Increased fat accumulation in the liver in HIV-infected patients with antiretroviral therapy-associated lipodystrophy

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Objective: To determine liver fat content in patients with highly active antiretroviral therapy (HAART)-associated lipodystrophy.

Background: Lipodystrophy in several animal models is associated with fat accumulation in insulin-sensitive tissues, such as the liver. This causes hyperinsulinaemia, dyslipidaemia and other features of insulin resistance.

Design: A cross-sectional study.

Subjects and methods: Three age- and weight-matched groups were compared: 25 HIV-positive men with HAART-associated lipodystrophy (HAART+LD+), nine HIV-positive men receiving HAART, but without lipodystrophy (HAART+LD−), and 35 HIV-negative healthy men (HIV−). Liver fat content was measured using proton spectroscopy. Intra-abdominal and subcutaneous fat were determined using magnetic resonance imaging.

Results: Liver fat content was significantly higher in the HAART+LD+ (8 ± 10%) than the HIV− (5 ± 7%; $P < 0.05$) or the HAART+LD− (3 ± 5%; $P < 0.01$) group. Liver fat content correlated with serum fasting insulin in the HAART+LD+ ($r = 0.47$; $P < 0.05$) and HIV− groups ($r = 0.65$; $P < 0.001$), but not with the amount of intra-abdominal fat. Within the HAART+LD+ group, serum insulin did not correlate with the amount of intra-abdominal fat. The HAART+LD+ group had a lower serum leptin concentration when compared to the two other groups. Features of insulin resistance, including hepatic fat accumulation, were not found in HAART+LD− group.

Conclusions: The severity of the insulin resistance syndrome in patients with HAART-associated lipodystrophy is related to the extent of fat accumulation in the liver rather than in the intra-abdominal region. Fat accumulation in the liver may therefore play a causative role in the development of insulin resistance in these patients.

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Keywords: liver fat, antiretroviral therapy, lipodystrophy, proton spectroscopy, insulin resistance, intra-abdominal fat, leptin

Introduction

Highly active antiretroviral therapy (HAART) has dramatically reduced morbidity and mortality of HIV-infected patients [1,2]. However, adverse metabolic effects associated with HAART might compromise improved prognosis by increasing the risk for cardiovascular disease [3–5]. The spectrum of the HAART-

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associated metabolic adverse events includes several features typical of insulin resistance such as hyperinsulinaemia, an increased amount of intra-abdominal fat, hypertriglyceridaemia and hypertension [6–11]. On the other hand, in contrast with typical insulin resistant individuals, who have either normal or increased amount of subcutaneous fat, patients with HAART-associated metabolic abnormalities often have subcutaneous lipoatrophy [12–14].

In mouse models of lipodystrophy, lack of subcutaneous fat is accompanied by fat deposition and insulin resistance in key insulin target tissues, i.e., the liver and skeletal muscle [15–19]. Retransplantation of fat subcutaneously reverses whole body insulin resistance and all insulin-signaling defects in insulin target tissues [20]. Consistent with these data in mice, the features of insulin resistance increase in severity with the extent of fat loss in HIV-negative lipodystrophic patients [21]. In non-diabetic subjects, fat accumulation in the liver and skeletal muscles has been shown to be much more closely correlated with whole body insulin resistance than with any measure of obesity, including the amount of visceral fat [22–25]. Taken together, these data imply that accumulation of fat in insulin sensitive tissues may cause insulin resistance. Whether patients with HAART-associated lipodystrophy have increased amount of fat in the liver is unknown. The possibility that HAART causes accumulation of fat in the liver is supported by studies in mice, in which ritonavir caused hepatic steatosis [26].

In the present study, we determined whether patients with HAART-associated lipodystrophy have an increased amount of fat in the liver when compared to healthy weight-matched subjects or to HIV-positive patients who have not developed lipodystrophy during antiretroviral therapy. The latter group was studied to determine whether features of insulin resistance can be observed in patients using HAART in the absence of lipodystrophy. For this purpose, we studied a total of 34 HIV-positive men receiving HAART and 35 healthy men, who were characterized with respect to various features of insulin resistance. Liver fat was quantified non-invasively using proton spectroscopy, and the volumes of intra-abdominal and subcutaneous adipose tissue with magnetic resonance imaging (MRI).

Methods

Subjects and study design

Thirty-four HIV-positive men receiving antiretroviral therapy were recruited from the HIV-outpatient clinic of the Helsinki University Central Hospital. The men were subgrouped according to the presence or absence of lipodystrophy. Lipodystrophy was defined as self-reported symptoms of subcutaneous fat loss with/without increased girth or buffalo hump. Patients were included in the lipodystrophy group (HAART+LD+, n = 25) after the investigators confirmed the self-reported findings. HIV-positive patients without lipodystrophy were receiving HAART, but had no symptoms or signs of lipodystrophy (HAART+LD−, n = 9). The HIV-negative healthy control group (HIV−) consisted of 35 age- and weight-matched men who were recruited for the study from occupational health services in Helsinki (Table 1). These men were healthy as judged by history and physical examination, and did not use any regular medication. None of the subjects in the HAART+LD+, the HAART+LD− or the HIV− group had serological evidence of hepatitis B or C, autoimmune hepatitis, clinical signs or symptoms of inborn errors of metabolism, or a history of use of toxins (other than alcohol) or drugs associated with liver steatosis [27]. In each subject, liver fat content was determined using proton spectroscopy and intra-abdominal and subcutaneous fat using MRI. In addition, various other measurements of body composition were performed (*vide infra*), and a blood sample was withdrawn after an overnight fast for biochemical measurements as detailed below.

