitazone may partly correct lipoatrophy, metformin improves visceral adiposity, lipid profile and endothelial function function.

In our patient groups, waist-to-hip ratio and visceral abdominal fat were high, but subcutaneous abdominal fat was low, consistent with the presence of lipodystrophy in both study arms. Rosiglitazone significantly increased subcutaneous abdominal fat in patients with HIV-lipodystrophy, despite ongoing HAART. Whether the outcomes are similar when rosiglitazone is used longer-term remains to be shown. The increase in subcutaneous abdominal fat by rosiglitazone was similar in magnitude as observed in studies of switching antiretroviral regimens[35,36]. Subjective improvement of lipodystrophy was reported in almost half of the rosiglitazone-treated patients, and was corroborated by clinical examination.

Our results are similar to those observed by Hadigan and colleagues[17]. However, in the study of Carr et al., rosiglitazone for 48 weeks did not improve lipoatrophy in a large patient group[18]. Similarly, rosiglitazone did not improve lipoatrophy in a smaller study by Sutinen and co-workers[19]. It is clinically relevant to identify those patients that are most likely to benefit from treatment with rosiglitazone. In our study, the increase in subcutaneous abdominal fat by rosiglitazone was related to low body weight and high insulin AUC at baseline, suggesting that especially those patients with marked lipoatrophy and insulin resistance may benefit the most from treatment with rosiglitazone. Stavudine, which was used by only four rosiglitazone-treated patients in our study, has been put forward as a factor that could hinder rosiglitazone-induced improvement of lipoatrophy. In our study, the patients on stavudine therapy did not show an increase in subcutaneous adipose tissue with rosiglitazone, 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[18,19].

In our study as well as in other published reports[17-19], a detrimental effect on plasma lipid levels was seen with rosiglitazone in some patients with lipodystrophy. Rosiglitazone should, therefore, be used with caution in patients with HIV-lipodystrophy and, possibly, avoided in those patients who are already hyperlipidemic, or given in conjunction with lipid-lowering agents. Metformin reduced subcutaneous and visceral abdominal fat, a finding which has been previously reported in HIV-infected patients[14-16]. Hence, metformin may be best for the viscerally obese, overweight, dyslipidemic patient, whereas it might not be appropriate for the patient with predominantly lipoatrophy, as they may suffer a further loss of subcutaneous fat. Rosiglitazone increased subcutaneous abdominal fat, an important benefit among patients with lipoatrophy, as shown previously by Hadigan et al.[17]. Clearly, these findings emphasize the importance of individualized treatment in HIV-infected patients.

Therapeutic modulation of insulin resistance may become an important aspect of the management of HIV-infected patients[37]. The treatment doses in our study were somewhat higher than those used in prior studies in HIV-infected patients[14,15,17], but similar to the doses used by others[18,19]. In this study, metformin and rosiglitazone showed similar benefits on postchallenge glucose and insulin levels, despite different mode of actions[11,12]. Our patients were all males and not particularly hyperinsulinemic. Whether the results can be extrapolated to females or a more hyperinsulinemic group remains to be shown. The impact of the two metformin-treated patients that discontinued the study on the results is probably minor, as revealed by a sensitivity analysis (data not shown). We did not find adverse events or drug interactions that might limit long-term use of these agents. However, caution should be taken in patients with renal- or liver disease or elevated lactic acid levels, which were exclusion criteria in our study. In these patients, treatment with metformin may not be recommended.

In our study, fasting FFA levels tended to be lower after treatment with rosiglitazone,

as previously reported by Hadigan et al[17]. Besides improved FFA storage, rosiglitazone may also improve insulin sensitivity indirectly by means of altered adipocytokine release[12,17,18]. Adiponectin, an adipocytokine, is reduced in patients with HIV-lipoatrophy and insulin resistance, possibly secondary to therapy with NRTIs[38,39]. Moreover, low adiponectin levels have been associated with a moderately increased cardiovascular disease risk in diabetic men[40]. Interestingly, we observed a marked increase in adiponectin with rosiglitazone, but not with metformin, despite reductions in the AUC for insulin with both agents. In our opinion, the increase in adiponectin may be explained by improved adipocyte function by rosiglitazone, and may convey increased protection from atherosclerosis.

Insulin resistance in patients with HIV-lipodystrophy can be associated with hepatic steatosis and increased aminotransferase levels[41,42]. We not only found a decrease in aminotransferase levels in the rosiglitazone-group, as observed previously[19], but also in the metformin-group. This may suggest that both drugs have a beneficial effect on hepatic inflammation and steatosis. However, this remains speculative as hepatic fat content was not directly assessed in this study.

Among HIV-negative adults, hyperinsulinemia and glucose intolerance are independent predictors of cardiovascular disease[43-45]. Therefore, the reductions in postchallenge glucose and insulin levels with metformin and rosiglitazone may result in improved cardiovascular risk profile. Previously, metformin also reduced markers of fibrinolysis in HIV-infected patients[46]. So far, prior studies have not examined whether in HIV, modulation of insulin resistance translates into vascular benefit. At baseline, the total group of HIV-infected patients had two-fold impaired endothelial function compared with 15 age-matched healthy males (data on file). In this study, only metformin improved fasting and postprandial endothelial function. Whether this improvement is sufficient to produce clinical benefit is an open issue, but it might be relevant. Previously, pravastatin did not improve endothelial function in a similar population[24].

Our results with respect to endothelial function are in contrast with a recent study that suggested a greater beneficial effect on endothelial function with rosiglitazone in comparison with metformin in patients with type 2 diabetes[26], possibly due to some additional anti-inflammatory effect[47]. Apparently, this finding in diabetic patient can not simply be extrapolated to HIV-infected patients. Several factors may explain this discrepancy. First, atherosclerosis is considered a low-grade inflammatory disease. Plasma CRP is a marker of inflammation that predicts future cardiovascular events[48]. Unexpectedly, rosiglitazone did not change CRP levels in our study, while prior studies in type 2 diabetic patients have shown substantial CRP reductions with rosiglitazone[49,50]. Presumably, it may be difficult for rosiglitazone to exert significant effects on inflammation in HIV-infected patients, although adiponectin (which was anti-inflammatory properties) increased with rosiglitazone. Second, the population studied was receiving HAART, which may have direct effects on endothelial function. Finally,

metformin showed greater benefits on lipid profile than rosiglitazone.

