Effects of rosiglitazone on gene expression in subcutaneous adipose tissue in highly active antiretroviral therapy-associated lipodystrophy

Jussi Sutinen,1 Katja Kannisto,1 Elena Korsheninnikova,3 Rachel M. Fisher,4 Ewa Ehrenborg,4 Tuulikki Nyman, Antti Virkamäki,5 Tohru Funahashi,5 Yuji Matsuzawa,6 Hubert Vidal,7 Anders Hamsten,4 and Hannele Yki-Järvinen.1,4

1Divisions of Diabetes and Infectious Diseases, Department of Medicine, Helsinki University Central Hospital, FIN-00029 HUS, Helsinki; 2Inminera Research Institute, FIN-00250 Helsinki, Finland; 3Atherosclerosis Research Unit, Department of Medicine, King Gustaf V Research Institute, Karolinska Institutet, S-17176 Stockholm, Sweden; 4Department of Internal Medicine and Molecular Science, Osaka University Graduate School of Medicine, Osaka 565-0871, Japan; and 5Faculty de Medicina R Laennec, Institut National de la Santé et de la Recherche Medicale, Unité 449, F-69370 Lyon, France

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Sutinen, Jussi, Katja Kannisto, Elena Korsheninnikova, Rachel M. Fisher, Ewa Ehrenborg, Tuulikki Nyman, Antti Virkamäki, Tohru Funahashi, Yuji Matsuzawa, Hubert Vidal, Anders Hamsten, and Hannele Yki-Järvinen. Effects of rosiglitazone on gene expression in subcutaneous adipose tissue in highly active antiretroviral therapy-associated lipodystrophy. Am J Physiol Endocrinol Metab 286: E941–E949, 2004. First published January 28, 2004; 10.1152/ajpendo.00490.2003.—Highly active antiretroviral therapy (HAART) has improved the prognosis of human immunodeficiency virus (HIV)-infected patients but is associated with severe adverse events, such as lipodystrophy and insulin resistance. Rosiglitazone did not increase subcutaneous fat in patients with HAART-associated lipodystrophy (HAL) in a randomized, double-blind, placebo-controlled trial, although it attenuated insulin resistance and decreased liver fat content. The aim of this study was to examine effects of rosiglitazone on gene expression in subcutaneous adipose tissue in 30 patients with HAL. The mRNA concentrations in subcutaneous adipose tissue were measured using real-time PCR. Twenty-four-week treatment with rosiglitazone (8 mg/day) compared with placebo significantly increased the expression of adiponectin, peroxisome proliferator-activated receptor-γ (PPARγ), and PPARγ coactivator 1 and decreased IL-6 expression. Expression of other genes involved in lipogenesis, fatty acid metabolism, or glucose transport, such as acyl-CoA synthase, adipocyte lipid-binding protein, CD45, fatty acid transport protein-1 and -4, GLUT1, GLUT4, keratinocyte lipid-binding protein, lipoprotein lipase, PPARβ, and sterol regulatory element-binding protein-1c, remained unchanged. Rosiglitazone also significantly increased serum adiponectin concentration. The change in serum adiponectin concentration was inversely correlated with the change in fasting serum insulin concentration and liver fat content. In conclusion, rosiglitazone increased significant changes in gene expression in subcutaneous adipose tissue and ameliorated insulin resistance in patients with HAL. Increased expression of adiponectin might have mediated most of the favorable insulin-sensitizing effects of rosiglitazone in these patients.

human immunodeficiency virus; adiponectin; real-time polymerase chain reaction; liver fat

HUMAN IMMUNODEFICIENCY VIRUS (HIV)-associated mortality and morbidity have dramatically decreased in industrialized countries since the introduction of highly active antiretroviral therapy (HAART) in 1996 (41). HAART usually consists of two nuclease reverse transcriptase inhibitors (NRTI) combined with either a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor (NNRTI) (63). Because eradication of HIV is not possible with current antiretroviral agents, patients need to continue HAART permanently. Unfortunately, HAART is also associated with severe side effects, such as lipodystrophy and insulin resistance (57), which may lead to increased cardiovascular morbidity in the future (25). Approximately one-half of HAART-treated patients develop at least one lipodystrophy-related symptom after 12–18 mo of therapy (9). HAART-associated lipodystrophy (HAL) has therefore become by far the most common form of human lipodystrophy (22). Lipodystrophy, especially facial lipodystrophy, can be disfiguring and stigmatizing. Currently there is no pharmacological therapy for lipodystrophy.