HIV-infection and antiretroviral-related characteristics of the HIV-positive subjects are shown in Table 2. All HIV-positive patients were receiving a stable HAART regimen with no changes in antiretroviral medication

Table 1. Characteristics of the study groups (mean ± SD).

	HAART+LD+ (n = 25)	HAART+LD− (n = 9)	HIV− (n = 35)
Age (years)	44 ± 10	40 ± 8	42 ± 9
Height (cm)	176 ± 7	178 ± 6	179 ± 7
Body weight (kg)	75 ± 12	73 ± 13	76 ± 8
Body mass index (kg/m^2)	23.9 ± 3.0	23.3 ± 4.2	23.7 ± 1.9
Waist (cm)	91 + 10	87 + 12	89 + 6
Waist : hip ratio	1.00 ± 0.07	0.93 ± 0.09**	0.94 ± 0.05*
Alcohol consumption (g/week)	107 ± 132	153 ± 168	78 ± 65

HAART+LD+, HIV-positive patients with HAART-associated lipodystrophy; HAART+LD−, HIV-positive patients using HAART but without lipodystrophy; HIV−, HIV-negative normal subjects.* $P < 0.01$ between HAART+LD+ and HIV−. ** $P < 0.05$ between HAART+LD+ and HAART+LD−.

Table 2. HIV-related characteristics of the patients (mean ± SD).

Variable	HAART+LD+	HAART+LD−
Number of patients	25	9
Time since diagnosis of HIV (months)	100 ± 8	103 ± 23
Currently using PI (%)	76%	56%
Currently using NNRTI (%)	32%	44%
Total duration of PI therapy (months)	39 ± 4	29 ± 8
Total duration of NNRTI therapy (months)	6 ± 2	9 ± 4
Total duration of NRTI therapy (months)	69 ± 5	45 ± 11; $P < 0.05$
Most recent CD4 cell count ($\times 10^6$/l)	561 ± 319	504 ± 305
CD4 cell count nadir ($\times 10^6$/l)	159 ± 129	199 ± 108
Most recent HIV-1 RNA log10 (copies/ml)	1.9 ± 0.9	1.8 ± 1.1
Most recent HIV-1 RNA < 50 copies/ml (%)	68%	89%
Blood lactate (mmol/l)	1.3 ± 0.6	1.1 ± 0.5

HAART+LD+, HIV-positive patients with HAART-associated lipodystrophy; HAART+LD−, HIV-positive patients using HAART but without lipodystrophy. PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

during the 8 weeks before entering the study. The HIV-positive patients were currently receiving the following protease inhibitors: indinavir (32% of patients in the HAART+LD+ versus 22% in the HAART+LD− group), nelfinavir (20% versus 11%), ritonavir (8% versus 11%), lopinavir (8% versus 0%), lopinavir + amprenavir (4% versus 0%), amprenavir (4% versus 0%), saquinavir (0% versus 11%). None of differences in the frequencies of currently used or ever used (data not shown) protease inhibitors were statistically significant between the HAART+LD+ and the HAART+LD− groups. The total duration of protease inhibitor therapy is given in Table 2. The total duration of the use of indinavir was 32 ± 18 months in the HAART+LD+ and 33 ± 26 months in the HAART+LD− group, nelfinavir 23 ± 12 versus 21 months (only one patient in the HAART+LD− group had ever used nelfinavir), ritonavir 21 ± 21 versus 24 ± 32 months, saquinavir 11 ± 13 versus 31 ± 36 months; none of the these durations were significantly different between the groups. In addition, in the HAART+LD+ group two patients had used lopinavir for 6 ± 2 months, one amprenavir for 23 months and one patient a combination of amprenavir and lopinavir for 2 months. The duration of nucleoside analogue therapy was significantly longer in the HAART+LD+ than in the HAART+LD− group. The groups were comparable with respect to the time since diagnosis of HIV, the most recent and nadir blood CD4 cell count, as well as HIV viral load (HIV-1 Monitor Test, Roche Diagnostics, Branchburg, New Jersey, USA). All patients were infected through sexual contact.

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 review committee of the Helsinki University Central Hospital.

Liver fat content (proton spectroscopy)

Localized single voxel ($2 \times 2 \times 2$ cm^3) 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. T1-weighed high resolution magnetic resonance images were used for localization of the voxel of interest within the right lobe of the liver. Vascular structures and subcutaneous fat tissue were avoided in localization of the voxel. Subjects were lying on their stomach on the surface coil, which was embedded in a mattress in order to minimize abdominal movement due to breathing. The single voxel spectra were recorded by using the stimulated-echo acquisition mode sequence with an echo time of 20 ms, a repetition time of 3000 ms, a mixing time of 30 ms, 1024 data points over 1000 kHz spectral width with 32 averages. Water suppressed spectra with 128 averages were also recorded to detect weak lipid signals. The short echo time and the long repetition time were chosen to ensure a fully relaxed water signal, which was used as an internal standard. Chemical shifts were measured relative to water at 4.80 p.p.m. The methylene signal, which represents intracellular triglyceride, was measured at 1.4 p.p.m. Signal intensities were quantified by using an analysis program VAPRO-MRUI (http://www.mrui.uab.es/mrui/). Spectroscopic intracellular triglyceride content was expressed as methylene/(water + methylene) signal area ratio × 100. This measurement of hepatic fat by proton spectroscopy has been validated against histologically determined lipid content of liver biopsies in humans [28] and against estimates of fatty infiltration by computed tomography [22,29]. All spectra were analysed by a physicist (A.H.) who was unaware of any of the clinical data. The reproducibility of repeated measurement of liver fat in non-diabetic subjects studied on two

occasions in our laboratory is 11% (n = 10, unpublished data).

