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Low plasma level of adiponectin is associated with stavudine treatment and lipodystrophy in HIV-infected patients

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SUMMARY

This study tested the hypothesis that in patients with HIV-associated lipodystrophy, adiponectin levels were related to insulin resistance, TNF-α and IL-6 and treatment with nucleoside analaogues. HIV seropositive men undergoing highly active antiretroviral treatment were enrolled into three predetermined clinical groups: lipodystrophy with central fat accumulation (n = 12); lipodystrophy without central fat accumulation (n = 15); no lipodystrophy (n = 15). HIV-negative healthy men served as controls (n = 12). Both lipodystrophic groups had a low percentage of limb fat compared to the two control groups. Patients with lipodystrophy with fat accumulation had increased truncal fat compared with controls. Levels of adiponectin did not correlate with either TNF-α or IL-6. Low levels of adiponectin were found in both lipodystrophic groups and were associated with current or previous treatment with stavudine. Furthermore, the adiponectin level correlated with the percentage of limb fat. Patients with lipodystrophy with fat accumulation were more insulin resistant, measured by HOMA-IR, compared with controls. However, HOMA-IR did no correlate to adiponectin or other cytokines. In conclusion, the finding of no difference between the two lipodystrophic groups with regard to adiponectin, indicates that low levels of adiponectin reflects fat atrophy, whereas the insulin resistance was best explained by increased truncal fat mass.

Keywords HIV lipodystrophy insulin resistance adiponectin cytokines

INTRODUCTION

Antiretroviral therapy of HIV-infected patients has been associated with the development of lipodystrophy (LD). LD is characterized by peripheral fat loss (lipoatrophy) and central fat accumulation [1,2], but a recent prospective study has demonstrated that highly active antiretroviral therapy (HAART) may induce selective loss of limb fat [3]. The changes of fat distribution are often associated with dyslipidaemia [1,4,5], increased lipolysis [6], insulin resistance [1,4], and less often with diabetes mellitus [1,7], recently rewieved by Chen *et al*. [8]. Initially this syndrome was regarded as an effect of protease inhibitors (PI) [1,7], but recent reports have also demonstrated fat redistribution in nucleoside reverse transcriptase inhibitors (NRTI-treated), PI-naïve patients [9–13]. In a number of studies stavudine has been associated with a higher risk to develop lipodystrophy [9–11,14,15].

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Recently, the adipose tissue has been identified as an important metabolic organ, which plays an important role in synthesizing and secreting a number of hormones and cytokines, including TNF-α [16], IL-6 [17], leptin and adiponectin [18].

Adiponectin is a 30-kD protein, synthesized and secreted exclusively by adipose tissue and shares homology to complement factor C1q and to collagen [19,20]. The functional role of adiponectin is not known, but recent evidence has suggested an important role for adiponectin in the regulation of insulin action and energy homeostasis [21]. *In vitro* and *in vivo* studies in rodents have shown that adiponectin reduces plasma levels of glucose [22–25], increases the ability of subphysiological levels of insulin to suppress gluconeogenesis [24], accelerates the oxidation of nonesterified fatty acids by muscle [22,23,26], lowers circulating fatty acid [22,23], reduces the plasma levels of triglycerides [22–24] and prevents lipid accumulation in skeletal muscle and liver [23]. Adiponectin might be regulated by TNF-α and IL-6. Thus, adiponectin knockout mice furthermore display high levels of TNF-α mRNA in adipose tissue and high concentrations in plasma [27]. Incubation of adipocytes with TNF-α result in a decrease of adiponectin [28], suggesting that TNF-α is a regulator of adiponectin [29] although it should be noted that the levels of

TNF-α used were 1000-fold greater than those reported in other studies investigating the role of TNF-α in metabolic conditions. In addition, a recent study demonstrated that adiponectin gene expression and secretion is inhibited by IL-6 [30]. Finally, plasma adiponectin concentration is decreased in humans with obesity [31,32], type 2 diabetes [32,33], coronary artery disease [33,34] and dyslipidaemia [35]. Plasma level of adiponectin is also low in humans with congenital and acquired lipodystrophies, characterized by selective loss of body fat [36].