Thiazolidinediones would theoretically seem ideal to treat HAART-associated insulin resistance and lipodystrophy, since they increase both insulin sensitivity and subcutaneous fat mass in patients with type 2 diabetes (34, 37). We have previously reported the results of the only controlled study on the use of rosiglitazone for the treatment of HAL (52). Rosiglitazone at 8 mg/day for 24 wk did not change subcutaneous or intra-abdominal fat mass measured using magnetic resonance imaging (MRI) and anthropometry but appeared to attenuate insulin resistance as judged from the decrease in fasting serum insulin concentration and liver fat content measured by spectroscopy.

Thiazolidinediones are ligands for nuclear peroxisome proliferator-activated receptor-γ (PPARγ), which is critical for adipocyte differentiation (50). Thiazolidinediones have been hypothesized to enhance insulin sensitivity via the “fatty acid steal phenomenon,” i.e., by retaining free fatty acids (FFAs) in adipose tissue and thereby decreasing FFA flux to the liver (34). This was suggested to be the mechanism in an uncontrolled study in patients with type 2 diabetes in which rosiglitazone decreased serum FFA, hepatic fat content, and insulin resistance and increased subcutaneous fat mass (34). However, the effects of rosiglitazone in patients with HAL, i.e., improvement in insulin sensitivity in the absence of changes in fat mass (52), suggest that the fatty acid steal phenomenon is not sufficient to explain the effects of rosiglitazone.

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Adiponectin has been suggested to be a mediator of the insulin-sensitizing effects of thiazolidinediones (13). Adi- ponectin is a protein secreted from adipocytes, the concentra- tion of which is decreased in type 2 diabetic patients (3). Treatment of normal subjects, subjects with glucose intoler- ance, and patients with type 2 diabetes with thiazolidinediones increases serum adiponectin concentrations (13, 27, 33, 61). In vitro and in vivo studies in mice suggest that adiponectin enhances hepatic insulin sensitivity (2, 12, 60).

On the basis of animal studies, however, it is not certain whether adipose tissue is required for thiazolidinediones to exert their insulin-sensitizing effects. Treatment of fatless A-ZIP/F-1 mice with rosiglitazone or troglitazone did not improve insulin resistance (11), whereas treatment of fatless aP2/DTA mice with troglitazone did (7). Currently there are no data on in vivo treatment effects of thiazolidinediones on gene expression in human adipose tissue in patients with type 2 diabetes or any other human group.

The present study was undertaken to examine the effects of a 24-wk treatment with rosiglitazone compared with placebo on the expression of adiponectin and other genes in subcuta- neous fat and whether these effects are associated with changes in features of insulin resistance, including liver fat content.

METHODS

Study design. The study protocol, which was investigator initiated and not supported by the manufacturer of rosiglitazone, consisted of a 24-wk randomized, double-blind treatment period with either rosiglitazone (8 mg once a day) or an identical-looking placebo in a parallel fashion. The primary clinical results of the study have been reported previously (52).

All patients were studied at 8:00 AM after an overnight fast. A needle aspiration biopsy of subcutaneous fat was taken under local anesthesia at baseline and after 24 wk. Fat biopsies were taken from abdominal subcutaneous fat from the same site in all participants by a single investigator. The fat sample was immediately frozen and stored in liquid nitrogen until analysis.

Intra-abdominal and subcutaneous fat depots were quantified using MRI and liver fat by MRI proton spectroscopy at baseline and after 24 wk of treatment. A fasting blood sample was taken at baseline and at weeks 6, 12, 18, and 24 for measurement of serum concentrations of glucose, insulin, triglycerides, total and HDL cholesterol, C-reactive protein (CRP), and FFA.

Study subjects. The patients were recruited from the HIV outpatient clinic of the Helsinki University Central Hospital. They had to have been treated with HAART for ≥18 mo before enrollment and to have developed lipodystrophy while receiving HAART (HAART + LD” group). Lipodystrophy was defined 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 con- firmed by a single investigator (J. Sutinen) before inclusion in the study. Exclusion criteria included serum transaminase concentrations higher than three times the upper limit of normal, heart failure, severe hypertriglycerideremia (serum triglycerides >10 mmol/l), diabetes, and pregnancy. Baseline characteristics of the HAART + LD” group were compared with a group of HIV-positive, HAART-treated pa- tients who had not developed lipodystrophy (HAART + LD”) during antiretroviral therapy. Results of the cross-sectional study on the gene expression in subcutaneous fat in HAART-treated patients with and without lipodystrophy have been described earlier (29, 53).

The purpose, nature, and potential risks of the study were explained to the patients before their written informed consent was obtained. The protocol was approved by the ethics committee of the Helsinki University Central Hospital.