Intra-abdominal and subcutaneous fat (MRI)

A single T1-weighted trans-axial scan was analysed for the determination of intra-abdominal and subcutaneous fat at the level of the fourth and fifth lumbar interspace (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 areas were measured using an image analysis software (Alice 3.0, Parexel, Waltham, Massachusetts, USA) as described previously [22]. 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%.

Other measurements

Alcohol consumption was quantified using a structured interview prior to participation. Waist circumference was measured midway between the lower rib margin and the iliac crest, and hip circumference over the great trochanters [30]. Serum-free insulin concentrations were determined with radioimmunoassay (Phadeseph Insulin RIA, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) after precipitation with polyethylene glycol [31]. The cross-reactivity of insulin-antibody is, by weight, 41% with proinsulin, $< 0.1\%$ for insulin-like growth factors I and II, and $< 0.1\%$ for C-peptide. Serum leptin concentrations were determined by radioimmunoassay using a commercial kit (Human leptin RIA kit, Linco Research, St. Charles, Missouri, USA). Serum C-peptide concentrations were determined with time-resolved fluoroimmunoassay (AUTOdelfia C-peptide, Wallac, Turku, Finland). HbA_{1c} was measured by high pressure liquid chromatography using the fully automated Glycosylated Hemoglobin Analyzer System (BioRad, Richmond, California, USA) [32]. Plasma glucose concentrations were measured using a hexokinase method, 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). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltransferase (GGT) activities were determined according to recommendations of the European Committee for Clinical Laboratory Standards using the Roche Diagnostics Hitachi 917 autoanalyser. Venous blood gas analysis was performed using specific electrodes with a blood gas analyser (Ciba Corning 850, Medfield, Massachusetts, USA) and blood lactate was determined using an enzymatic method (Dade Behring ACA Analytical Test Packs, Dade Behring, Deerfield, Illinois, USA).

Statistical analysis

Analysis of variance followed by pair-wise comparison using Fisher's Least-Significant-Difference test was used to compare differences between the groups. Logarithmic transformation was performed on data that were not normally distributed. Correlation analyses were performed using Spearman's non-parametric rank correlation analysis. Categorical variables were compared using Fisher's exact test. All calculations were performed using the Systat statistical package, version 10.0 (Systat, Evanston, Illinois, USA) or GraphPad Prism version 2.01 (GraphPad Inc, San Diego, California, USA). Data are shown as mean ± standard deviation (SD) unless indicated otherwise. A P value < 0.05 was considered statistically significant.

Results

Body composition

Age, body mass index (BMI) and alcohol consumption were comparable between all groups (Table 1). The total amount of fat in the abdominal region (by MRI) was comparable between the groups, but its distribution was different (Fig. 1). The HAART+LD+ group had significantly more intra-abdominal fat than the HAART+LD− or the HIV− group, and also significantly less subcutaneous fat than the HIV− group (Fig. 1). The ratio of intra-abdominal to subcutaneous fat was 4.4-fold higher in the HAART+LD+ group (3.1 ± 2.7) than in the HAART+LD− group (0.7 ± 0.4; $P < 0.001$) and 6.2-fold higher than in the HIV− group (0.5 ± 0.2; $P < 0.001$). The waist : hip ratio was significantly higher in the HAART+LD+ group (1.00 ± 0.07) than in the HIV− (0.94 ± 0.05; $P < 0.01$) or the HAART+LD− (0.93 ± 0.09; $P < 0.05$) group (Table 1).

Biochemical characteristics

Serum insulin and C-peptide concentrations were significantly higher in the HAART+LD+ group than either in the HAART+LD− or the HIV− group (Fig. 2). Serum insulin concentrations did not correlate with the amount of intra-abdominal fat within HAART+LD+ group ($r = 0.26$, not significant). The HAART+LD+ patients had significantly lower concentrations of serum HDL cholesterol and higher concentrations of serum triglycerides than the HAART+LD− patients or the HIV-negative subjects (Fig. 2). Serum total cholesterol was significantly higher in the HAART+LD+ group (5.9 ± 1.1 mmol/l) than in the HAART+LD− group (4.8 ± 0.8 mmol/l; $P < 0.01$) or in the HIV− group (5.1 ± 1.0 mmol/l; $P < 0.01$). Serum ALT and AST concentrations were significantly higher in the HAART+LD+ group than in the HAART+LD− or the HIV− group, and serum GGT was significantly higher in the HAART+LD+