This study has several limitations. The study was not blinded or placebo-controlled and did not measure clinical outcomes. Participants were included on the basis of subjective criteria for lipodystrophy, while anthropometric and/or metabolic inclusion criteria were absent. Also, we did not measure peripheral subcutaneous fat. Some minor differences in baseline characteristics were notable between groups, which were generally due to few extreme values. Finally, it should be noted that a large number of statistical tests was conducted, and results should be interpreted with caution.

In conclusion, the findings of the present study reinforce the importance of individualized care in HIV-infected patients. Although rosiglitazone may partly correct lipoatrophy, metformin improves visceral adiposity, lipid profile and vascular function.

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9

General discussion

Cardiovascular disease (CVD) is the main cause of mortality in Western societies. Important risk factors include insulin resistance, dyslipidemia, hypertension, unfavorable body fat distribution and a prothrombotic and proinflammatory state. Most of these risk factors are strongly interrelated and cluster in the metabolic syndrome, which has been associated with an increased risk for CVD. The incidence of the metabolic syndrome is rapidly increasing in Western societies due to the increasing prevalence of obesity. However, mounting evidence suggests that absolute or partial lack of body fat (lipodystrophy) is associated with a similar metabolic risk profile. During the last years, much attention has been directed to lipodystrophy among HIV-infected patients receiving highly active antiretroviral therapy (HAART). The focus of this thesis is on two aspects of metabolic dysregulation, type 2 diabetes mellitus and HIV-lipodystrophy, and the effects of insulin-sensitizing agents, as described in **chapter 1**.

Thiazolidinediones in type 2 diabetes mellitus

Thiazolidinediones (TZDs) have received increasing attenttion for the treatment of insulin resistance and hyperglycemia in patients with type 2 diabetes as several benefits on cardiovascular risk factors beyond glycemic control are surfacing. Currently, there are two TZDs available: rosiglitazone and pioglitazone. **Chapter 2** gives a summary analysis of published double-blind, placebo-controlled studies evaluating the effects of rosiglitazone and pioglitazone on blood lipids in patients with type 2 diabetes. We reported that studies with pioglitazone show greater benefits on fasting plasma triglycerides (TG), LDL-cholesterol and HDL-cholesterol than studies with rosiglitazone. Whether the magnitude of these differences are sufficient to produce clinically relevant cardiovascular benefits is an open question. Several factors may explain our observations. First, it has been shown that, at the same clinical dose, pioglitazone is associated with greater PPAR-α activation than rosiglitazone[1]. PPAR-α is the main target for fibrates, a class of lipid-lowering drugs, which mainly reduce TG and increase HDL-cholesterol. Second, we found that the pioglitazone-treated subjects were characterized by a more pronounced dyslipidemia (increased TG and decreased HDL-cholesterol) at baseline than the rosiglitazone-treated subjects. It is well recognized that the lipid-lowering responses of fibrates and statins are enhanced in patients with more pronounced dyslipidemia at baseline[2]. Why rosiglitazone and pioglitazone exert different effects on LDL-cholesterol is an open issue. Recently, it has been shown that pioglitazone reduces VLDL-TG levels by increasing the fractional clearance rate of VLDL-TG from the circulation, almost certainly due to increased LPL-mediated lipolysis, without changing direct removal of VLDL particles[3]. The effects of rosiglitazone on the production and fractional clearance rates of VLDL-TG and apolipoprotein B are not known.

Since humans are non-fasting most part of the day, this period may be of particular importance in the pathogenesis of atherosclerosis. Delayed clearance of postprandial TG-rich lipoproteins and their remnants is an important characteristic of diabetic dyslipidemia, and is linked to accelerated atherosclerosis, even in fasting normolipidemic subjects[4-6]. In one study, postprandial TG levels distinguished even better between cases with future myocardial infarction and controls than fasting plasma TG levels[7]. In **chapter 3**, the effects of rosiglitazone on postprandial TG and FFA metabolism are described. Rosiglitazone did not change fasting plasma TG levels, but decreased the postprandial TG rise in plasma (-37%), chylomicrons (-20%) and VLDL1 (-27%). It is tempting to hypothesize that the anti-atherosclerotic effects observed with rosiglitazone may involve improvement of postprandial lipemia[8]. Potential explanations for the postprandial TG reductions by rosiglitazone are increased LPL-mediated lipolysis, decreased postprandial hepatic FFA delivery, increased insulin sensitivity, and improved glycemic control. Interestingly, the postprandial TG and FFA reductions by rosiglitazone were related to the reductions of hepatic aminotransferases, suggesting reduced liver fat content[9], which may be due to preferential adipocyte FFA storage. However, this remains only speculative since hepatic fat was not directly assessed.

One of the potential mechanisms by which postprandial lipemia may promote atherosclerosis is inflammation and oxidative stress, leading to endothelial dysfunction[10,11]. Remnants of TG-rich lipoproteins accumulate in the subendothelial space[12]. The oxidative modification of lipoproteins in the subendothelial space induces the production of chemoattractants and adhesion molecules by different cell types in the arterial wall, the recruitment of leukocytes, transmigration of monocytes into the arterial wall, and the formation of foam cells. An elevated blood leukocyte count is an independent predictor of cardiovascular disease (CVD)[13]. The best association with CVD has been demonstrated for blood neutrophils, despite the fact that these cells are absent in the atherosclerotic lesion until it is ruptured[13]. However, upon activation, resident and recruited neutrophils may affect endothelial function via the production of pro-inflammatory cytokines and generation of oxidative stress[11]. Postprandial leukocyte recruitment and activation has been described in healthy subjects[11,14]. In **chapter 4**, we also determined the effects of rosiglitazone on postprandial leukocytes, pro-inflammatory cytokines (IL-6 and IL-8), CRP and MCP-1 in our randomized double-blind, placebo-controlled cross-over trial in patients with type 2 diabetes. 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, 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[15,16], which is probably clinically relevant. IL-6 is the major cytokine responsible for hepatic CRP production and is also independently associated with CVD[17]. 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 man lives in a postprandial period most part of the day, a reduced inflammatory response after a meal may contribute to cardiovascular risk reduction.