Total RNA and cDNA preparation. Frozen fat tissue (50–150 mg) was homogenized in 2 ml of RNA STAT-60 (Tel-Test, Friendswood, TX) and total RNA isolated according to the manufacturer’s instruc- tions. After DNase treatment (RNase-free DNase set; Qiagen, Hilden, Germany), RNA was purified using the RNeasy Mini Kit (Qiagen). RNA concentrations were measured using the RiboGreen fluorescent nucleic acid stain (RNA quantification kit; Molecular Probes, Eugene, OR). The quality of RNA was checked by agarose gel electrophoresis. Average yields of total RNA were 3 ± 1 µg/100 mg adipose tissue wet wt and did not differ between the groups. Isolated RNA was stored at −80°C until quantification of the target mRNAs. A total of 0.1 µg of RNA was transcribed into cDNA by use of MMLV reverse transcriptase (Life Technologies, Paisley, UK) and oligo(dT)12-18 primer.

Quantification of β-actin, PPARγ, sterol regulatory element-bind- ing protein-1c, adiponectin, and lipoprotein lipase gene expression. Quantification of the mRNAs was performed by real-time PCR using LightCycler technology (Roche Diagnostics, Mannheim, Germany). Two microliters of 1:10 diluted cDNA were brought to a final volume of 20 µl, which contained 3 mM MgCl2, 2 µl of LightCycler-FastStart DNA SYBR Green 1 Mix (Roche Diagnostics), and 0.5 µM of primers. After initial activation of the DNA polymerase at 95°C for 10 min, the amplification conditions were as follows: 40 cycles consisting of denaturation at 95°C for 15 s, annealing for 5 s at 57°C (β-actin), 56°C (PPARγ), 58°C (adiponectin), 58°C [lipoprotein lipase (LPL)], or for 10 s at 60°C [sterol regulatory element-binding protein-1c (SREBP-1c)], and extension at 72°C. The extension times (seconds) were calculated from the amplification size (base pairs/25). Fluorescent data were acquired at the end of each extension phase. After amplification, a melting curve analysis from 65 to 95°C with a heating rate of 0.1°C/s with a continuous fluorescence acquisition was made.

The primers for PPARγ, SREBP-1c, adiponectin, LPL, and β-actin are listed in Table 1. A standard curve for PPARγ was created using purified cloned plasmid cDNA (QIaquick PCR purification kit; Qia- gen). For human β-actin, SREBP-1c, adiponectin, and LPL expres- sion, standard curves were created from a specific PCR product. To account for differences in RNA loading, PPARγ, SREBP-1c, adi- ponectin, and LPL mRNA concentrations were expressed relative to β-actin. The mRNA concentrations of human β-actin were not dif- ferent between the HAART + LD” and HAART + LD” groups or between rosiglitazone and placebo groups either at baseline or after 24 wk of treatment (data not shown).

Quantification of human β2-microglobulin, GLUT1, GLUT4, PPARγ coactivator-1, PPARβ, adipocyte lipid-binding protein, kera- tinoocyte lipid-binding protein, fatty acid transport protein-1 and -4, acyl-CoA synthase, CD45, and IL-6 gene expression. TaqMan real-time semiquantitative PCR was performed according to the manufac- turer’s protocol by use of the ABI PRISM 7000 Sequence Detection System instrument and software (PE Applied Biosystem, Foster City, CA). Primer and probe sets were designed using the manufacturer’s software and sequences available in GenBank (Table 1). IL-6 was measured using Pre-Developed TaqMan Assay Reagents (PE Applied Biosystem). The GLUT4 primer set has been published (43). Differences in loading of RNA were adjusted for by expression results relative to β2-microglobulin. mRNA concentrations of β2-micro- globulin were not different between the HAART + LD” and HAART + LD” groups or between rosiglitazone and placebo groups either at baseline or after 24 wk of treatment (data not shown).

Intra-abdominal and subcutaneous fat (MRI) and liver fat (MRI proton spectroscopy). A series of T1-weighted transaxial scans for the determination of intra-abdominal and subcutaneous fat were acquired from a region extending from 8 cm above to 8 cm below the 4th and 5th lumbar interspace (16 slices, field of view 375 × 500 mm2, slice thickness 10 mm, breath-hold repetition time 138.9 ms, echo time 4.1 ms) and analyzed as described in detail (54). The reproducibility of
repeated measurements of subcutaneous and intra-abdominal fat volumes as determined on two separate occasions in our laboratory is 3 and 5% (n = 10) (51). For measurement of liver fat, localized single-voxel (2 x 2 x 2 cm³) 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 and signal intensities quantified using an analysis program as previously described (54). Spectroscopic single-voxel (2 cm³) 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 and signal intensities quantified using an analysis program as previously described (54).