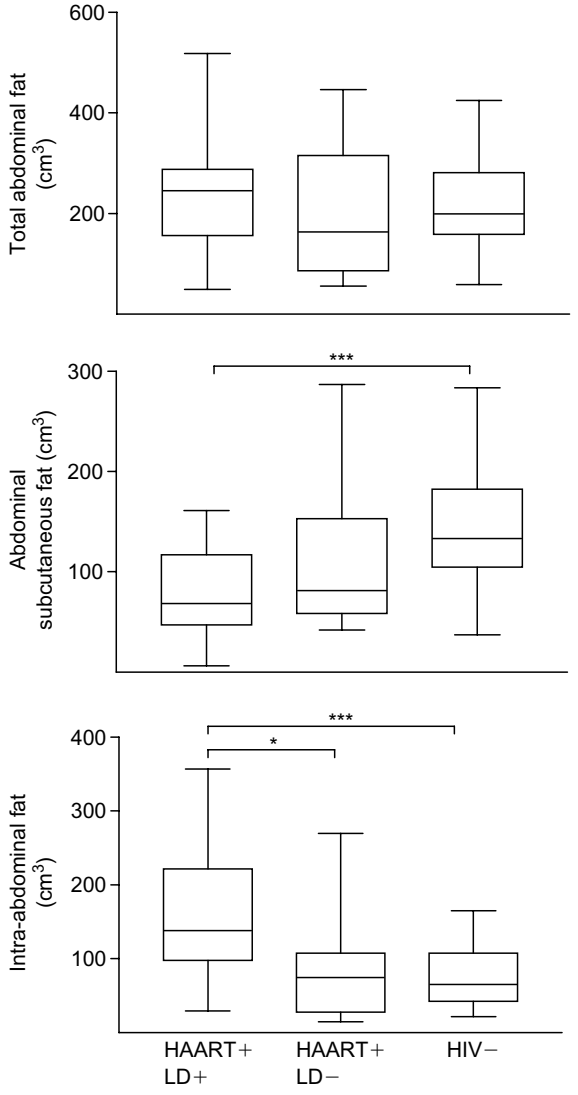

Fig. 1. Box and whiskers plots of total abdominal fat (MRI), abdominal subcutaneous fat (MRI), and intra-abdominal fat (MRI) in the three study groups. The box shows the interquartile range, with a line at the median (the 50th percentile). The whiskers extend above and below the box to show the highest and lowest values. * $P < 0.05$, *** $P < 0.001$ for comparisons between the groups as indicated.

group than in the HIV-negative subjects (Fig. 3). Blood lactate concentrations were similar in both HIV-positive groups and none of the patients had acidosis (Table 2).

Liver fat

Liver fat content in the HAART+ LD+ patients ($8.1 \pm 9.9\%$) was 53% higher than in the normal subjects ($5.3 \pm 6.6\%$; $P < 0.05$) and 179% higher than in the HAART+ LD− patients ($2.9 \pm 4.7\%$; $P < 0.01$). Liver fat content did not differ between the HAART+LD− and HIV-negative groups ($P = 0.31$). Liver fat content was highly significantly correlated with fasting serum insulin concentration in the HIV-negative subjects (Fig. 4; r, 0.65; $P < 0.001$) and in the HAART+LD+ group (r, 0.47; $P < 0.05$). Similar highly significant relationships were observed between liver fat and serum C-peptide concentrations (Fig. 4). The slopes of the regression lines relating liver fat and fasting insulin concentration were similar in HAART+LD+ and HIV− groups. The intercepts of the regression lines were, however, significantly different between the HAART+LD+ group and the HIV-negative subjects ($P < 0.001$) implying that for a given percentage of liver fat, serum fasting insulin concentrations were significantly higher in the HAART+LD+ group than in the HIV-negative subjects (Fig. 4). Liver fat did not correlate with the amount of intra-abdominal fat or waist : hip ratio in the HAART+LD+, HIV− group (Fig. 4) or the HAART+LD− group (data not shown).

Relationship between measures of body composition and serum leptin concentrations

The HAART+LD+ group had significantly lower leptin concentrations than the two other groups (Fig. 5 upper panel). Serum leptin concentrations were closely correlated with the amount of subcutaneous fat in both the HAART+LD+ and the HIV− groups (Fig. 5 middle panel). Within the groups of HAART+LD+ and HIV−, serum leptin was correlated with BMI, but the slopes of these relationships were different (Fig. 5 lower panel). For the same BMI above approximately 20 kg/m^2, the HAART+LD+ group had a lower leptin concentration than the HIV− group.

Discussion

Liver fat content has not been quantified previously in patients with HAART-associated lipodystrophy. We found liver fat content to be higher in the HAART+ LD+ patients than in the age- and weight-matched HIV-negative subjects or the HAART+LD− patients. Within the HAART+LD+ group, serum insulin concentration correlated with the percentage of liver fat but did not correlate with the amount of intra-abdominal fat. This finding suggests that fat accumulation in insulin sensitive tissues, such as the liver, rather than accumulation of intra-abdominal fat is critical for the development of features of insulin resistance in HAART-associated lipodystrophy.

Common non-invasive methods for evaluating liver fat content, such as ultrasound are not specific and are at best only semi-quantitative [33]. In the present study, we used magnetic resonance proton spectroscopy,

