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Rosiglitazone in the treatment of HAART-associated lipodystrophy – a randomized double-blind placebo-controlled study

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Highly active antiretroviral therapy (HAART) is associated with metabolic adverse events such as insulin resistance and lipodystrophy, that is, atrophy of subcutaneous fat and accumulation of intra-abdominal fat. Currently, there is no pharmacological treatment for lipoatrophy. Glitazones, a novel class of insulin-sensitizing anti-diabetic agents, increase subcutaneous fat in patients with type 2 diabetes. There are no controlled studies of glitazones in patients with HAART-associated lipodystrophy (HAL). In this randomized, double-blind, placebo-controlled study, 30 patients with HAL received either rosiglitazone (8 mg daily) or placebo for 24 weeks. Baseline characteristics were compared to a group of 30 age-, sex- and weight-matched HIV-negative controls. At baseline, patients with HAL had 1.8-fold (P<0.001) more intra-abdominal and 2.4-fold (P<0.05) more liver fat than HIV-negative controls, who had 1.8-fold (P<0.001) more subcutaneous fat than the patients. After 24 weeks of treatment, rosiglitazone had no effect on body weight, subcutaneous or intra-abdominal fat (magnetic resonance imaging), total body fat (bioimpedance analysis), anthropometric measurements or serum leptin concentrations (a circulating marker of adipose tissue mass). However, rosiglitazone decreased % liver fat (spectroscopy) and serum insulin concentrations, and normalized liver function tests. During the first 12 weeks of rosiglitazone treatment, serum triglycerides increased from 3.5 ±0.5 to 6.5 ±2.0 mmol/l (from 310 ±44 to 575 ±177 mg/dl) (P<0.05) and serum cholestrol from 6.0 ±0.4 to 7.8 ±0.7 mmol/l (from 232 ±15 to 301 ±27 mg/dl) (P<0.01). Contrary to data in other patient groups, rosiglitazone did not increase subcutaneous fat in patients with HAL after 24 weeks of treatment. Rosiglitazone seemed to ameliorate insulin resistance judged by the decreased serum insulin concentrations and % liver fat. Rosiglitazone unexpectedly caused significant increases in serum triglyceride and cholesterol concentrations, which must be carefully monitored if glitazones are used in these patients.

Introduction

Highly active antiretroviral therapy (HAART) has dramatically reduced HIV-associated morbidity and mortality in the developed world [1,2]. However, HAART is also associated with various adverse events including lipodystrophy, that is, atrophy of subcutaneous fat, hypertrophy of intra-abdominal fat or development of a buffalo hump [3–5]. Patients have also features of insulin resistance, such as hyperinsulinaemia and hyperlipidaemia [3,6]. These adverse events may compromise improved prognosis of HIV infection by increasing cardiovascular morbidity [7–9] and by reducing compliance to HAART [10]. In addition, severe forms of lipodystrophy, especially lipoatrophy, can be disfiguring and stigmatizing. Currently, there is no pharmacological therapy for HIV lipoatrophy. Abacavir substitution of stavudine or zidovudine showed improvement in lipoatrophy after 24 weeks, however, this limited change was clinically not recognized by either patients themselves or their treating physicians [11].

Thiazolidinediones (glitazones) are novel insulin-sensitizing anti-diabetic agents. The first glitazone, troglitazone, was withdrawn due to hepatotoxicity [12]. The newer compounds, rosiglitazone and pioglitazone, are available for treatment of type 2 diabetes both in Europe and the USA. These agents are ligands for the nuclear peroxisome-proliferator activated receptor-gamma (PPARγ), activation of which is critical for adipocyte differentiation [13,14]. In patients with type 2 diabetes, treatment with rosiglitazone improves insulin sensitivity despite increasing body weight and fat mass [15–19]. The increase in fat mass amounts to 3.5–4.0 kg in 12 weeks [16,19] and appears to occur almost exclusively in subcutaneous

adipose tissue [17,18,20], an effect that would be desirable in patients with lipodystrophy. The expression of PPARγ in subcutaneous adipose tissue was recently shown to be decreased in HIV-positive patients with lipodystrophy [21]. *In vitro*, rosiglitazone increases PPARγ expression [13] and prevents the block in adipocyte differentiation induced by protease inhibitors [22].