Other measurements. Serum adiponectin concentrations were measured using a commercial enzyme-linked immunoassay (Human Adiponectin ELISA Kit; B-Bridge International, San Jose, CA). Serum IL-6 concentration was measured using an enzyme-linked immunoassay (Quantikine; R&D Systems, Minneapolis, MN). Serum CRP was analyzed using a commercial kit (Ultralight sensitive CRP Kit; Orion Diagnostica, Espoo, Finland). Serum FFAs were measured by a fluorometric assay (36). Serum free insulin concentrations were determined by radioimmunoassay (Phadeseph Insulin RIA; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) after precipitation with polyethylene glycol. Plasma glucose concentrations were measured using a hexokinase method. Serum cholesterol and triglyceride concentrations were determined with respective enzymic kits from Roche Diagnostics by use of an autoanalyzer (Roche Diagnostics Hitachi 917; Hitachi, Tokyo, Japan). Serum alanine aminotransferase (ALT) activities were determined according to recommendations of the European Committee for Clinical Laboratory Standards by use of the Roche Diagnostics Hitachi 917 autoanalyzer.

Statistical analysis. The unpaired t-test was used to compare differences between the groups. Correlations were calculated using Spearman’s rank correlation coefficient. Categorical variables were compared using Fisher’s exact test. 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. All data are given as means ± SE. Sample size was calculated on the basis of the effects of troglitazone on abdominal subcutaneous fat measured by MRI in patients with non-HIV lipodystrophy (1). In that study, subcutaneous fat in the abdominal region increased by 3 ml after 6 mo 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 when it is assumed that the common standard deviation (= ¼ liver fat). The reproducibility of repeated measurements of liver fat in nondiabetic subjects studied on two occasions in our laboratory (n = 10) is 11% (51).

Table 1. Primers and probes used for mRNA analyses

<table>
<thead>
<tr>
<th>mRNA GenBank No.</th>
<th>Sense Primers</th>
<th>Antisense Primers</th>
<th>Probe</th>
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<tbody>
<tr>
<td>PPARγ</td>
<td>5'-CTCATATCCGGAGGACC 3'</td>
<td>5'-TGCCGAATCTCGCTATCC 3'</td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td>5'-CAGACTGTCGACATCATCAG 3'</td>
<td>5'-GCTAACCTGACTGCTTACC 3'</td>
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<tr>
<td>SREBP-1c</td>
<td>5'-GCGAGAGCATTAGTTGCAA 3'</td>
<td>5'-GTTCCTTTCTGATACAGGCCC 3'</td>
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</tr>
<tr>
<td>Adiponectin</td>
<td>5'-CAAGAGATGGACCTGGTTGAC 3'</td>
<td>5'-TTTGACCGATGCTGCTTACT 3'</td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>5'-GTCGAAGCTTTGGAATCCCAG 3'</td>
<td>5'-TAGGGCATCGAAGAAGATTC 3'</td>
<td></td>
</tr>
<tr>
<td>β2-Microglobulin</td>
<td>5'-GGCTGCGGATTTGACGAT 3'</td>
<td>5'-TTATAGCTGTCTGATCCCCATAT 3'</td>
<td>5'-TGACTTTGTCAGAACAGCCA</td>
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<tr>
<td>GLUT1</td>
<td>5'-CTTGTGGAAGGCTTCAAA 3'</td>
<td>5'-TCATAAGCAACAGGAGGATCTT 3'</td>
<td>5'-CAGCTGTCGAAAGAC</td>
</tr>
<tr>
<td>GLUT4</td>
<td>5'-GCTACCTCGATCATCAGGATCTC 3'</td>
<td>5'-CGCAAAAGATCGGCCCA 3'</td>
<td>5'-CTGCCGAAAGAGTCGAAAAACCT</td>
</tr>
<tr>
<td>PGC-1</td>
<td>5'-AGACACGGGAGGCTTCTTGG 3'</td>
<td>5'-AACGTGCTGACCTGGTTGA 3'</td>
<td>5'-AAGCTCAGACATGGAGAAGC</td>
</tr>
<tr>
<td>PPARα</td>
<td>5'-CAGACGCGGGGCTTCTTGG 3'</td>
<td>5'-CCTTCTCTGCTGCGCCAA 3'</td>
<td>5'-ATCAGACGATCCGAGAC</td>
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<td>CD34</td>
<td>5'-TCTCGGAATTTGCTTCGGC 3'</td>
<td>5'-GGAGACTGCTGCTGGTGTA 3'</td>
<td>5'-CTGGACACAGAACTTAT</td>
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<td>ALBP</td>
<td>5'-TGATAATCGTGCTGGAATGC 3'</td>
<td>5'-CCCTTCGGTTATCGTCTCCA 3'</td>
<td>5'-TCTGAAAGGCGTCATCTTGACAGA</td>
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<tr>
<td>KLBP</td>
<td>5'-GGAGAGAATCTGCAACAGAAGAAGAAGA 3'</td>
<td>5'-GGATACAGGATGCACTTTGATG 3'</td>
<td>5'-CAGACTGCGGACTAATTGGCCACCTTTGAATT</td>
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<tr>
<td>FATP-1</td>
<td>5'-AGATGGCCGCTACTCTCG 3'</td>
<td>5'-GCTAGGGCCGCTTCTCTGGA 3'</td>
<td>5'-CCACCGGGGCGACCTTTCTTC</td>
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<tr>
<td>FATP-4</td>
<td>5'-CAAGACCCTACGGGGG 3'</td>
<td>5'-GGACCATTTTGGCTCCATCC 3'</td>
<td>5'-TCTTTGCGGGGCTGTCCTCCT</td>
</tr>
<tr>
<td>ACS</td>
<td>5'-CAAGAGGCGCTTGAATTTGAAGGT 3'</td>
<td>5'-CCATTTGGTAACATTTCGAGAGGCCC 3'</td>
<td></td>
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PPAR, peroxisome proliferator-activated receptor; SREBP-1c, sterol regulatory element binding protein-1c; LPL, lipoprotein lipase; PGC-1, PPARγ coactivator 1; ALBP and KLBP, adipocyte and keratinocyte lipid-binding proteins; FATP, fatty acid transport protein; ACS, acyl-CoA synthase.
RESULTS