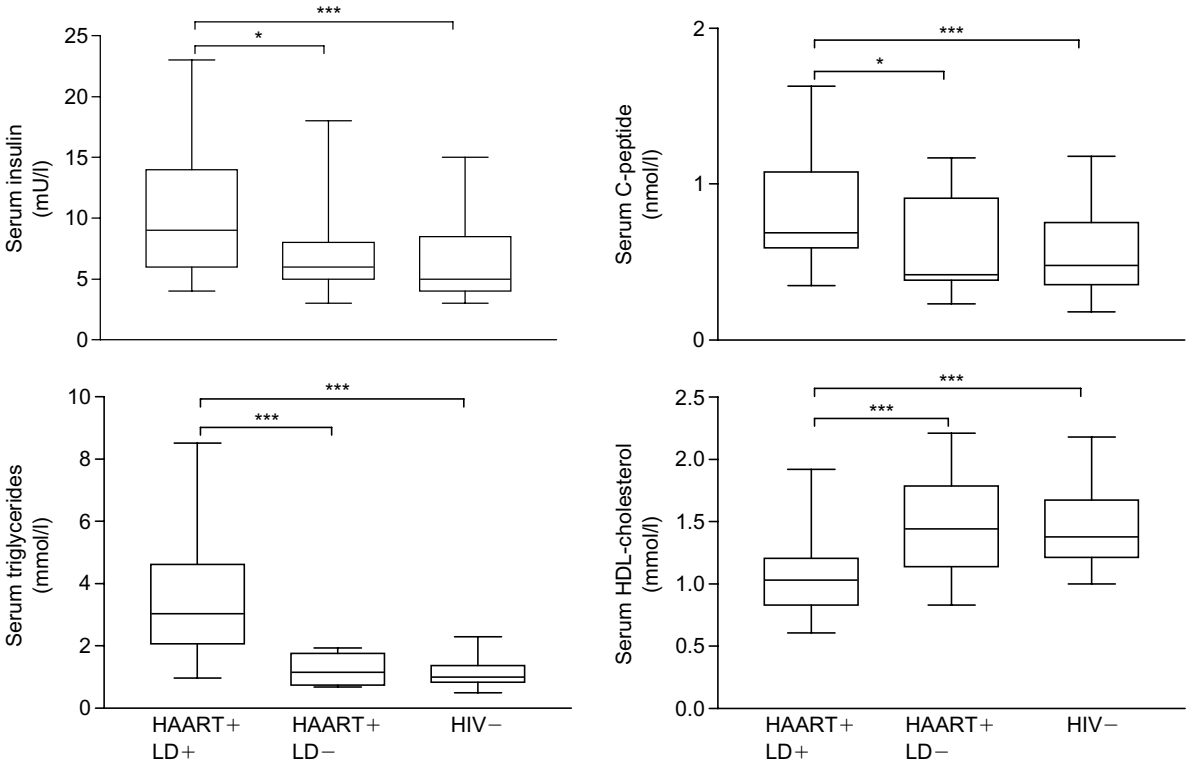

Fig. 2. Box and whiskers plots of serum fasting insulin, C-peptide, triglyceride and HDL-cholesterol concentrations in the three study groups. * $P < 0.05$, *** $P < 0.001$ for comparisons between the groups as indicated.

which allows non-invasive quantification of liver fat without radiation exposure. Liver fat content measured using proton spectroscopy correlates closely with that determined histologically from liver biopsies and with liver density measurements calculated by computed tomography [22,28,29]. Furthermore, by spectroscopic determination of liver fat a larger volume of liver tissue can be evaluated than by liver biopsies [34]. However, it is important to emphasize that quantification of liver fat with proton spectroscopy does not allow distinction between micro- and macrovesicular steatosis or evaluation of the presence or absence of fibrotic or inflammatory changes or mitochondrial abnormalities. Such information would be important for the understanding of the aetiology of steatohepatosis but could not be obtained in the present study because liver biopsies were not clinically indicated.

The increased amount of hepatic fat in the HAART+ LD+ group could be explained neither by alcohol consumption, which was comparable between the study groups, nor by autoimmune causes. Co-infection with hepatitis C virus has recently been shown to increase the risk for severe liver damage during HAART treatment [35]. This association cannot account for our results as none of the subjects in this study were carriers of hepatitis C or B virus. Mitochondrial toxicity induced by nucleoside analogues has been suggested to cause lactic acidosis and hepatic steatosis in some patients using HAART [36–38]. However, this mechanism for liver abnormalities is unlikely to explain our findings, as the lactate concentrations were similar between the HAART+LD+ and the HAART+LD− groups (Table 2) and none of the patients had acidosis. On the other hand, in the absence of liver biopsies, we cannot exclude the possibility that at least some patients had mitochondrial abnormalities typical of those described in patients with non-alcoholic steatohepatitis, who also are known to be insulin resistant [39].

Although liver fat has not been quantified previously in patients with HAART-associated lipodystrophy, Ariogolu *et al.* found fatty infiltration of the liver in 12 out of 20 HIV-negative patients with various forms of lipodystrophy [40]. In addition, scattered case reports have described hepatosplenomegaly in patients with various forms of acquired or congentital lipodystrophies [21] and liver fat content was also increased in a patient with acquired generalized lipoatrophy (Lawrence syndrome) [41]. These human data resemble those of genetically modified mice. Mice in which adipose tissue has been genetically ablated develop insulin resistant diabetes, hypertriglyceridaemia, fatty liver and have reduced levels of leptin [17,42].