These data support the hypothesis that glitazones could improve subcutaneous lipoatrophy in HIV-infected patients receiving HAART. We conducted a 24-week, randomized, double-blind, placebo-controlled study to determine the efficacy and safety of rosiglitazone in HIV-lipodystrophy syndrome.

Methods

Study design

The study protocol, which was investigator-initiated and not supported by the manufacturer of rosiglitazone, consisted of a 24-week randomized double-blind treatment period with either rosiglitazone (8 mg once a day) or an identical-looking placebo in a parallel fashion. The primary aim was to determine whether rosiglitazone increases the amount of subcutaneous fat in patients with HIV lipodystrophy. Secondary aims included evaluation of effects of rosiglitazone on features of insulin resistance and on safety parameters in patients using HAART. Measurements of body composition were performed at 0 and 24 weeks and included quantification of: i) intra-abdominal and subcutaneous fat using magnetic resonance imaging (MRI); ii) total body fat by bioimpedance analysis (BIA); iii) liver fat by magnetic resonance proton spectroscopy; iv) serum leptin concentrations as a marker of adipose tissue quantity [23]; and v) anthropometric measurements (waist to hip ratio, skinfold thicknesses). To monitor drug safety and features of insulin resistance, blood samples were taken after an overnight fast at outpatient visits at baseline, 2 (blood counts and liver enzymes only), 6, 12, 18 and 24 weeks.

Study subjects

The patients were recruited from the HIV outpatient clinic of Helsinki University Central Hospital. Inclusion criteria included: i) positive HIV-antibodies; ii) age older than 18 years; iii) treatment with HAART for at least 18 months with no changes in the treatment regimen during 8 weeks prior to randomization; and iv) lipodystrophy, defined clinically as self-reported symptoms of loss of subcutaneous fat with or without increased abdominal girth, breast size or development of a buffalo hump. These findings were confirmed by the investigator (JS) before enrolment. This definition may be considered a limitation, but currently there are no uniformly approved objective criteria for diagnosing lipodystrophy. Exclusion criteria included serum transaminase concentrations higher than three times the upper limit of normal, heart failure, severe hypertriglyceridaemia (serum triglycerides above 10 mmol/l, 885 mg/dl), diabetes and pregnancy.

Physical and biochemical characteristics of the HIV-positive patients (25 men, five women) were compared to those of an HIV-negative group (25 men, five women), which was recruited from occupational health services (Table 1). This group was healthy as judged by history and physical examination, and standard laboratory tests, and did not use any regular medications. The purpose, nature and potential risks of the study were explained to the study subjects before their written informed consent was obtained. The protocol was approved by the ethics review committee of the Helsinki University Central Hospital.

Intra-abdominal and subcutaneous fat (MRI)

A total of 16 T1-weighted trans-axial scans reaching from 8 cm above to 8 cm below the 4th and 5th lumbar interspace, were analysed for the determination of intra-abdominal and subcutaneous fat (field of view 375×500 mm^2, slice thickness 10 mm, breath-hold repetition time 138.9 ms, echo time 4.1 ms). Intra-abdominal and subcutaneous fat volumes were measured by a single investigator in a blinded fashion using an image analysis software (Alice 3.0, Parexel, Waltham, Mass., USA) as previously described [24]. The reproducibility of repeated measurements of subcutaneous and intra-abdominal fat as determined on two separate occasions in our laboratory in non-diabetic subjects (n=10, unpublished data) is 3 and 5%.

Liver fat (proton spectroscopy)

Localized single voxel (2×2×2 cm) proton spectra were recorded using a 1.5 T whole body system (Siemens Magnetom Vision, Erlangen, Germany), which consisted of the combination of whole body and loop surface coils for radiofrequency transmitting and signal receiving. The single voxel spectra were recorded as previously described [24]. The signal intensities were quantified using an analysis program VAPRO-MRUI (www.mrui.uab.es/mrui/). Spectroscopic intracellular triglyceride content was expressed in % and calculated from methylene-to-(water+methylene) signal area ratio ×100 (=% liver fat). All spectra were analysed by a physicist who was unaware of patient assignment. The reproducibility of repeated measurement of liver fat in non-diabetic subjects studied on two occasions in our laboratory (n=10, unpublished data) is 11%. This measurement of liver fat by proton spectroscopy has been validated against histologically determined lipid content of liver biopsies in humans

[25] and against estimates of fatty infiltration by computed tomography [24,26].