Physical and biochemical characteristics of the study groups. As previously reported, the HAART + LD + group had approximately one-half the amount of subcutaneous, twice the amount of intra-abdominal, and three times the amount of liver fat compared with the HAART + LD − group (53). The HAART + LD + group had several laboratory abnormalities indicative of insulin resistance. The results remained unchanged if women were excluded from the analysis (data not shown). Duration of antiretroviral combination therapy was comparable in both groups [4.3 ± 0.2 vs. 3.8 ± 0.4 yr, HAART + LD + vs. HAART + LD −, not significant (NS)].

All patients in both the HAART + LD + and HAART + LD − groups were currently receiving NRTI, 33% in the HAART + LD + and 38% in the HAART + LD − (NS) received NNRTI, and 73 and 62%, respectively (NS), used protease inhibitors.

Effects of rosiglitazone vs. placebo on body composition and features of insulin resistance. All patients in both the rosiglitazone and the placebo groups were currently receiving NRTI, 20% in the placebo and 47% in the rosiglitazone group (NS) were receiving NNRTI, and 93 and 67% of the patients (NS), respectively, were currently receiving a protease inhibitor-containing regimen (52). Of the NRTI class of antiretroviral agents, lamivudine was used by 14 patients in the placebo and 9 in the rosiglitazone group, stavudine by 11 and 10, zidovudine by 3 and 4, didanosine by 1 and 6, and abacavir by 1 and 3, respectively; one patient in the rosiglitazone group used zalcitabine. Of the NNRTIs, efavirenz was used by 3 patients in both groups and nevirapine by 4 patients in the rosiglitazone group. The most common protease inhibitor used was indinavir, used by 5 patients in the placebo and 4 in the rosiglitazone group, nelfinavir was used by 4 and 2, lopinavir by 2 and 2, amprenavir by 1 and 2, respectively; ritonavir was used by 2 patients in the placebo group. None of the patients changed any of the antiretroviral agents during the 24-wk study period.

At baseline, the rosiglitazone and placebo groups were matched for the laboratory and body composition characteristics. As previously described, after 24 wk of treatment, body weight and subcutaneous and intra-abdominal fat remained unchanged in both groups (52). Rosiglitazone did not change body composition even if baseline subcutaneous fat, which was slightly but not significantly higher in the placebo group, was included as a covariate. Liver fat content and serum fasting insulin concentration decreased significantly in the rosiglitazone group compared with the placebo group. Serum ALT concentrations decreased significantly in the rosiglitazone group but did not change in the placebo group. Serum triglyceride (from 3.5 ± 0.5 to 6.5 ± 2.0 mmol/l, P < 0.05) and total cholesterol concentrations (from 6.0 ± 0.4 to 7.8 ± 0.7 mmol/l, P < 0.01) increased markedly in the rosiglitazone group during the first 12 wk of treatment but remained unchanged in the placebo group [serum triglycerides from 3.2 ± 0.5 to 3.3 ± 0.5 mmol/l (NS) and total cholesterol from 5.9 ± 0.2 to 6.2 ± 0.2 mmol/l (NS)]. In the rosiglitazone group, serum CRP (from 1.5 ± 0.3 to 1.0 ± 0.3 mg/l, P < 0.05) and FFA (from 550 ± 35 to 422 ± 36 μmol/l, P < 0.05) concentrations, and total white blood cell count (from 5.6 ± 0.3 to 5.0 ± 0.3 × 10^9/l, P < 0.05) decreased significantly, but they all remained unchanged in the placebo group [1.6 ± 0.4 vs. 1.6 ± 0.3 (NS), 572 ± 64 vs. 516 ± 55 (NS), 5.9 ± 0.6 vs. 6.1 ± 0.6 (NS), respectively]. Serum IL-6 concentration did not change in either group [2.0 ± 0.3 vs. 1.8 ± 0.3 pg/ml (NS) in the rosiglitazone group and 2.4 ± 0.4 vs. 2.1 ± 0.4 (NS) in the placebo group]. Serum adiponectin concentration increased significantly in the rosiglitazone group (from 3.6 ± 0.5 to 6.2 ± 1.1 μg/ml, P < 0.05) but remained unchanged in the placebo group [3.1 ± 0.6 vs. 3.6 ± 0.6 μg/ml (NS)]. The results regarding the effects of rosiglitazone vs. placebo on body composition and laboratory parameters remained unchanged, even if women were excluded from the analysis (data not shown).