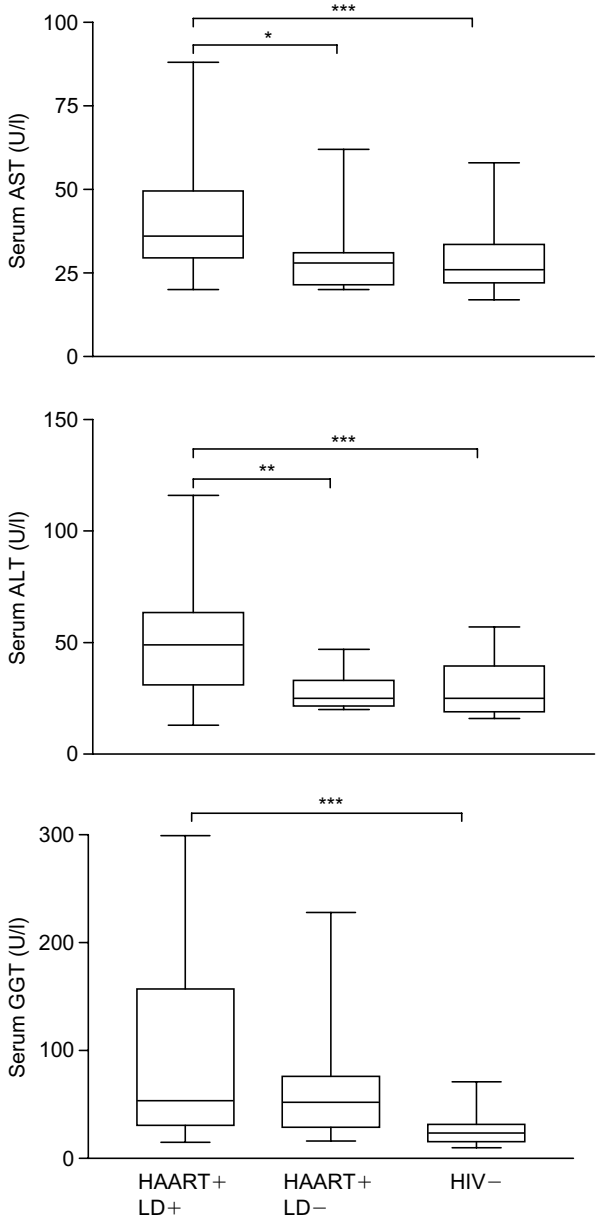

Fig. 3. Box and whiskers plots of serum AST, ALT and GGT concentrations in the study groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for comparisons between the groups as indicated.

In the present study, the HAART+LD+ group had significantly lower leptin concentrations than the other two groups. Serum leptin was similarly correlated with subctaneous fat in both HAART+LD+ and HIV− groups (Fig. 5, middle panel). Serum leptin concentrations also correlated with BMI significantly within both groups, but the slopes of the these relationships were significantly different. For a given BMI, approximately above 20 kg/m^2, the HAART+LD+ group had a lower leptin concentration than the HIV− group (Fig. 5, lower panel). This result suggests that subcutaneous fat is the major, if not the exclusive source of circulating leptin, and also that the quantity of leptin produced per unit of subcutaneous fat mass is unaltered in HAART+LD+ patients. These data are consistent with previous data demonstrating low plasma leptin concentrations in patients with HAART-associated lipodystrophy [43] and with *in vitro* studies of human adipose tissue, which have shown that subcutaneous adipose tissue produces two- to threefold more leptin than visceral fat [44]. In the lipodystrophic SREBP-1c (sterol regulatory element binding protein-1c) overexpressing mouse, a low dose leptin infusion completely normalizes insulin sensitivity and depletes the liver of its massive fat deposits [45]. In another lipodystrophic mouse model characterized by severely reduced peroxisome proliferator activator receptor-γ activity, leptin alone has been insufficient to completely normalize insulin sensitivity [46]. In the latter model, a combination of leptin and adiponectin, which is another hormone produced exclusively by adipose tissue, completely reversed insulin resistance and normalized the fat content of the liver. It is not known whether similar manoeuvres would be helpful in HAART-associated lipodystrophy.

For a given amount of liver fat, serum-free insulin concentrations were higher in the HAART+LD+ than the HIV− group (Fig. 4) implying that liver fat alone was insufficient to explain all of the variation in serum insulin concentrations. Fat cannot be deposited only in the liver but also intramyocellularly in skeletal muscles in humans. Intramyocellular fat correlates negatively with insulin sensitivity in the muscle [23–25,47,48]. It is thus possible that insulin resistance in skeletal muscles might explain some of the variation in fasting insulin concentrations. As we did not perform euglycemic insulin clamp studies combined with infusion of glucose tracers in the present study, it is not possible to determine the contribution of individual tissues to the increase in fasting insulin concentrations. On the other hand, after an overnight fast, the liver rather than skeletal muscle is the key target for insulin action [49,50]. At least in mice, selective deletion of the insulin receptor from skeletal muscle does not lead to hyperinsulinaemia or abnormal glucose tolerance [51], while tissue-specific deletion of the insulin receptor from the liver induces dramatic fasting and postprandial hyperinsulinaemia and severe glucose intolerance as well as fatty degeneration of the liver [52]. An alternative potential explanation for the disproportionate hyperinsulinaemia is that liver fat content is not a perfect measure of hepatic insulin sensitivity. Information of the rates of free fatty acid (FFA) influx into the liver and their metabolism, or of other processes possibly interfering with insulin action in the liver, would be helpful in this regard.