So far, there are no data on the effects of TZDs on cardiovascular endpoints, but studies using surrogate markers of vascular disease provide preliminary evidence that TZDs delay progression of atherosclerosis, as described in **chapter 5**. In nondiabetic patients with documented coronary artery disease, rosiglitazone reduced common carotid intima-media thickness progression after 48 weeks of treatment compared with placebo[8]. Interestingly, only minor effects on insulin sensitivity and glucose and lipid homeostasis were observed in that study. These data could be interpreted as a strong argument in favour of direct anti-atherosclerotic effects of rosiglitazone, due to suppression of inflammation in the vasculature. Direct anti-atherosclerotic effects of TZDs include increased nitric oxide bio-availability, decreased leukocyte-endothelial cell interaction, reduced vascular smooth muscle cell migration and proliferation, increased cholesterol efflux from macrophages, and reduced levels of pro-inflammatory molecules. The anti-atherosclerotic effects of TZDs are not confined to diabetic patients, but can be extrapolated to patients with documented coronary artery disease without manifest diabetes[8]. Moreover, rosiglitazone retarded carotid IMT progression on top of statins and anti-hypertensive agents[8], which is important since many high-risk patients are using this medication.

Current perspectives and future directions

Clearly, TZDs interfere with key processes in atherogenesis and may, therefore, offer additional opportunities to improve cardiovascular risk beyond treatment of glycemic control and insulin resistance in patients with and without type 2 diabetes. So far, there are no data on the effects of TZDs on cardiovascular endpoints in patients with type 2 diabetes or other insulin-resistant conditions. Two studies, the Prospective Pioglitazone Clinical Trial in Macrovascular Events and the Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycaemia in Diabetes trial, are currently investigating the effects of pioglitazone and rosiglitazone, respectively, on cardiovascular endpoints in patients with type 2 diabetes. The results of these studies may offer more clues regarding the position of TZD therapy in clinical medicine in patients with type 2 diabetes. Moreover, results on combination therapy with metformin need to be awaited. Whereas PPAR-γ agonists can markedly improve insulin resistance and glycemic control, PPAR-α agonists (fibrates) can be used in the treatment of diabetic dyslipidemia[18]. Hence, the combined actions of the dual PPAR-α/γ agonists appear

ideally suited to decrease the risk for CVD in patients with type 2 diabetes[19]. 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.

Metabolic dysregulation and interventions in HIV-lipodystrophy

HAART improves the survival of patients with HIV infection, but is associated with changes in body fat distribution (lipodystrophy) and metabolic risk factors. The etiology of lipodystrophy and metabolic alterations is multifactorial, including inhibitory effects of HIV drugs on adipocyte differentiation, alterations of mitochondrial functions in adipocytes, and altered expression of leptin, adiponectin and cytokines in adipose tissue of patients[20]. NRTIs may induce mitochondrial dysfunction and apoptosis of adipocytes by inhibition of mitochondrial DNA polymerase-γ, and depletion of mitochondrial DNA[20]. PI-induced disruption of adipogenesis occurs through altered expression of sterol regulatory element-binding protein-1c (SREBP-1c) and PPAR-γ, which are essential for adipocyte differentiation[20]. The NNRTI efavirenz also induces a strong inhibition of the SREBP-1c-dependent lipogenic pathway that might contribute to lipoatrophy[21]. The metabolic toxicity associated with antiretroviral therapy seems to be class-specific, but also partly drug-specific. The effects of newer antiretroviral agents, such as atazanavir, on adipocyte differentiation should therefore be awaited. In addition, central adipocytes (intra-abdominal and dorsocervical) are more metabolically active than subcutaneous adipocytes. It would be interesting to know whether those two fat compartments respond differently to antiretroviral agents. Finally, it has recently been proposed that antiretroviral treatment induces an autonomic dysbalance in the central nervous system, resulting in redistribution of adipose tissue[22].

Adipose tissue may thus play a key role in disorganized lipid metabolism and insulin resistance associated with antiretroviral therapy. In **chapter 6**, we postulated that patients with HIV-lipodystrophy have impaired adipocyte FFA trapping. For this purpose, we investigated FFA, hydroxybutyric acid (HBA), as a measure of hepatic FFA oxidation, and TG changes after a high-fat meal 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. 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 indicate increased hepatic FFA delivery, which may aggravate insulin resistance and dyslipidemia, ultimately leading to an increased cardiovascular risk in these patients.

Clearly, as survival of patients with HIV increases, it is becoming increasingly important to identify those patients that are at increased risk for accelerated atherosclerosis. However, there is still a lack of studies on cardiovascular risk assessment using established intermediate endpoints in HIV. In **chapter 7**, we determined functional (flow-mediated vasodilation) and structural (carotid intima-media thickness and aortic pulse-wave velocity) markers of atherosclerosis and related those parameters to clinically easily obtainable anthropometric, haemodynamic and laboratory parameters in HIV-infected patients. The NCEP-ATPIII guidelines were used to identify HIV-infected patients with the metabolic syndrome[23]. The metabolic syndrome is present when at least three out of five risk determinants (increased waist circumference, increased blood pressure, elevated fasting TG, low HDL-cholesterol and elevated fasting glucose) are present. Remarkably, HIV-infected patients without the metabolic syndrome showed endothelial dysfunction comparable to that of age-matched type 2 diabetic patients, suggesting increased cardiovascular risk in HIV-infected patients, even in the absence of metabolic risk variable clustering. Several factors may explain this observation. Firstly, in HIV-infected patients, the endothelium could be activated either directly by HIV-associated proteins or by cytokines secreted in response to leukocyte activation by HIV[24]. Secondly, HIV-infected patients without the metabolic syndrome had high apolipoprotein B, and high apolipoprotein B was closely related to endothelial dysfunction. Thirdly, antiretroviral agents may also directly induce endothelial dysfunction, independent of lipid profile[25]. As expected, the presence of the metabolic syndrome in HIV-infected patients was associated with more severe endothelial dysfunction, as well as increased intima-media thickness, in our cohort of HIV-infected males, suggesting more advanced functional and structural atherosclerotic changes. Hence, the NCEP-ATPIII criteria of the metabolic syndrome may also be used in HIV-infected patients in order to identify subjects at risk for accelerated atherosclerosis. Finally, IGT was also related to functional and structural markers of atherosclerosis in HIV-infected patients.