Recently, a relationship between low circulating levels of adiponectin and lipodystrophy in HIV patients has been described [37]. The purpose of this study was to test the hypothesis that in patients with HIV-associated lipodystrophy, adiponectin levels were related to the cytokines TNF-α and IL-6 as well as treatment with nucleoside analaogues. In addition, by defining two lipodystrophy groups, one with and one without central fat accumulation, we would obtain information about whether it was the peripheral fat loss or the central fat accumulation which would be linked with the low adiponectin levels.

MATERIALS AND METHODS

Subjects

Forty-two HIV-positive men receiving antiretroviral therapy were recruited from the outpatient clinic of the Department of Infectious Diseases, Rigshospitalet in Copenhagen. Lipodystrophy (LD) was defined clinically by physical examination of peripheral lipoatrophy (fat loss from face, arms, buttocks or legs) with or without central fat accumulation (abdomen, dorsocervical fat pad) [38]. Patients were enrolled into the following three predetermined clinical groups of fat distrubution: Patients with lipodystrophy with central fat accumulation (LD-FA) ($n = 12$); Patients with lipodystrophy without central fat accumulation (lipoatrophy) (LD-LA) ($n = 15$); Patients without lipodystrophy (no-LD) ($n = 15$). Twelve age-matched HIV-seronegative healthy individuals served as controls. Body mass index (BMI) were similar for the groups except for the HIV-patients with lipodystrophy without central fat accumulation (LD-LA), who had a significantly lower BMI (Table 1). Demographic data were colleted for each patient: age, duration of HIV infection, weight, height, CD4 count and HIV-RNA copies. Information was obtained regarding duration and type of antiretroviral therapy. All patients were taking nucleoside reverse transcriptase inhibitors (NRTI). In the LD-FA group 11 patients were taking protease inhibitors (PI), and 4 were taking non-nucleoside reverse transcriptase inhibitors (NNRTI). In the LD-LA group 10 patients were taking PI, and 4 were taking NNRTI. In the no-LD group 9 patients were taking PI, and 5 were taking NNRTI.

All HIV-positive patients received a stable HAART regimen with no changes in antiretroviral therapy during the preceding 8 weeks, had no signs of ongoing infections and had fasting glucose under 7 mmol/l. Informed consent was obtained from all patients according to the requirements of the local ethical committee.