Adipose tissue gene expression at baseline. The baseline characteristics of the gene expression in HAART-treated patients with and without lipodystrophy have been previously described (29, 53). Briefly, the expression of PPARγ, SREBP-1c, PPARδ, PGC-1, adiponectin, GLUT4, LPL, and ACS were significantly decreased, whereas the expression of IL-6 and CD45 (a surface marker expressed on leukocytes) were increased in the HAART + LD + group compared with the HAART + LD − group. Expression of fatty acid transport (FATP-1, FATP-4) and binding proteins (KLBP, ALBP) and GLUT1 were comparable between the groups. There were no significant differences between the mRNA concentrations for any of the genes at baseline between the placebo and rosiglitazone groups (Table 2).

Effect of rosiglitazone on adipose tissue gene expression. Rosiglitazone compared with placebo induced a significant increase in the expression of adiponectin and PGC-1 and a significant decrease in the expression of IL-6. In addition, the expression of PPARγ was increased in the rosiglitazone group compared with the placebo group. Expression of other genes involved in lipogenesis, fatty acid metabolism, or glucose transport remained unchanged in both groups (Table 2).

Interrelationships between gene expression and features of insulin resistance. Among lipodystrophic patients at baseline, the adiponectin mRNA concentration in subcutaneous adipose tissue had an inverse correlation with the percent liver fat (r = −0.39, P < 0.05) and positively correlated with SREBP-1c (r = 0.69, P < 0.0001) and LPL (r = 0.95, P < 0.0001) mRNA concentrations in subcutaneous adipose tissue. Similar correlations were also found after 24 wk between adiponectin expression and the percent liver fat (r = −0.34, P = 0.07), and between adiponectin and SREBP1-c (r = 0.79, P < 0.0001) and LPL (r = 0.94, P < 0.0001) expression. Serum adiponectin concentration at 24 wk, but not at baseline, correlated significantly with the percent liver fat (r = −0.36, P < 0.05) and the fasting serum insulin concentration (r = −0.38, P < 0.05).

In all patients with HAL, the change in adiponectin mRNA concentration in subcutaneous adipose tissue was closely correlated with the corresponding changes in the mRNA concentrations of LPL (r = 0.89, P < 0.0001) and SREBP1-c (r = 0.47, P < 0.05). The change in serum adiponectin concentration was inversely correlated with the change in fasting serum insulin concentration (r = −0.49, P < 0.01), the percent liver fat (r = −0.45, P < 0.05; Fig. 1) and serum ALT (r = −0.38, P < 0.05). The change in fasting serum insulin concentration and the change in percent liver fat correlated significantly (r = 0.45, P < 0.05).
The change in the mRNA concentration of IL-6 in adipose tissue correlated positively with the changes in fasting serum FFA ($r = 0.52, P < 0.01$) and CRP ($r = 0.40, P < 0.05$) concentrations. The change in the serum concentration of CRP also correlated inversely with the changes in mRNA concentrations of SREBP-1c ($r = -0.45, P < 0.05$) and LPL ($r = -0.45, P < 0.05$) in adipose tissue.

**DISCUSSION**

In the present study, rosiglitazone induced changes in gene expression in subcutaneous adipose tissue and decreased both the fasting serum insulin concentration and the liver fat content without changing the sizes of subcutaneous or intra-abdominal fat depots in patients with HAART-associated lipodystrophy. Rosiglitazone significantly increased the expression of adiponectin and PGC-1, increased circulating adiponectin concentration, and decreased the expression of IL-6. Rosiglitazone also increased PPARγ expression, but the increase reached statistical significance only compared with the decreased expression in the placebo group, suggesting that lipoatrophic tissue may be more refractory to this effect of rosiglitazone than healthy adipose tissue. The increase in serum adiponectin by rosiglitazone correlated significantly with the decrease in serum insulin concentration and liver fat content. These data demonstrate that rosiglitazone can have insulin-sensitizing effects without increasing the mass of subcutaneous adipose tissue.