In order to determine more clearly which risk factors should be targeted to reduce cardiovascular risk in these patients we investigated whether agents that improve insulin resistance could ameliorate endothelial function. The results of this study are described in **chapter 8**. In this chapter 8, we also compared the effects of rosiglitazone and metformin on insulin sensitivity and body fat distribution in patients with HIV-lipodystrophy in a randomized clinical trial. Metformin and rosiglitazone are used in clinical medicine to improve insulin resistance and glycemic control in patients with type 2 diabetes[26,27]. However, these agents may also be useful in nondiabetic patients with insulin resistance, such as patients with HIV-lipodystrophy. We observed that 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[28], but in contrast to those observed by others[29,30]. 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[29,30]. 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[31-33]. 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[34].

Current perspectives and future directions

During the last years, much attention has been directed to the management of lipodystrophy and cardiovascular risk in HIV-infected patients. In this regard, development and use of less toxic antiretroviral agents to treat HIV with fewer adverse events on adipose tissue and cardiovascular risk is the first step. Promising preliminary results have been observed with PI-sparing regimens[35,36]. Alternatively, the novel PI atazanavir is, contrary to the other PIs, not associated with deteriorations in glucose and lipid metabolism[37]. NRTI-sparing regimens (consisting of a PI and NNRTI) may delay the occurence of body fat distribution[38]. Finally, in the following years new classes of antiretroviral agents, including integrase and fusion inhibitors as well as chemokine receptor blockers, will be available. These classes may have less adverse effects on

adipose tissue metabolism and cardiovascular risk. Although the implications of insulin resistance and dyslipidemia in HIV-infected patients are not fully known, preliminary data indicate increased cardiovascular mortality among HIV-infected individuals[39], suggesting that measures to reduce cardiovascular risk should be provided. Recently, guidelines for the evaluation and management of dyslipdemia in HIV-infected adults receiving antiretroviral therapy have been published by members of the Cardiovascular Subcommittee of the Adult AIDS Clinical Trials Group[40]. It was recommended that HIV-infected adults should undergo evaluation and treatment on the basis of NCEP-ATPIII guidelines for dyslipidemia, with particular attention to potential drug interactions with antiretroviral agents and maintenance of virologic control of HIV infection. Pravastatin or atorvastatin were recommended for initial therapy for increased LDL-cholesterol, and gemfibrozil or fenofibrate for hypertriglyceridemia[40]. Whether an oral glucose tolerance tests and the NCEP-ATPIII definition of the metabolic syndrome are useful for routine screening of HIV-infected patients remains to be shown. Despite normal fasting glucose levels, many HIV-infected patients have IGT, and IGT was closely linked to vascular abnormalities in chapter 7. The presence of the metabolic syndrome according to the NCEP–ATPIII criteria in HIV-infected patients was also associated with functional and structural atherosclerotic changes. 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 paitents on HAART. Until these issues have been investigated, evaluation and management of individual cardiovascular risk factors using the currently available guidelines is recommended[23,40].

The impact of switching antiretroviral therapy on lipodystrophy and metabolic complications has also been reviewed recently[41]. Switching therapy from PIs to NNRTIs may partly reverse dyslipidemia and insulin resistance, whereas the morphologic alterations remain unchanged. In studies in which NRTIs are switched to other HIV drugs, dyslipidemia appears unaffected, but a modest improvement in lipoatrophy has been reported. The results of chapter 8 suggest that insulin-sensitizing agents can be used for treatment of lipodystrophy and insulin resistance in HIV-infected patients, for example in patients in which switching is not desirable. Besides patients with manifest hyperglycemia, HIV-infected patients with the metabolic syndrome and/or IGT may also benefit from insulin-sensitizing agents, although it has not been established whether this treatment strategy will reduce the onset of diabetes or cardiovascular complications in this population. Moreover, the results as described in chapter 8 reinforce the importance of individualized care in HIV-infected patients. Metformin may be best for the viscerally obese, overweight, dyslipidemic patient, whereas it may not be appropriate for the patient with predominantly lipoatrophy, as they may suffer

a further loss of subcutaneous fat. Our data also suggest that in patients with HIV-lipodystrophy, metformin has greater benefits on vascular function than rosiglitazone. Rosiglitazone may be best for lipoatrophic patients, but rosiglitazone should be used with caution in hyperlipidemic patients. If used, this must be accompanied by careful monitoring of the lipid profile. We did not find adverse events or drug interactions that might limit long-term use of these insulin-sensitizing agents. However, treatment with metformin may not be recommended in patients with renal- or liver disease or elevated lactic acid levels, which were exclusion criteria in our study. Regarding TZDs, it is important to note that rosiglitazone is a substrate for CYP2C8 and is unlikely to affect CYP3A4 metabolism on concomitantly administrated 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-α/γ agonists may be useful to treat both dyslipidemia and lipodystrophy in HIV-infected patients in the future. Taken together, in HIV-infected patients, maintaining viremic control remains the overriding factor, because short-term absolute rates of CVD are significantly lower than death rates from AIDS in inadequately suppressed HIV-infected patients[39]. However, prevention and management of lipodystrophy and cardiovascular risk is a major challenge for HIV practitioners in the future.

Main conclusions of this thesis

- Studies conducted with pioglitazone show greater benefits on blood lipids, but also different study population characteristics in comparison with studies conducted with rosiglitazone.
- Rosiglitazone improves the metabolism of large triglyceride-rich lipoproteins and decreases postprandial fatty acid concentrations in type 2 diabetes. These effects may contribute to cardiovascular risk reduction.
- Rosiglitazone attenuates the postprandial increases of neutrophils and cytokines in patients with type 2 diabetes. These effects may be part of the overall attenuation of the low-grade inflammatory state, and may convey increased protection from atherosclerosis.
- Rosiglitazone reduces atherosclerotic disease progression in non-diabetic patients with coronary artery disease, despite only minor effects on glucose and lipid homeostasis. These findings could be interpreted as a strong argument in favour of direct anti-atherosclerotic effects of rosiglitazone, due to repression of inflammation in the vasculature.
- Patients with HIV-lipodystrophy have disturbed postprandial fatty acid metabolism, most likely due to inadequate incorporation of fatty acids into triglycerides in subcutaneous adipose tissue. The higher postprandial hydroxybutyric acid levels reflect increased hepatic fatty acid delivery, and may aggravate insulin resistance and dyslipidemia, leading to an increased cardiovascular risk in these patients.