Other measures of body composition

Percentage of body fat and total body fat mass were determined using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model #BIA-101A, RJL Systems, Detroit, Mich., USA). Waist circumference was measured midway between the lower rib margin and the iliac crest, and hip circumference over the great trochanters [27]. Skinfold thicknesses (sum of mean values of triplicate measurements) were determined at six sites (triceps, biceps, subscapular, iliac crest, thigh and cheek) [28]. Bioelectrical impedance analyses and all anthropometry measurements were performed by a single investigator blinded for treatment randomization.

Analytical procedures

Serum-free insulin concentrations were determined with radioimmunoassay (Phadeseph® Insulin RIA, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) after precipitation with polyethylene glycol [29]. Plasma glucose concentrations were measured using a hexokinase method, and serum total and high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations with respective enzymatic kits from Roche Diagnostics using an autoanalyser (Roche Diagnostics Hitachi 917, Hitachi Ltd, Tokyo, Japan). HbA_{1c} was measured by high pressure liquid chromatography using the fully automated Glycosylated Hemoglobin Analyzer System (BioRad, Richmond, Calif., USA) [30]. Serum leptin concentrations were determined by radioimmunoassay using a commercial kit (Human leptin RIA kit, Linco Research, St. Charles, Mo., USA). Serum alanine aminotransferase (ALT) activities were determined according to recommendations of the European Committee for Clinical Laboratory Standards using the Roche Diagnostics Hitachi 917 autoanalyser. Venous blood gas analyses were performed using specific electrodes with a blood gas analyser (Ciba Corning 850, Medfield, Mass., USA). HIV viral load was measured using the HIV-1 Monitor Test (Roche Diagnostics, Branchburg, NJ, USA) with a detection limit of 50 copies/ml. Serum protease inhibitor trough concentrations were determined using liquid chromatography; the assay was available for indinavir, nelfinavir, ritonavir and saquinavir.

Statistical analysis

The paired t-test was used for comparisons of changes before and after placebo and rosiglitazone treatment. Logarithmic transformation was performed on data that were not normally distributed. Repeated measurements over time were compared using analysis of variance followed by Fisher's Least-Significant-Difference test. All data are given as mean ±SEM. Sample size was calculated based on the effects of troglitazone on abdominal subcutaneous fat measured by magnetic resonance imaging in patients with non-HIV lipodystrophy [31]. In this study, subcutaneous fat in the abdominal region increased by 837 ml after 6 months of troglitazone treatment. In the present study, a sample size of 15 in each group has 95% power to detect a difference in means of abdominal subcutaneous fat of 450 ml assuming that the common standard deviation is 300 ml using a two-group t-test with a significance level of 0.05.

Results

Thirty HIV-positive patients were screened and eligible for the study. All 30 patients were randomized, 15 were assigned to receive rosiglitazone and 15 placebo. At baseline, the placebo and rosiglitazone groups were similar with respect to age, gender, body weight and composition (Table 1). The mean time from diagnosis of HIV was 8.4 ±0.6 years. The mean duration of a combination antiretroviral therapy was 4.3 ±0.2 years. All patients in both groups were currently receiving nucleoside reverse transcriptase inhibitors (NRTIs), 20% in the placebo and 47% in the rosiglitazone group (NS) were receiving a non-nucleoside reverse transcriptase inhibitor (NNRTI), and 93% and 67% of the patients (NS), respectively, were currently receiving a protease inhibitor-containing regimen. Of the NRTI class of antiretroviral agents, lamivudine was used by 14 patients in the placebo and nine in the rosiglitazone group, stavudine by 11 and 10, zidovudine by three and four, didanosine by one and six, abacavir by one and three, respectively; one patient used zalcitabine in the rosiglitazone group. Of the NNRTIs, efavirenz was used by three patients in both groups and nevirapine by four patients in the rosiglitazone group. The most common protease inhibitor was indinavir, used by five patients in the placebo and four in the rosiglitazone group, nelfinavir was used by four and two, lopinavir by two and two, amprenavir by one and two, respectively; ritonavir was used by two patients in the placebo group. The doses used were standard ones with the exception of one patient in the rosiglitazone group who received indinavir 1000 mg three times a day due to previously measured low serum indinavir concentration. The mean CD4 count was 572 ±54 cells/mm^3 and 70% of the patients had a viral load below 50 copies/ml at baseline. All patients had contracted HIV through sexual contact. None of the patients was positive for hepatitis C antibodies or hepatitis B surface antigen. There were no significant