Table 1. Charateristics of the study groups

Characteristics	LD-FA $n = 12$	LD-LA $n = 15$	No-LD $n = 15$	Healthy controls $n = 12$
Age (years)	46·5 (33–63)	48 (33–72)	45 (31–62)	45 (27–52)
Duration of HIV-infection (years)	8·5 (6–12·5)	11 (8–14·5)	9 (6·0–14·5)	
HIV RNA, undetectable HIV RNA, No. (%)	10 (83)	12 (80)	14 (93)	
CD4 cell count (cells/μl)	645 (365–815)	460 (375–615)	450 (315–700)	
Antiretroviral therapy				
Duration of antiretroviral therapy (months)	71·0 (48·0–82·0)	90·0 (79·0–111·0)††	67·0 (50·5–71·0)	
Duration of PI (months)	53·0 (32·0–69·0)	65·0 (59·0–69·5)	65·0 (61·0–69·0)	
Duration of NNRTI (months)	27·5 (11·5–41·5)	18·0 (8·5–27·5)	25·0 (13·0–29·0)	
Duration of NRTI (months)	71·0 (48·0–82·0)	90·0 (79·0–111·5)††	67·0 (50·5–71·0)	
Duration of stavudine (month)	27·0 (17·0–47·0)*	61·0 (49·0–70·0)	25·0 (22·0–49·5)	
Stavudine: current/previous, no (%)	2.0 (16·7)/3(25)	7 (46·7)/7(46·7)	2 (13·3)/0	
Body composition				
Weight (kg)	80 (74·5–84·3)**	66 (61·8–73)‡‡‡	70·5 (57·3–75·5)	81 (74–90·1)
BMI (kg/m2) (22·5–27·1)	23·7 (23·1–25·8)**	21·3 (20·4–21·9)‡‡‡‡		23·0 (22·3–23·4) 24·9
Total fat mass (kg)	16·7 (15·9–22·3)**	7·23 (7·0–10·4)‡‡‡‡	14·1 (11·5–17·1)‡	19·1 (15·1–22·9)
Total fat mass (%)	21 (19–26)***	13 (10·5–15)†‡‡‡	19·5 (17–23)	23 (20–24)
Total lean mass (kg) (57·8–66·4)	59·3 (57·3–61·0)	53·3 (51·9–58·6)	51·9 (47·4–59·2)	59·7
Truncal fat mass (kg)	11·2 (9·7–13·2)**††	4·7 (3·5–6·9)‡‡	6·67 (5·7–9·6)	9·9 (7·1–12·5)
Truncal fat mass (%)	61·9 (59·3–64·3)†‡‡‡	59·9 (52·5–65·9)	52·7 (46·7–55·6)	50·9 (45·9–54·4)
Limb fat mass (kg)	5·57 (4·53–7·65)**‡‡	2·49 (2·23–2·76)†‡‡‡‡‡	4·65 (3·89–0·10)‡‡	8·12 (7·1–9·6)
Limb fat mass (%)	33·3 (28·5–35·7)†‡‡‡‡	28·3 (26·2–36·4)†‡‡‡‡‡	40·2 (37·8–44·9)	45·3 (41·6–47·2)
Trunk fat mass/limb fat mass	1·76 (1·73–1·26)†‡‡‡	2·17 (1·45–2·54)†‡‡‡‡	1·32 (1·08–1·48)	1·13 (0.97–1·29)

Data expressed are median and 25% and 75% quartiles/[range]. LD, lipodystrophy. LD-FA, lipodystrophy with fat accumulation; LD-LA, lipodystrophy without fat accumulation; no-LD, patients without lipodystrophy. NRTI, nucleoside reversetranscriptase inhibitors; NNRTI, non-nucleoside reversetranscriptase inhibitors; PI, protease inhibitors. Previous indicate previous therapy. *indicates significant difference between the indicated subgroup and LD-LA (*: $P < 0·05$, **: $P < 0·01$) † indicates significant difference between the indicated subgroup and no-LD (†: $P < 0·05$, ††: $P < 0·01$) ‡indicates significant difference between the indicated subgroup and controls (‡: $P < 0·05$, ‡‡: $P < 0·01$, ‡‡‡: $P < 0·001$, ‡‡‡‡: $P < 0·0001$).

Blood sampling

Peripheral blood samples were obtained after an overnight fasting. Measurements of plasma glucose (mmol/l), insulin (pmol/l), total cholesterol (mmol/l), HDL-cholesterol (mmol/l), LDL-cholesterol (mmol/l) and triglycerides (mmol/l) were determined immediately using routine methods.

Laboratory tests

Cytokines were measured in plasma. Ethylenediaminetetraacetate (EDTA) was used as an anticoagulant. Plasma was stored at –80°C until analysed. TNF-α and IL-6 were determined by enzyme-linked immunosorbent assay (ELISA) kits (Quantikine High Sensitivity, R & D systems, Minneapolis, USA). Thresholds of detection were 0·094 pg/ml for TNF-α and 0·18 pg/ml for IL-6. Adiponectin was determined by a human adiponectin Radio Immuno Assay (RIA) kit (The LINCO Research, Inc., St. Charles, MO, USA). Samples were diluted 1 : 500 in assay-buffer prior to measurements. Threshold of dectection was 1 ng/ml. The intra- and inter-assay coefficients of variation were between 1·78% and 6·21% and 9·25 and 6·90%, respectively. All samples and standards were run as duplicates and the mean of duplicates was used in the statistical analyses.