- Endothelial function is similarly disturbed in HIV-infected patients without the metabolic syndrome and type 2 diabetic patients, suggesting increased cardiovascular risk in HIV-infected patients, even in the absence of metabolic risk variable clustering. The presence of the metabolic syndrome in HIV is associated with even more advanced functional and structural vascular abnormalities.
- Insulin-sensitizing agents can be used for treatment of lipodystrophy and insulin resistance in HIV-infected patients. Whereas rosiglitazone may partly correct lipoatrophy, metformin improves visceral fat accumulation, fasting lipid profile, and endothelial function.

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10

Nederlandse samenvatting

Dankwoord

List of publications

Curriculum vitae

Hart- en vaatziekten (HVZ) vormen doodsoorzaak nummer één in de westerse samenleving. Belangrijke risicofactoren voor HVZ zijn diabetes mellitus, hoge bloedvetten (cholesterol en triglyceriden) en hoge bloeddruk. Deze risicofactoren zijn nauw aan elkaar verwant en vormen samen het metabool syndroom. Overgewicht en onvoldoende lichaamsbeweging zijn de belangrijkste risicofactoren voor het metabool syndroom. Overgewicht veroorzaakt insulineresistentie: ongevoeligheid van de lichaamscellen voor de werking van het hormoon insuline, waardoor er minder glucose (bloedsuiker) wordt verbrand in de cellen dan normaal. Insulineresistentie gaat vaak gepaard met hoge bloedvetwaarden en hoge bloeddruk. Met name vetophoping in de buik is sterk gerelateerd aan het metabool syndroom. Er zijn echter aanwijzingen dat ook een sterk onevenredige vetverdeling geassocieerd is met het metabool syndroom. Een voorbeeld hiervan is lipodystrofie bij HIV-geïnfecteerde patiënten ten gevolge van de antiretrovirale combinatietherapie (medicatie om HIV infectie te onderdrukken), zoals in **hoofdstuk 1** uitgebreid wordt besproken. Lipodystrofie wordt gekenmerkt door onderhuids vetverlies (in het aangezicht, de armen en benen, en de billen), alsmede vetophoping in de buik en de nek. Patiënten met lipodystrofie hebben vaak insulineresistentie en verhoogde concentraties cholesterol en triglyceriden. In dit proefschrift worden de metabole risicofactoren en de effecten van medicamenteuze interventies bestudeerd bij patiënten met type 2 diabetes mellitus en HIV-geïnfecteerde patiënten met lipodystrofie.

Type 2 diabetes mellitus

Diabetes mellitus is een veelvoorkomende chronische ziekte die gekenmerkt wordt door een te hoog bloedsuikergehalte. Ongeveer 90% van de diabetes patiënten heeft type 2 diabetes mellitus. Type 2 diabetes wordt veroorzaakt door een combinatie van insulineresistentie en een afname van de insuline afgifte uit de alvleesklier door uitputting van de bèta-cellen. Met de toename van overgewicht komt ook type 2 diabetes steeds meer voor. De verwachting is dat het aantal patiënten met type 2 diabetes in Nederland zal stijgen tot meer dan 500.000 in 2010. Voldoende lichaamsbeweging en afvallen vormen de hoeksteen voor de behandeling van type 2 diabetes, maar zijn zelden afdoende om de bloedsuikers adequaat te reguleren. De thiazolidinedionen vormen een relatief nieuwe klasse bloedsuikerverlagende medicijnen. Thiazolidinedionen verhogen de insulinegevoeligheid, en pakken dus de primaire oorzaak van type 2 diabetes aan. Het belangrijkste doelorgaan van thiazolidinedionen is het vetweefsel. Thiazolidinedionen stimuleren de opname van triglyceriden in het onderhuidse vetweefsel, maar niet in het vetweefsel in de buikholte, en zorgen dus voor een gunstige vetverdeling in het lichaam. Thiazolidinedionen blijken tevens invloed te hebben op de concentraties bloedvetten. Dit is van groot belang, omdat afwijkingen in de bloedvetten een belangrijke risicofactor zijn voor HVZ bij patiënten met type 2 diabetes. Er zijn momenteel twee thiazolidinedionen

beschikbaar: rosiglitazon en pioglitazon. Beide middelen vertonen een vergelijkbaar effect op de bloedsuiker, maar lijken te verschillen in de effecten op de bloedvetten.

In **hoofdstuk 2** wordt een overzicht gegeven van de effecten van rosiglitazon en pioglitazon op de bloedvetwaarden bij patiënten met type 2 diabetes. In dit overzicht wordt gebruik gemaakt van de resultaten van wetenschappelijke placebo-gecontroleerde studies die met deze middelen zijn uitgevoerd. Uit de analyse blijkt dat er inderdaad een aantal verschillen is tussen rosiglitazon en pioglitazon. Pioglitazon verlaagt de triglyceriden, verhoogt het HDL-cholesterol (goede cholesterol), maar heeft geen effect op het LDL-cholesterol (slechte cholesterol). Rosiglitazon heeft geen effect op de triglyceriden, maar verhoogt zowel het HDL-cholesterol als het LDL-cholesterol. Pioglitazon lijkt dus gunstigere effecten op de bloedvetten te hebben vergeleken met rosiglitazon. Er zijn twee mogelijke verklaringen voor deze bevindingen. Ten eerste hebben rosiglitazon en pioglitazon enkele verschillende farmacologische eigenschappen, die een deel van de bevindingen kunnen verklaren. Echter, een tweede opmerkelijke bevinding is dat de patiënten die met pioglitazon behandeld worden gemiddeld dikker zijn en metabool slechter ingesteld (hogere bloedsuikers en bloedvetten) dan de patiënten die met rosiglitazon behandeld worden. Het is bekend dat er bij patiënten met een slechte metabole regulatie meer winst te behalen is met medicamenteuze behandeling. Kortom, zowel de verschillen in farmacologische eigenschappen als de verschillen in metabole regulatie dragen waarschijnlijk bij aan onze bevindingen.