differences between the rosiglitazone and the placebo group with respect to HIV-related characteristics at baseline. None of the patients had any changes in the antiretroviral therapy during the study.

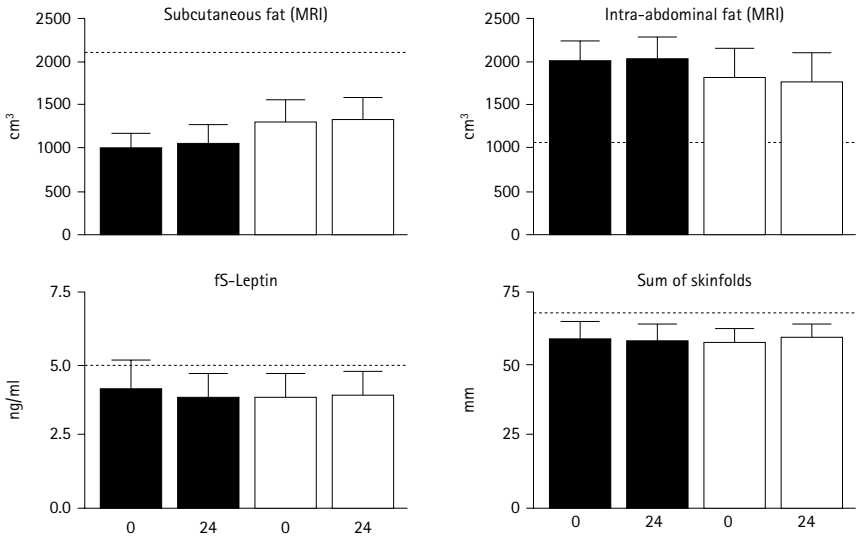
Compared to age- and weight-matched HIV-negative subjects, the HIV-positive patients with lipodystrophy had significantly less subcutaneous (1140 ±160 vs 2080 ±140 cm^3; $P<0.001$) and more intra-abdominal (1920 ±210 vs 1070 ±110 cm^3; $P<0.001$) fat, and higher waist to hip and intra-abdominal to total fat ratios (Table 1). Fasting serum insulin (11 ±1 vs 5 ±1 μU/ml; $P<0.001$) and triglyceride [3.4 ±0.4 vs 1.0 ±0.1 mmol/l (301 ±35 vs 88 ±9 mg/dl); $P<0.001$] concentrations were significantly higher and HDL cholesterol [1.1 ±0.1 vs 1.5 ±0.1 mmol/l (42 ±4 vs 58 ±4 mg/dl); $P<0.001$] concentrations lower in the patients with HIV lipodystrophy than the HIV-negative subjects. Plasma glucose (5.5 ±0.3 vs 5.3 ±0.1 mmol/l in the HIV-lipodystrophy versus HIV-negative subjects, NS) or HbA_{1c} (5.2 ±0.2 vs 5.5 ±0.1%, respectively, NS) concentrations were not different between the groups.

After 24 weeks of treatment, there were no significant changes in body weight or in the amount of intra-abdominal or subcutaneous fat as determined by MRI, serum leptin concentrations, the sum of skinfold thicknesses or other measures of adiposity in either placebo or rosiglitazone group (Figure 1 and Table 1).