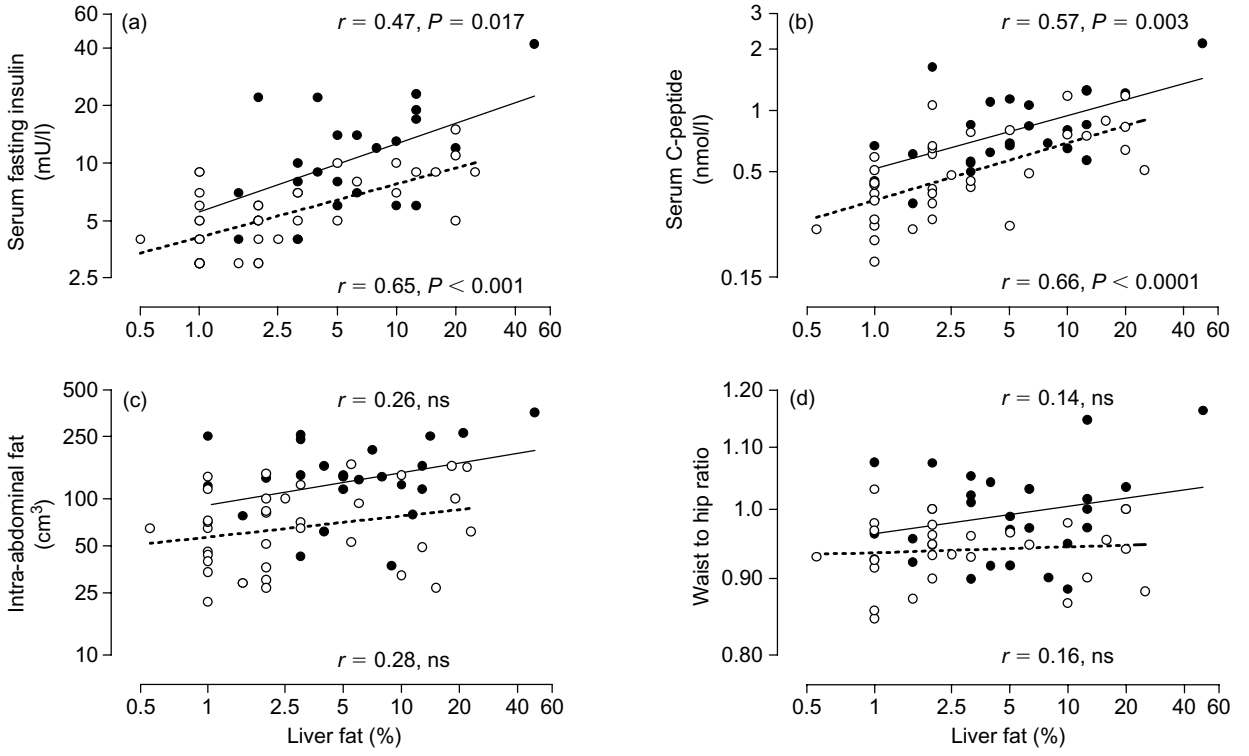

Fig. 4. Relationships between liver fat and serum fasting insulin (a), serum C-peptide (b), intra-abdominal fat (c) and the waist : hip ratio (d) in patients with HAART-associated lipodystrophy (filled circles, solid line) and in HIV-negative subjects (open circles, dotted line).

The correlation of serum fasting insulin with liver fat content, but not with the amount of intra-abdominal fat in HAART+LD+ patients challenges the idea that intra-abdominal fat, at least alone, is responsible for features of insulin resistance in HAART-associated lipodystrophy. Our finding is in accordance with a previous report, in which insulin sensitivity measured by euglycaemic clamp technique was not different between two groups of patients with HAART-associated lipodystrophy regardless the strikingly different amount of truncal fat (19.0 versus 6.8 kg) measured by dual energy X-ray absorptiometry (DEXA) [53]. Treatment with protease inhibitors for 3 months has been reported to increase fasting serum insulin, glucose and triglyceride concentrations in the absence of changes in weight or regional body fat distribution [54]. However, in these studies intra-abdominal versus subcutaneous adipose tissue could not be differentiated as these two fat compartments can only be distinguished by MRI or computed tomography and not by DEXA. In the present study, regardless of the similar amount of total abdominal fat, the ratio intra-abdominal : subcutaneous fat measured by MRI was 6.2-fold higher in the HAART+LD+ group than in the HIV− group ($P < 0.001$) and 4.4-fold higher than in the HAART+LD− group ($P < 0.001$). At least theoretically, the increased amount of intra-abdominal fat could contribute to insulin resistance by three mechanisms. First, if visceral fat doesn't produce as much leptin as subcutaneous fat, this might promote fat deposition in the liver and insulin resistance [44,45]. Visceral fat also appears to produce more interleukin-6 (IL-6) than subcutaneous fat [55]. IL-6 has recently been shown to cause insulin resistance by down-regulating key insulin signalling molecules such as insulin receptor substrate-1 [56]. Finally, according to the 'portal hypothesis', visceral fat may cause hepatic insulin resistance via releasing FFA at high rates. As discussed by K. Frayn, this hypothesis remains unproven and is weakened by data from hepatic catheterization studies which have shown that maximally 10% of FFA reaching the liver originate from visceral fat [57]. In the present study we can, however, neither confirm nor negate the role for visceral fat in at least contributing to fasting hyperinsulinaemia or hepatic fat accumulation. In a previous study, we found a very close correlation between directly measured hepatic insulin sensitivity and liver fat content while there was no correlation between intra-abdominal and liver fat [22].