CD4 cell counts were calculated by flowcytometry and HIV-RNA copies were measured by the Amplicor Hiv Monitor (Roche Molecular Systems, Branchburg, NJ, USA) (lower limit of dectection: 20 copies/ml).

Insulin resistance

Insulin resistance was assessed from fasting plasma insulin and glucose by using the homeostasis model (HOMA) [39]. The model assumes that normal weight subjects younger than 35 years have an insulin resistance of 1. The formulae for HOMA method is as follow: HOMA-IR = insulin (μU/ml) × glucose (mmol/l)/22·5 [39].

Body composition analysis

Fat and fat-free tissue masses for the whole body, trunk and extremities were measured using dual-energy X-ray absorptiometry (DEXA) scanner, Norland XR 36 (Norland Corporation, Fort Atkinson, WI, USA). The pixel size is $6{\cdot}5 \times 13{\cdot}0\ mm^2$ (width × height). Software version 3·94. Patients were placed as previously described [40]. The scanner was calibrated daily against the standard calibration block supplied by the manufacturer. All measurements were done in a single laboratory. Whole-body and regional fat measurements (trunk and extremity) were determined as previously described [40,41].

Statistics

Statistical calculations were performed using SYSTAT statistical software 8·0 (SYSTAT, Evanston, IL, USA). Data are presented as medians and 25% and 75% quartiles. $P < 0{\cdot}05$ was considered significant in all analyses. Differences between independent groups were evaluated by Fischer's exact test (categorical variables) and a two–tailed *t*-test (continuous variables) or an analysis of variance (ANOVA) if more than two groups were compared. If the ANOVA had a significant *P*-value, pair-wise comparisons were subsequently performed by a Tukey test. In all statistical analyses, groups were considered independent because they were included only by the presence of an HIV diagnosis and the presence of lipodystrophy. The HIV-controls and the healthy controls were randomly selected according to their age and lack of lipodystrophy. Adiponectin, TNF-α and IL-6 were normally distributed after log transformations. Correlations were evaluated by Spearman's rank correlation coefficient (Rs).

RESULTS

The demographic and body compostion characteristics and antiretroviral therapy are shown in Table 1. There were no significant differences in the duration of the HIV infection, CD4 + cell count and HIV amount between the three HIV-infected groups. The subjects in the LD-LA group had received therapy (NRTI-therapy) for a longer period than subjects in the no-LD group and had a lower BMI compared to patients in the LD-FA group and the healthy controls. The dual PI therapy was saquinavir-ritonavir, indinavir-saquinavir or lopinavir-ritonavir and one patient had indinavir-nelfinavir. The LD-LA group compared to the no-LD group and the LD-FA group were more likely to receive stavudine and abacavir (46·7% *versus* 13·3% (no-LD) and *versus* 16·7% (LD-FA)) and had higher cumulative months of stavudine use (Table 1). Nearly all patients currently or previously receiving stavudine had LD (90%) and among LD patients 70% received or had received stavudine ($P = 0{\cdot}001$). 77% of patients ever treated with abacavir had LD, but all of these also had received or received stavudine. Only 37% of LD patients had received or received abacavir. Two patients received abacavir without stavudine and none had LD (data not shown).

Patients in the LD-LA group had reduced percentage fat compared to the LD-FA group, the no-LD group and the controls. The lean body mass did not differ between the groups. The LD-LA group had reduced total limb fat (kg) compared to the LD-FA group, the no-LD group and the healthy controls. The LD-FA group had increased total truncal fat (kg) compared to the LD-LA group and the no-LD group, but not compared to healthy controls. However, the LD-FA group had higher percentage of truncal fat compared to healthy subjects, whereas the percentage of truncal fat did not differ between the LD-LA group, the no-LD group and the healthy controls. The percentage of limb fat did not differ between the two LD groups, but it was lower than both the no-LD group and controls. The truncal fat to limb fat ratio did not differ between the two LD groups, but was increased compared to both the no-LD group and the controls (Table 1).

Adiponectin, TNF-α and IL-6

Levels of plasma adiponectin, TNF-α or IL-6 did not differ between the LD-FA group and the LD-LA group. Accordingly, the two groups were pooled into one group, named lipodystrophy (LD-group).