Serum insulin concentrations and % liver fat decreased in the rosiglitazone group, but increased in the placebo group (Figure 2); the changes between the groups were statistically significant. In the rosiglitazone group, the change in serum insulin concentration correlated with the change in the % liver fat ($r=0.52$, $P<0.05$), but not with any other measure of body composition. Serum triglyceride concentrations increased markedly during rosiglitazone treatment (Figure 2). Serum total cholesterol concentrations also increased significantly (Figure 2). Serum HDL cholesterol did not change significantly in either group: 1.0 ±0.1 at baseline vs 0.9 ±0.1 mmol/l (37 ±4 vs 35 ±4 mg/dl) after 24 weeks in the rosiglitazone and 1.1 ±0.1 vs 1.2 ±0.1 mmol/l (42 ±4 vs 46 ±4 mg/dl) in the placebo group. Plasma glucose concentration decreased non-significantly in both groups: by 0.01 ±0.2 in the placebo and 0.15 ±0.1 mmol/l in the rosiglitazone group.

Serum ALT and haemoglobin concentrations decreased significantly in the rosiglitazone group and remained stable in the placebo group (Figure 3). One patient in the rosiglitazone group discontinued the study after 12 weeks of treatment due to increased triglyceride concentration [32.5 mmol/l (2876 mg/dl)]. None of the patients developed hypoglycaemia or clinically detectable oedema. Venous blood pH and bicarbonate concentrations did not change significantly in either group. CD4 counts and protease inhibitor concentrations did not change significantly in either group (data not shown). None of the patients lost virological control during the study.

Figure 1. The effect of rosiglitazone versus placebo on the volume of subcutaneous and intra-abdominal adipose tissue determined by magnetic resonance imaging (MRI) (upper panels) and on the serum leptin concentration and the sum of skinfold thicknesses measured at six body sites (lower panels)



Black columns, rosiglitazone; open columns, placebo; error bars, SEM; 0, baseline; 24, after 24 weeks on therapy; dashed line, mean value of HIV-negative normal subjects.

Table 1. Effects of rosiglitazone and placebo on measures of body composition in patients with HIV lipodystrophy syndrome as compared to HIV-negative normal subjects

	HIV lipodystrophy syndrome				
	Rosiglitazone (*n*=15)		Placebo (*n*=15)		HIV-negative normal subjects (*n*=30)
	0 week	24 weeks	0 week	24 weeks	
Age (years)	44 ±3		42 ±2		43 ±2
Male/female	12/3		13/2		25/5
Weight (kg)	72.9 ±2.5	73.7 ±2.5	73.3 ±3.4	74.4 ±3.6	72.9 ±1.6
Body mass index (kg/m^2)	23.7 ±0.7	23.9 ±0.6	23.6 ±0.8	23.9 ±0.9	23.6 ±0.4
Total body fat mass (kg)	13.0 ±1.3	13.4 ±1.3	13.1 ±1.9	14.0 ±1.9	14.4 ±0.9
Waist to hip ratio	0.98 ±0.02 †	0.98 ±0.02 †	0.99 ±0.02 †	0.99 ±0.02 †	0.92 ±0.01
Intra-abdominal to total fat ratio	0.68 ±0.06 §	0.68 ±0.06 §	0.59 ±0.05 §	0.57 ±0.05 §	0.34 ±0.02
Liver fat (%)	7.3 ±1.6*	6.2 ±1.3* #	8.0 ±3.1*	10.1 ±3.3*	3.2 ±0.9
fS-Insulin (µU/ml)	12.6 ±1.5 §	9.3 ±0.6 § ǂ #	9.6 ±1.8 †	16.3 ±5.7 †	5.3 ±0.5
fS-Triglycerides (mmol/l)	3.5 ±0.5 §	5.8 ±2.0 §	3.2 ±0.5 §	3.8 ±1.0 §	1.0 ±0.1
fS-Cholesterol (mmol/l)	6.0 ±0.4*	7.4 ±0.7 § ǂǂ	5.9 ±0.2*	5.9 ±0.3*	5.3 ±0.2
S-ALT (U/l)	46 ±7 §	32 ±4 ǂ	45 ±6 §	42 ±5 †	26 ±2

*P<0.05, † P<0.01 and § P<0.001 for comparisons between HIV-negative normal subjects and patients with HIV lipodystrophy syndrome. ǂP<0.05, ǂǂP<0.01 for comparisons between baseline and at 24 weeks within rosiglitazone or placebo group. #P<0.05 for comparisons of change in the rosiglitazone versus the placebo group. Data are shown as mean ±SEM.