Na elke maaltijd stijgt het voedingsvet (triglyceriden) in het bloed. Mensen die na de maaltijd het voedingsvet vertraagd uit het bloed verwerken, hebben gedurende de dag een hoog vetgehalte in het bloed. Een verhoogd bloedvetgehalte houdt direct verband met een verslechterde werking van de bloedvaten, en is een risicofactor voor HVZ. De zogenoemde postprandiale (na de maaltijd) vetverwerking kan worden onderzocht na inname van een standaard slagroommaaltijd ("vetbelasting"). Het is bekend dat patiënten met type 2 diabetes een vertraagde vetverwerking hebben vergeleken met gezonde proefpersonen. In **hoofdstuk 3** worden de effecten van rosiglitazon op de postprandiale vetverwerking onderzocht bij patiënten met type 2 diabetes. De belangrijkste resultaten van dit onderzoek zijn dat rosiglitazon de vetverwerking verbetert. Deze resultaten zijn klinisch van belang, omdat een verbeterde vetverwerking na een maaltijd het proces van slagaderverkalking waarschijnlijk kan vertragen. Uit de triglyceriden die na een maaltijd in het bloed komen, worden vetzuren vrijgemaakt. Deze vetzuren worden gebruikt als energiebron of opgeslagen in vetcellen. Rosiglitazon blijkt de postprandiale vetzuurconcentraties te verlagen, waarschijnlijk door de opslag van vetzuren in het onderhuidse vetweefsel te stimuleren.

HVZ worden tegenwoordig gezien als een ontstekingsziekte, waarbij de bloedvatwand en ontstekingscellen in het bloed zijn betrokken. Deze ontstekingsreactie kan worden uitgelokt door verschillende risicofactoren, zoals bloedsuikers en bloedvetten. Belangrijke markers van ontsteking zijn witte bloedcellen en het "C-reactief proteïne" (een ontstekingsfactor die door de lever wordt gemaakt). Bij patiënten met een verhoogd aantal witte bloedcellen en verhoogde waarden van het C-reactief proteïne is verhoogde sterfte aan HVZ vastgesteld. Uit eerder onderzoek binnen onze afdeling is gebleken dat jonge gezonde vrijwilligers na een slagroommaaltijd een stijging van de hoeveelheid witte bloedcellen vertonen. Tegelijkertijd verminderde de werking van de bloedvaten. De postprandiale periode lijkt dan ook het ontstekingsproces te bevorderen, en draagt zo mogelijk bij aan het ontstaan van slagaderverkalking. In **hoofdstuk 4** laat rosiglitazon een gunstig effect zien op de stijging van het aantal witte bloedcellen na de slagroommaaltijd. Het C-reactief proteïne vertoont geen stijging na de maaltijd, maar rosiglitazon zorgt wel voor een forse afname van de nuchtere waarde. Deze resultaten duiden op een gunstig effect van rosiglitazon op de ontstekingsreactie in zowel de nuchtere als postprandiale periode, en bieden mogelijk bescherming tegen het optreden van HVZ bij patiënten met type 2 diabetes. Uit recent wetenschappelijk onderzoek bij patiënten met bewezen HVZ, maar zonder type 2 diabetes, is inderdaad gebleken dat rosiglitazon het proces van slagaderverkalking kan remmen. De resultaten van dit onderzoek worden in **hoofdstuk 5** uitgebreid besproken. In dit onderzoek kreeg de helft van de deelnemers een jaar lang rosiglitazon, de andere helft een placebo (neppil). Bij aanvang van het onderzoek en aan het einde van het onderzoek werd de vaatwanddikte van de halsslagader (als maat voor de hoeveelheid slagaderverkalking) met behulp van ultrageluidsgolven gemeten. Rosiglitazon bleek bij deze patiënten het proces van slagaderverkalking te remmen vergeleken met placebo. Opvallend was dat er slechts weinig verschillen in bloedsuikers en bloedvetten werden gevonden, wat duidt op een gunstig effect van rosiglitazon op de vaatwanddikte, mogelijk door de remming van het ontstekingsproces.

Lipodystrofie bij HIV-geïnfecteerde patiënten

Momenteel zijn er in Nederland ongeveer 7.000 tot 10.000 mensen geïnfecteerd met het humaan immunodeficiëntie virus (HIV). HIV breekt het afweersysteem af en maakt het lichaam dus vatbaar voor infecties en bepaalde vormen van kanker. De standaard behandeling bestaat uit een combinatie van verschillende antiretrovirale middelen. De introductie van deze combinatietherapie heeft gezorgd voor een drastische reductie in de sterfte ten gevolge van AIDS. Echter, de combinatietherapie wordt gecompliceerd door het optreden van een aantal bijwerkingen, waaronder lipodystrofie. Lipodystrofie wordt gekenmerkt door onderhuids vetverlies (in het aangezicht, de armen en benen, en de billen), alsmede vetophoping in de buik en de nek. Deze veranderingen in de vetverdeling zijn voor veel patiënten een belangrijk cosmetisch bezwaar. Daarnaast

gaat lipodystrofie vaak gepaard met een aantal risicofactoren voor HVZ, zoals insulineresistentie en verhoogde concentraties cholesterol en triglyceriden. Deze combinatie van risicofactoren vertoont grote gelijkenis met het metabool syndroom, zoals beschreven bij patiënten met type 2 diabetes. De antiretrovirale middelen hebben een direct verband met het optreden van de vetstofwisselingsstoornissen.

In **hoofdstuk 6** wordt beschreven dat HIV-geïnfecteerde patiënten met lipodystrofie een vertraagde vetverwerking hebben na een slagroommaaltijd vergeleken met patiënten zonder lipodystrofie en gezonde controles. Tevens is de capaciteit van het vetweefsel om postprandiale vetzuren op te slaan verminderd bij HIV-geïnfecteerde patiënten met lipodystrofie. Dit leidt tot verhoogde vetzuurconcentraties in het bloed, wat een aantal belangrijke gevolgen heeft. Ten eerste verminderen hoge vetzuurconcentraties de insulinegevoeligheid. Ten tweede kunnen vetzuren ook naar de lever worden getransporteerd, waar ze als bouwstof dienen voor nieuwe vetdeeltjes, die weer door de lever worden uitgescheiden. Een deel van de vetzuren wordt in de lever omgezet in ketonlichamen, die daardoor gebruikt kunnen worden als maat voor de hoeveelheid vetzuren die naar de lever gaan. De patiënten met lipodystrofie hebben een sterk verhoogde hoeveelheid ketonlichamen in het bloed vergeleken met de patiënten zonder lipodystrofie en gezonde controles. Tevens blijken zowel de vetzuurconcentraties als ketonlichamen direct gerelateerd te zijn aan een laag onderhuids vetgehalte, welke met computertomografie (CT scan) is gemeten. Een tekort aan onderhuids vetweefsel draagt dus mogelijk bij aan een gestoorde vetstofwisseling in het bloed en insulineresistentie bij HIV-geïnfecteerde patiënten. Al de hierboven beschreven verschijnselen zijn belangrijke risicofactoren voor HVZ. De vrees is dan ook dat HIV-geïnfecteerde patiënten in de toekomst steeds meer te maken zullen krijgen met HVZ.