Most, but not all, patients were receiving a protease inhibitor-containing HAART regimen. The number of patients studied was, however, too small to allow separate analysis of the response of patients using and not using protease inhibitors (5 in the rosiglitazone group and 1 in the placebo group). Such analysis would have been of interest given the different metabolic effects of the different classes of drugs (8, 21, 45, 55, 56).

A 23% decrease in FFA concentration in the rosiglitazone group in the present study is in keeping with previous findings of 20–30% decreases in FFA concentration in patients with type 2 diabetes treated with rosiglitazone (37, 42). The decrease in serum FFA can be due to decreased production or increased clearance. In patients with type 2 diabetes, rosiglitazone seems to lower fasting FFAs by decreasing lipolysis (37). In patients with HAART-associated lipodystrophy, rates of lipolysis have been suggested to be increased (24). Because the
sizes of adipose tissue depots remained unchanged, it is not possible to explain the decrease in FFA by a decrease in lipolysis in adipose tissue. FFA originating from intravascular lipolysis is unlikely to be decreased, since serum triglycerides increased and expression of LPL remained unchanged. Other possibilities, which cannot be resolved on the basis of the present study, include increased FFA utilization in skeletal muscle as has been found in the rat (62). In mice, thiazolidinediones induce expression of fatty acid transport proteins (FATP-1, CD36), intracellular adipocyte fatty acid-binding protein (aP2) and acyl-CoA synthetase in white adipose tissue (4). Such changes could increase the clearance of FFA. Human data are limited regarding effects of rosiglitazone on expression of genes involved in FFA utilization. In isolated human adipocytes, rosiglitazone has been reported to have no effect on expression of FATP-1 (44). The lack of induction of these genes and of other genes involved in lipogenesis (SREBP-1c, FATP-1, CD36), intracellular adipocyte fatty acid-binding protein (aP2) and acyl-CoA synthetase in white adipose tissue (4). Such changes could increase the clearance of FFA. Human data are limited regarding effects of rosiglitazone on expression of genes involved in FFA utilization. In isolated human adipocytes, rosiglitazone has been reported to have no effect on expression of FATP-1 (44). The lack of induction of these genes and of other genes involved in lipogenesis (SREBP-1c, FATP-1, CD36), intracellular adipocyte fatty acid-binding protein (aP2) and acyl-CoA synthetase in white adipose tissue (4). Such changes could increase the clearance of FFA. Human data are limited regarding effects of rosiglitazone on expression of genes involved in FFA utilization. In isolated human adipocytes, rosiglitazone has been reported to have no effect on expression of FATP-1 (44). The lack of induction of these genes and of other genes involved in lipogenesis (SREBP-1c, FATP-1, CD36), intracellular adipocyte fatty acid-binding protein (aP2) and acyl-CoA synthetase in white adipose tissue (4).

Contrary to previous studies in which rosiglitazone has had modest effects on blood lipids (19, 31, 37), serum triglycerides increased markedly in the present study. Blood lipids must therefore be monitored carefully in all future trials using rosiglitazone in patients with HAART-associated lipodystrophy. When indicated, hypertriglyceridemia can be treated with fibrates (15). If statins are used, their potentially hazardous interactions with protease inhibitors must be taken into account (15). As discussed above, rosiglitazone did not change expression of LPL as has been described in rat adipose tissue (46) and in human subcutaneous adipose tissue in vitro (35). The lack of induction of LPL expression could have contributed to the increase in serum triglycerides but cannot explain why triglycerides increased in the first place. Possibly, rosiglitazone mobilized triglycerides from the liver, the fat content of which significantly decreased.

Serum fasting insulin concentrations decreased in the rosiglitazone compared with the placebo group, in keeping with previous studies on troglitazone in non diabetic (polycystic ovary syndrome) (16, 17) and rosiglitazone in type 2 diabetic patients (31, 34). The decrease in insulin was observed in the absence of change in fasting glucose, which is indicative of enhanced insulin sensitivity. There are conflicting in vitro data regarding effects of thiazolidinediones on glucose transport proteins (39, 44, 58). The mRNA concentrations of GLUT1 and GLUT4 in adipose tissue remained unchanged in the present study and thus cannot explain the improved insulin sensitivity. These data do not exclude the possibility that rosiglitazone increased GLUT4 expression or translocation in muscle (18, 65). On the other hand, the major physiological function of fasting insulin is to control hepatic glucose production (64). Changes in liver fat have been closely correlated with changes in the ability of insulin to suppress hepatic glucose production (48). Consistent with these data and the idea that the decrease in serum fasting insulin was due, at least in part, to enhanced hepatic insulin sensitivity, the decrease in serum fasting insulin and liver fat content were significantly correlated in the present study.