Discussion

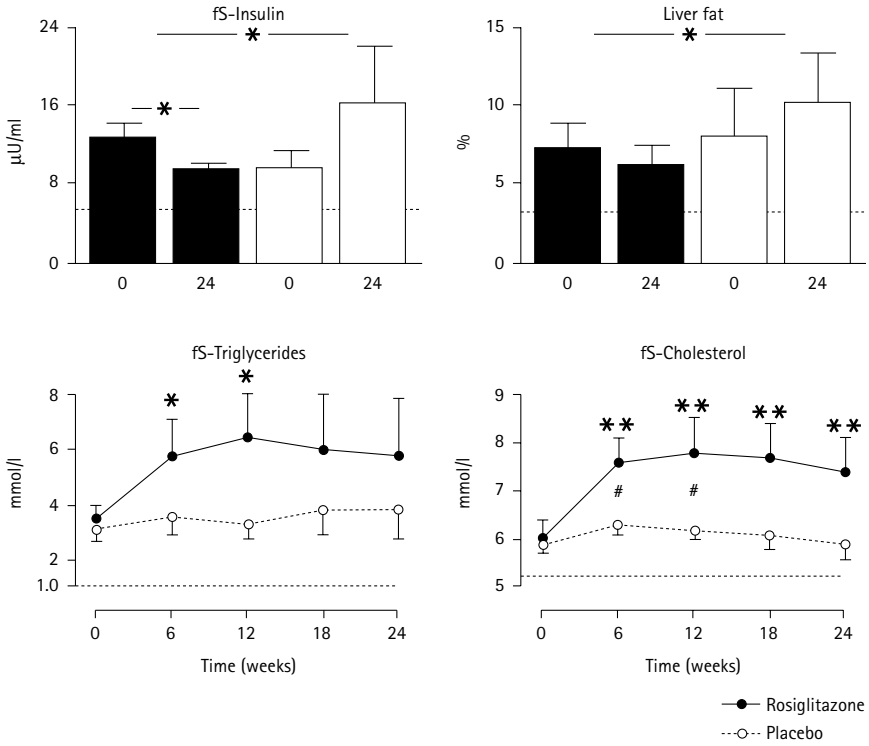
This is the first controlled study evaluating the efficacy and safety of a glitazone in patients with HIV lipodystrophy syndrome. In contrast to results in HIV-negative patients [15–19,31] and *in vitro* data [22], treatment with rosiglitazone did not increase any of the several measures of adiposity. Rosiglitazone did ameliorate insulin resistance as determined from decreases in serum insulin concentrations and % liver fat, and did improve liver function tests. Unexpectedly and again contrary to data in HIV-negative subjects, rosiglitazone induced a significant increase in serum triglyceride and cholesterol concentrations.

The failure of rosiglitazone to increase subcutaneous fat volume in HIV lipodystrophy syndrome is in striking contrast with previous reports in patients with type 2 diabetes [15–19] and HIV-negative patients with lipodystrophy [31]. While MRI scanning of the abdominal area showed no change in abdominal subcutaneous fat mass, one may ask whether there was an increase in peripheral subcutaneous fat. This seems unlikely because, in the face of unchanged body weight and abdominal volumes, peripheral subcutaneous fat could only have increased at the expense of a reduction in lean body mass. The unchanged sum of skinfold thicknesses and lean body mass (assessed by bioimpedance analysis, which has good reproducibility [32], but is unreliable in absolute terms in lipodystrophic patients, data not shown) argue against this possibility. Recently, in an uncontrolled study of eight patients with HAART-associated insulin resistance using rosiglitazone for 6–12 weeks, an increase in abdominal subcutaneous fat, as determined from a single computed tomography slice was observed by Gelato *et al.* [33]. Changes in body weight or peripheral subcutaneous fat mass after rosiglitazone treatment were not reported [33].