In **hoofdstuk 7** wordt onderzocht wat de beste voorspellers zijn van vroege vaatschade bij HIV-geïnfecteerde patiënten. Het endotheel vormt de binnenbekleding van de bloedvaten en heeft een aantal regulatoire functies. Een verminderde functie van het endotheel kan worden gemeten met ultrageluidsgolven en is een goede voorspeller voor het optreden van HVZ. De polsgolf snelheid, gemeten over het traject van de buikslagader, is een maat voor de stijfheid van de bloedvaten. De vaatwanddikte van de halsslagader is een anatomische maat voor de hoeveelheid slagaderverkalking. Omdat onze interesse uitgaat naar zowel de functionele als anatomische eigenschappen van de bloedvaten bij HIV-geïnfecteerde patiënten, hebben we gebruik gemaakt van deze drie vaatmetingen. De totale groep HIV-geïnfecteerde patiënten wordt onderverdeeld in een groep mét en een groep zónder het metabool syndroom. Een belangrijke bevinding in dit onderzoek is dat de endotheelfunctie bij HIV-geïnfecteerde patiënten zonder het metabool syndroom evenveel gestoord is als bij

patiënten met type 2 diabetes. Bij HIV-geïnfecteerde patiënten met het metabool syndroom is de endotheelfunctie nog meer gestoord, en de vaatwanddikte verhoogd. De stijfheid van de bloedvaten blijkt niet verhoogd te zijn bij de HIV-geïnfecteerde patiënten. In dit onderzoek zijn de kenmerken van vroege vaatschade gerelateerd aan metabole risicofactoren (bloedvetten en insulineresistentie), de HIV infectie, de antiretrovirale therapie en ontsteking (witte bloedcellen en het C-reactief proteïne). Deze bevindingen duiden op een verhoogd risico op HVZ bij deze patiëntengroep. Daarnaast lijken verschillende risicofactoren aan dit verhoogde risico bij te dragen. Het is dan ook van belang deze risicofactoren vroegtijdig op te sporen en te behandelen.

Rosiglitazon en metformine zijn geneesmiddelen die ontwikkeld zijn voor de behandeling van type 2 diabetes mellitus, en verhogen de insulinegevoeligheid. Rosiglitazon stimuleert de opname van bloedvetten in het onderhuidse vetweefsel, en kan zo mogelijk het onderhuidse vetverlies ten gevolge van de antiretrovirale therapie tegen gaan. Metformine blijkt bij patiënten met type 2 diabetes voornamelijk de hoeveelheid vetweefsel in de buikholte te verminderen. In **hoofdstuk 8** worden de effecten van rosiglitazon en metformine bij HIV-geïnfecteerde patiënten met lipodystrofie direct met elkaar vergeleken. De insulinegevoeligheid wordt gemeten met behulp van een orale glucose tolerantietest. Met behulp van deze test wordt de glucoseverwerking na inname van een suikerdrankje bestudeerd. De hoeveelheid onderhuids vetweefsel en vetweefsel in de buikholte worden gemeten met behulp van computertomografie (CT scan). Rosiglitazon als metformine blijken de door insuline gestimuleerde glucose verwerking vergelijkbaar te verbeteren. Echter, de effecten van beide medicamenten op de vetverdeling zijn tegenovergesteld: rosiglitazon verhoogt het onderhuidse vetweefsel, terwijl metformine zowel het onderhuidse vetweefsel als het vetweefsel in de buikholte verlaagt. Een andere opvallende bevinding is dat rosiglitazon de bloedvetwaarden verslechtert, terwijl metformine deze verbetert. Hier dient men rekening mee te houden, indien men bij deze patiëntengroep behandeling met rosiglitazon overweegt. Tot slot worden in dit onderzoek de effecten van beide medicamenten op de endotheelfunctie onderzocht. Alleen metformine verbeterde de endotheelfunctie bij deze patiëntengroep, mogelijk door de gunstige effecten op de bloedvetten. Geen van beide medicijnen verlaagde de ontstekingsreactie. Dit kan wellicht worden verklaard door de hoge onstekingsactiviteit door de HIV infectie, welke moeilijk door rosiglitazon en metformine is te onderdrukken. Rosiglitazon en metformine lijken dus beiden effectief om lipodystrofie en insulineresistentie bij HIV-geïnfecteerde patiënten te behandelen. Metformine lijkt de eerste keus bij patiënten met vetophoping in de buikholte en hoge bloedvetwaarden, rosiglitazone bij patiënten met voornamelijk onderhuids vetverlies.

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Graag wil ik iedereen die een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift bedanken. Alvorens mensen persoonlijk te noemen, wil ik eerst alle patiënten die aan dit onderzoek hebben deelgenomen bedanken voor de enthousiaste deelname. Ondanks de lange onderzoeksdagen, het vele bloedprikken en het slagroom drinken op de nuchtere maag was jullie inzet enorm. Zonder jullie medewerking was dit proefschrift nooit tot stand gekomen. Mijn dank hiervoor is groot.

Prof. dr. I.M. Hoepelman, geachte promotor, beste Andy; het heeft lange tijd geduurd voordat je de Vasculaire Geneeskunde en Diabetologie kon overtuigen van de metabole en vasculaire problematiek bij HIV-geïnfecteerde patiënten. Uiteindelijk is dit dan toch gelukt. Ik wil je hartelijk bedanken voor je vele inspanningen, het in mij gestelde vertrouwen en de enthousiaste begeleiding. Ik kon altijd bij je terecht.