Thiazolidinediones have anti-inflammatory effects in both animals and humans. In mice, troglitazone decreases tumor necrosis factor-α (TNF-α) and IL-6 expression in white adipose tissue and in the liver (49). In humans, treatment of patients with type 2 diabetes with rosiglitazone decreases serum CRP and matrix metalloproteinase-9 concentrations and total white blood cell count but does not seem to change serum IL-6 concentration (26). Similar effects of rosiglitazone, i.e., a decrease in serum CRP concentration and white blood cell count together with an unchanged serum IL-6 concentration, were found in the present study. IL-6 is a key regulator of CRP production in hepatocytes (47). In the present study, the decrease in CRP concentration cannot be explained by the unchanged serum IL-6 concentration. In mice, thiazolidinediones downregulate proinflammatory cytokines in Kupffer cells in the liver (6). Whether thiazolidinediones have similar local anti-inflammatory properties in the liver in humans is not known. In the present study, despite having no effect on the total circulating IL-6 concentration, rosiglitazone markedly decreased the expression of IL-6 in subcutaneous adipose tissue (Table 2). Very recently, expression of CRP has been demonstrated also in human adipose tissue (40). If IL-6 regulates the expression of CRP in adipose tissue, it is possible that the decreased IL-6 expression may have led to decreased local CRP expression in subcutaneous adipose tissue.

Rigoslitazone significantly increased adiponectin expression in subcutaneous adipose tissue (Table 2) and almost doubled its circulating concentration. The change in serum adiponectin concentration correlated inversely with the change in serum fasting insulin concentration and liver fat content (Fig. 1). Expression of adiponectin in subcutaneous adipose tissue in humans has not previously been reported during thiazolidinedione therapy. Incubation of isolated human adipocytes from omental but not from subcutaneous fat depots with rosiglitazone increases the secretion of adiponectin (38). The increase in circulating adiponectin concentration after rosiglitazone treatment is in keeping with recent data in patients with type 2 diabetes (61), glucose intolerance (33), and normal subjects (13).

The molecular mechanism by which PPARγ agonists enhance adiponectin expression is not fully understood but seems to include direct binding of PPARγ/1XR heterodimers to PPAR-responsive elements in the human adiponectin promoter (28). The decrease in IL-6 expression may also have contributed to the increase in adiponectin expression, since IL-6 has been shown to inhibit adiponectin expression in 3T3-L1 adipocytes (23). Finally, since the almost twofold increase in serum concentration of adiponectin was greater than the 16% increase in its expression in subcutaneous fat, it is possible that clearance of adiponectin changed during therapy.

Infusion of adiponectin decreases liver and skeletal muscle fat content in obese and lipodystrophic mice (60). Adiponectin enhances insulin sensitivity in these tissues by activating a 5'-AMP-activated protein kinase (59). This is accompanied by a decrease in the expression of enzymes regulating gluconeogenesis in the liver and an increase in enzymes of fatty acid oxidation in myocytes (59). In isolated hepatocytes, adiponectin increases the ability of subphysiological concentrations of insulin to suppress glucose production (2). In a mouse model of lipodystrophy, infusion of adiponectin increased β-oxidation in muscle and decreased liver and muscle triglycerides as well as serum FFA and triglycerides (20). Adiponectin also has anti-inflammatory properties. Adiponectin knockout mice express high levels of TNF-α in adipose tissue, which can be reversed by viral mediated adiponectin expression in this mouse model.
(32). In the present study, one can hypothesize that adiponectin may have mediated most of the favorable effects (the decrease in liver fat content, serum fasting insulin and FFAs concentrations, and the decrease in inflammatory markers) observed during rosiglitazone treatment. However, we cannot exclude a contribution of additional factors to the insulin-sensitizing effects of rosiglitazone, such as decreased expression of 11β-hydroxysteroid dehydrogenase type 1 (5).

Adipose tissue does not consist of adipocytes only but also of other cells, such as endothelial cells and macrophages (30). Consequently, RNA isolated from adipose tissue samples does not originate exclusively from adipocytes. Therefore, it remains unresolved whether the observed change, e.g., the decrease in the expression of IL-6, which is expressed both in adipocytes (14) and macrophages (10), actually is due to decreased expression in one or both of these cell lines within adipose tissue.

In conclusion, rosiglitazone induced significant changes in gene expression in subcutaneous adipose tissue in the absence of changes in subcutaneous or intra-abdominal adipose tissue mass in patients with HAART-associated lipodystrophy. The expression of adiponectin in subcutaneous adipose tissue and its serum concentration increased significantly and might have mediated most of the favorable effects of rosiglitazone in these patients.

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