Inadequate dosing of rosiglitazone cannot explain the lack of effect on subcutaneous adipose tissue mass, as we used the highest recommended daily dose [34]. In patients with type 2 diabetes, the mean increase in body fat mass was 3.5–4.0 kg after just 12 weeks of treatment with rosiglitazone [16,19]. In view of these data, the lack of effect of 24-week treatment with rosiglitazone on body fat in the present study demonstrates that either rosiglitazone is unable to increase adipose mass in patients with HIV lipodystrophy syndrome or these patients require much longer treatment than HIV-negative patients. It can also be hypothesized that rosiglitazone caused a stimulatory effect of adipocyte differentiation, but this beneficial effect was simultaneously neutralized by the 'lipotoxic' effect of the unchanged antiretroviral therapy. Noncompliance cannot explain the lack of rosiglitazone efficacy either, since a decrease in haemoglobin, a well-known side effect of glitazones [34], was observed in the rosiglitazone but not the placebo group. Of the two glitazones currently on the market, we chose to use rosi- rather than pioglitazone, since the former does not interact with cytochrome P450 3A4 substrates like HIV protease inhibitors [35,36].

Before the treatment, the HIV-positive patients with lipodystrophy had an increased % liver fat compared to the HIV-negative control subjects (Figure 2). This increase could be to due to the direct stimulatory effect

Figure 2. The effect of rosiglitazone versus placebo on the serum insulin concentration and the % liver fat determined by spectroscopy (upper panels) and on the serum triglyceride and the total cholesterol concentration (lower panels)



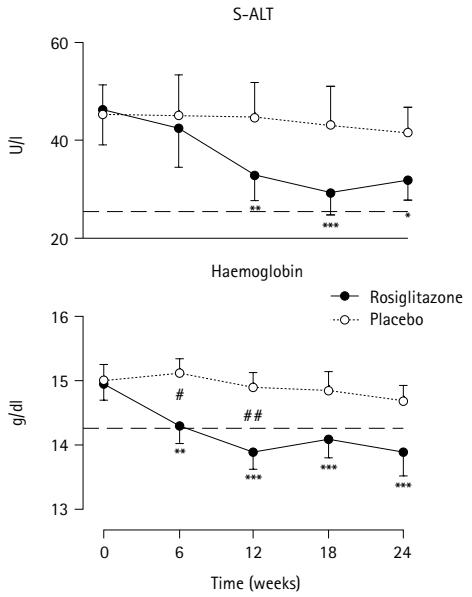
Liver fat content is expressed as % as described in methods. Black columns, rosiglitazone; open columns, placebo; error bars, SEM; 0, baseline; 24, after 24 weeks on therapy; dashed line, mean value of HIV-negative normal subjects; **P*<0.05; ***P*<0.01 for comparisons in the upper panels as indicated, in the lower panels vs baseline. #*P*<0.05 for comparisons between the rosiglitazone and the placebo groups. To convert triglycerides to mg/dl, divide by 0.0113. To convert total cholesterol to mg/dl, divide by 0.0259.

of protease inhibitors on liver fat [37] or secondary to subcutaneous lipoatrophy [38]. In mouse models, lipoatrophy resulting from inactivation of adipogenic transcription factors leads to ectopic fat accumulation in the liver and skeletal muscle [38–42]. The latter rather than loss of fat *per se* appears to be the principal cause of insulin resistance [43]. Also in humans, fat accumulation in the liver is associated with defects in insulin action independent of obesity [44].

In the present study, despite lack of effect on adipose tissue mass or distribution, rosiglitazone decreased fasting serum insulin concentrations and % liver fat (Figure 2). The decrease in % liver fat by rosiglitazone is similar to that reported in HIV-negative subjects in an uncontrolled study [19]. It has been suggested that glitazones reduce liver fat by redistributing lipid from liver to peripheral adipocytes [19]. Clearly, the mechanism by which rosiglitazone improves insulin sensitivity is more complex since in the present study insulin levels and liver fat decreased in the absence of changes in subcutaneous or intra-abdominal fat. Taken together, these data suggest that the action of rosiglitazone is preserved in the liver, but absent or diminished in adipose tissue in HIV lipodystrophy syndrome. The present study should be compared with effects of metformin in HIV lipodystrophy [45]. In the latter study, body weight, diastolic blood pressure and serum insulin concentration after a glucose tolerance challenge, but not in the fasting state, decreased significantly. Liver enzymes and serum triglycerides remained unchanged. Metformin also reduced plasminogen activator inhibitor-1 concentrations in these patients [46]. Although neither rosiglitazone nor metformin reversed lipodystrophy, metformin might be considered at the moment the drug of choice to treat insulin resistance in these patients in view of the potentially dangerous increases in serum lipids by rosiglitazone. On the other hand, the two drugs have not been compared in the same study and long-term data with glitazones would be interesting.