Prof. dr. T.J. Rabelink, geachte promotor, beste Ton; vanaf het begin van het onderzoek heb je me in alle opzichten gesteund. Het blijft voor mij een raadsel hoe je binnen je overvolle agenda toch altijd tijd voor mij wist vrij te maken. Mijn dank is groot voor je goede zorgen en het in de gaten houden van de grote lijnen van het onderzoek.

Dr. M. Castro Cabezas, geachte co-promotor, beste Manuel; tijdens mijn doctoraalstage op de afdeling vetstofwisseling heb je mijn enthousiasme voor het wetenschappelijk onderzoek gewekt. Jou enorme bevlogenheid heeft me de afgelopen jaren bijzonder geïnspireerd. Ik ben je veel dank verschuldigd voor je hulp en steun gedurende mijn periode als onderzoeker. Ik heb veel van je geleerd en hoop dan ook in de toekomst nog veelvuldig met je te kunnen samenwerken.

Dr. E.J.P. de Koning, geachte co-promotor, beste Eelco; met veel plezier heb ik de afgelopen tijd met je samengewerkt. Jou actieve inspanningen bij het opzetten van de studie, de analyse van de data en het reviseren van de manuscripten waren voor mij erg belangrijk. Hartelijk dank voor al je hulp en gezelligheid en veel succes met de eilandjestransplantaties in Boston.

De leden van de beoordelingscommissie Prof. dr. H.A. Koomans, Prof. dr. J.R.E. Haalboom, Prof. dr. P.A. Doevendans, Prof. dr. J. Lange en Prof. dr. J. Betteridge, wil ik bedanken voor alle inspanningen.

Beste Jos, jij hebt me gedurende het hele onderzoek enorm geholpen bij het uitvoeren van de vaatmetingen en slagroomproeven. Zonder jou grappen en grollen waren deze lange onderzoeksdagen waarschijnlijk oersaai geworden. Ik hoop dat je in Leiden weer helemaal je draai kunt vinden en dat we in de toekomst nog eens een project samen kunnen uitvoeren. Nogmaals, bedankt voor al je hulp en gezelligheid.

Dear Blai Coll, Jorge Joven and Josep Ribalta, Thank you for the critical reviews of the postprandial data and manuscripts. Soon, I hope to meet you in Reus and enjoy the Spanish tapas and wine. That our collaboration may continue!

Alle (ex)collega's, onderzoekers en laboranten van het laboratorium Vasculaire Geneeskunde: Albert, Arash, Berthil, Carolien, Cindy, Despina, Fabrice, Hetty, Jobien, Livio, Lonneke, Maarten, Peter, Petra, Sharon, Stijn, Tjeerd en vele studenten. Bedankt voor jullie belangstelling en ondersteuning. Zonder de vele hoogtepunten van de afgelopen jaren (labstapdagen, Vat-het-Vat congres en kerstdiners) was het een stuk minder leuk geweest. Ik wens jullie verder veel succes met de huidige projecten. Beste Arno, bedankt voor je hulp bij de slagroomproeven, en niet te vergeten de online-games. Succes met je opleiding tot cardioloog. Ik wil tevens Geesje Dallinga-Thie, Anton-Jan van Zonneveld, Marianne Verhaar en Frank Visseren bedanken voor hun kritische blik en suggesties. De buurtjes van de Nefrologie en Experimentele Cardiologie wil ik bedanken voor alle hulp en gezelligheid.

Het secretariaat van de afdeling Interne Geneeskunde en Infectieziekten (Jeanette en Els) en de polikliniek Infectieziekten (Carla, Marianne, Mariska en Martine) wil ik bedanken voor de interesse en hulp bij diverse logistieke zaken. Verder wil ik alle Infectiologen bedanken voor de hulp bij de patiëntenwerving.

Roy Sanders, bedankt voor je inzet en creativiteit om de lay-out van dit proefschrift te verzorgen.

Na mijn onderzoek ben ik gestart met de opleiding tot internist in ziekenhuis de Gelderse Vallei te Ede. Ik wil alle assistenten, internisten en gastro-enterologen bedanken voor de hulp bij de overgang naar de kliniek en de interesse naar het proefschrift. Dankzij jullie voel ik me in Ede helemaal op mijn plaats.

Beste jaarclubgenoten, het is goed te zien dat de vriendschappen ook na de studietijd blijven bestaan. Maarten en Koen, bedankt dat jullie me 11 oktober als paranimfen terzijde willen staan.

Lieve ouders, Ludo en Els, jullie hebben me altijd alle ruimte gegeven om te gaan studeren. Daarnaast leerden jullie me dat studeren veel meer is dan alleen een standaardprogramma afwerken. Ik besef me steeds meer dat jullie voor een uitstekende thuisbasis hebben gezorgd. Bedankt voor al jullie steun en interesse. Lieve Michiel, ook jou wil ik bedanken voor je interesse en positieve blik. Hopelijk hebben we nu weer wat meer tijd om elkaar vaker te zien.

Lieve Karin, ik had nooit gedacht zoiets dierbaars aan mijn promotie-onderzoek over te houden. Duizend maal dank voor al je steun en liefde!

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The author was born on January 25th, 1977 in Utrecht, the Netherlands. After graduation from secondary school at the Oosterlicht College in Nieuwegein in 1995, he started Medical School at the University of Utrecht. As a medical student he spent a year at the Laboratory of Lipidology at the University Medical Center Utrecht (Prof. dr. D.W. Erkelens and Dr. M. Castro Cabezas) where he investigated diurnal triglyceride metabolism in relation to insulin resistance and premature coronary sclerosis. Furthermore, as a modern medical student he inevitably visited a foreign hospital during his medical training (clerkship Internal Medicine, Parirenyatwa Hospital and Harare Central Hospital, Harare, Zimbabwe). He obtained his Medical degree in August 2002 and then started the work described in this thesis at the Department of Internal Medicine at the Laboratory of Vascular Medicine in the University Medical Center Utrecht, the Netherlands. In May 2003, he received educational grant support from the Dutch Organization for Scientific Research (NWO). In may 2005, he started specialist training in Internal Medicine in Hospital Gelderse Vallei, Ede, the Netherlands (supervised by Dr. R. Heiligenberg).