In the present study, only patients with lipodystrophy had features of insulin resistance, while those in the HAART+LD− group had not. This was also the case for the accumulation of hepatic fat. The HAART+

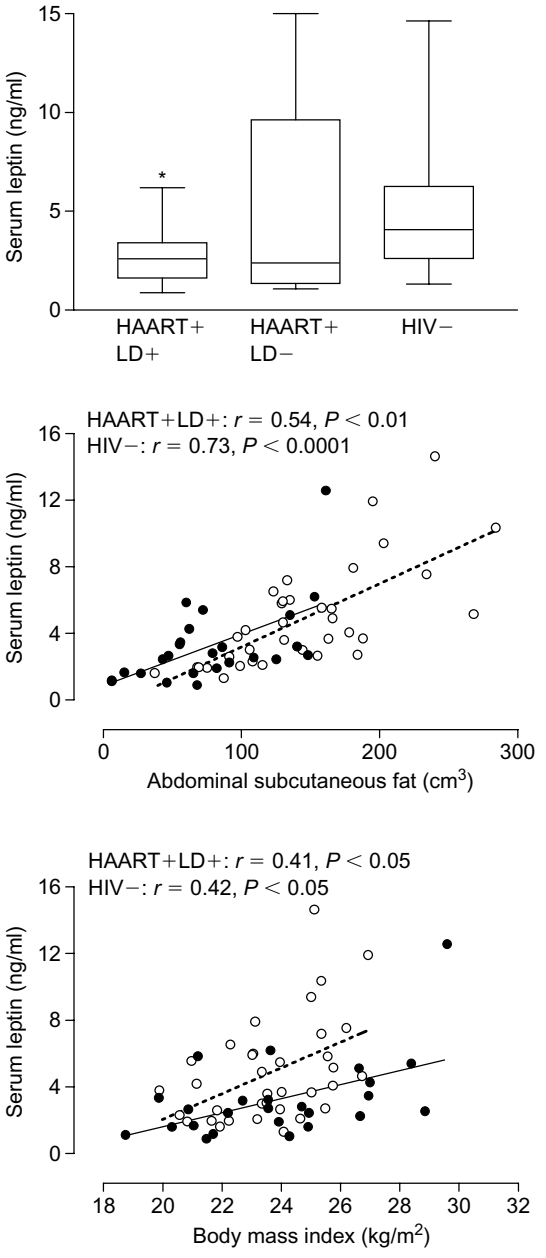

Fig. 5. Box and whiskers plots of serum leptin concentrations in the three study groups (upper panel). The relationship between serum leptin and abdominal subcutaneous fat (MRI) (middle panel) and BMI (lower panel) in patients with HAART-associated lipodystrophy (filled circles, solid line) and in HIV-negative subjects (open circles, dotted line). * $P < 0.05$ for comparison of HAART+LD+ group with the two other groups.

LD+ and HAART+LD− groups did not differ with respect to the duration of HIV-infection or the nadir CD4 cell count (Table 1). The duration of protease inhibitor and non-nucleoside treatment did not differ significantly between the groups. Furthermore, their immunologic and virologic response to HAART was comparable. The only significant difference in the treatment history was a longer duration of nucleoside analogue therapy in the HAART+LD+ than in the HAART+LD− group. However, this difference is unlikely to fully explain the lack of metabolic abnormalities in the HAART+LD− group, as the features of insulin resistance were similarly absent both in the HAART+LD− group and the HIV− group; yet the latter had never received HAART. On the other hand, the HAART+LD− group had slightly, but not significantly, lower amounts of subcutaneous fat than the HIV− group (Fig. 1). Whether continuation of nucleoside analogues for as long as in the HAART+LD+ group would result in a further decrease in subcutaneous fat can only be determined in a prospective study.

There are very limited data regarding the effects of HAART on lipogenesis in the liver. In a recent report, ritonavir caused liver enlargement and lipid accumulation in the liver of mice, especially when the mice were fed a high fat diet [26]. The proposed mechanism for the increased liver fat in this mouse model was excessive accumulation of the lipogenic transcription factors SREBP-1 and -2 in the nucleus of hepatocytes. Ritonavir was shown to cause this accumulation by suppressing the degradation of activated forms of SREBP in the nucleus of hepatocytes. If these data were relevant in humans, they would suggest that features of insulin resistance in patients susceptible to HAART-associated lipodystrophy were a consequence of a primary effect of HAART in the liver. However, as discussed above, it is also possible that accumulation of fat in the liver is a consequence of subcutaneous lipoatrophy because the liver seems to retain its ability to store triglycerides even when atrophic subcutaneous fat has lost this ability.

Liver fat appears to be an important target for the action of especially insulin-sensitizing anti-diabetic drugs. In a recent report, treatment of patients with non-alcoholic fatty liver disease with metformin for 4 months decreased liver volume and liver enzymes and improved insulin sensitivity [58]. In rats, metformin increased hepatic insulin sensitivity by decreasing activity and expression of a key lipogenic transcription factor (SREBP-1) in hepatocytes [59]. Metformin also reduced insulin resistance in patients with HAART-associated lipodystrophy without affecting the visceral : subcutaneous fat ratio [60]. The effect of metformin on the liver fat was not evaluated in this study. Glitazones, such as rosiglitazone have also been suggested to improve insulin sensitivity by reducing hepatic fat content in humans [61] and liver size was reported to decrease in a study on HIV-negative lipodystrophic patients treated with troglitazone for 6 months [40]. Data on the effect of glitazones on HAART-associated lipodystrophy are not yet available.

In conclusion, we have demonstrated increased hepatic fat content in HIV-positive men with HAART-associated lipodystrophy when compared to healthy HIV-negative men of similar age and BMI and to HIV-positive men who had received HAART but not developed lipodystrophy. Increased liver fat is closely correlated with features of insulin resistance and may play a causative role in the development of insulin resistance in these patients.

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