In HIV-negative subjects, rosiglitazone has neutral effects on serum triglyceride and cholesterol concentrations [15–19,47]. Unexpectedly, serum triglycerides increased markedly in patients in the rosiglitazone

Figure 3. The effect of rosiglitazone versus placebo on serum alanine aminotransferase (ALT) and haemoglobin concentration



Error bars, SEM; dashed line, mean value of HIV-negative normal subjects; *P<0.05, **P<0.01, ***P<0.001 for comparisons versus baseline. #P<0.05, ##P<0.01 for comparisons between the rosiglitazone and the placebo groups.

group but remained unchanged in the placebo group (Figure 2). The increase in serum triglycerides was not associated with use of any specific drug or combination of antiretrovirals. At baseline, serum triglycerides exceeded 5 mmol/l in 20% of the patients both in the rosiglitazone and the placebo groups. After 6 and 12 weeks of treatment with rosiglitazone this was 40 and 53%, respectively. These data imply, given the risk of pancreatitis and need of hypolipidaemic drugs when triglycerides exceed 5–10 mmol/l [48] that triglycerides need to be monitored closely in all future trials testing rosiglitazone efficacy in HIV lipodystrophy syndrome. The cause of the increase in serum triglycerides remains speculative as effects of rosiglitazone on very low-density lipoprotein (VLDL) kinetics are unknown even in HIV-negative individuals. Possibly, rosiglitazone mobilized triglycerides from the liver, but was unable to sufficiently enhance their clearance by adipose tissue.

Given the fear of hepatotoxicity because of the idiosyncratic, often fatal liver reactions induced by troglitazone [12], liver function tests were monitored frequently. Again contrary to expectations, liver function tests continuously improved in the rosiglitazone group, possibly as a consequence of the decrease in the % liver fat. Since rosiglitazone does not interact with cytochrome P450 3A4 substrates [35,36] and is not metabolized by this enzyme complex itself [49], we did not expect it to have interactions with antiretroviral agents. This indeed proved to be true as the protease inhibitor concentrations remained unchanged. Also, none of the patients lost virological control during the study. However, these safety data must be viewed in context of our exclusion criteria: patients with symptoms of heart failure, elevated transaminases (>3 times upper limit of normal) and those with marked hypertriglyceridaemia already at baseline [>10 mmol/l (885 mg/dl)] were not included in the study. In addition, none of the patients was a carrier of hepatitis B or hepatitis C, and all patients had a normal haemoglobin concentration at baseline.

In vitro, both protease inhibitors and nucleoside analogues can inhibit adipocyte differentiation [22,50–54]. The suggested mechanisms include both SREBP-1/PPARγ dependent [22,50] and independent mechanisms [52,53]. *In vitro*, this block in differentiation can be prevented by incubating preadipocytes with rosiglitazone [22]. The present data imply that this does not appear to happen *in vivo*. Since glitazones promote preadipocyte differentiation into mature adipocytes through the activation of PPARγ [14], decreased expression or dysfunction of PPARγ could account for the poor effect. This explanation is supported by very recent data showing both PPARγ mRNA and protein concentrations to be markedly reduced in subcutaneous fat tissue of patients with HIV lipodystrophy [21]. Another possibility is that the block in adipocyte differentiation could perhaps be prevented if patients were treated with glitazones before rather than after the development of lipodystrophy.

In conclusion, rosiglitazone had several unexpected effects in patients with HIV lipodystrophy syndrome. Most importantly, it had, contrary to HIV-negative subjects [15–19,31], during 6 months of treatment with a maximal dose, no effect on subcutaneous fat mass, fat distribution, leptin concentrations or body weight. In contrast to lack of clinically significant effects on adipose tissue mass or distribution, rosiglitazone appeared to have beneficial effects on the liver as judged from improvements in liver function tests and decreases in % liver fat and fasting serum insulin concentrations. Rosiglitazone induced marked hypertriglyceridaemia and hypercholesterolaemia. These data imply that rosiglitazone is unlikely to reverse HIV lipodystrophy syndrome with ongoing antiretroviral therapy and if used requires careful monitoring of especially serum triglycerides.

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