The LD-group had lower levels of adiponectin compared with the no-LD group and compared with controls. There was no significant difference between the no-LD group and the healthy subjects (Fig. 1a).

The IL-6 level was higher in the LD-group compared with the healthy control group (Fig. 1b). Furthermore, the TNF-α level was higher in the total HIV-positive group compared with controls, but there was no difference between the different HIV-positive groups (Fig. 1c).

Adiponectin and highly active antiretroviral therapy

Regarding the effect of antiretroviral drugs, the patients were divided into three new groups: (a) currently receiving the drug; (b) previously treated with the drug and not receiving it today; or

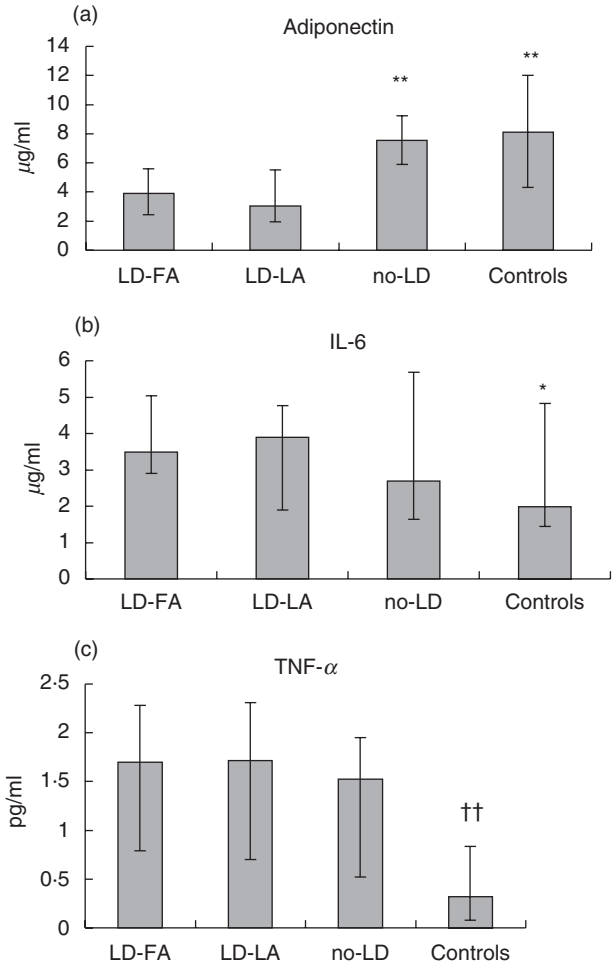

Fig. 1. Levels of circulating adiponectin, IL-6 and TNF-α in HIV-infected patients with lipodystrophy with fat accumulation (LD-FA), HIV-infected patients with lipodystrophy without fat accumulation (LD-LA), HIV-infected patients without lipodystrophy (no-LD) and in healthy, HIV negative controls (controls). LD-FA and LD-LA did not differ with regard to any parameters. Medians and quartiles are shown. *indicates significant difference (*$P < 0{\cdot}05$) (**$P < 0{\cdot}01$) between LD-FA/LD-LA and the indicated subgroup; †indicates significant difference (†† = $P < 0{\cdot}01$) between the total HIV group and controls. LD-FA $n = 12$; LD-LA $n = 15$; no-LD $n = 15$; Controls $n = 12$.

(c) never received the drug. Group a *versus* c was compared by a two-tailed *t*-test for independent groups. Futhermore, a + b (a and b pooled) *versus* c was compared. Groups were considered independent because they included different subjects, who were not matched by any epidemiological, clinical or chemical parameters. For PI the comparison between a + b *versus* c were not made because only two patients never received PI.

Statistical comparisons were performed for groups of antiretroviral drugs: protease inhibitors, nucleoside analogues and non nucleoside analogues. Thereafter, the effect of individual drugs was examined ($n > 8$): indinavir, saquinavir, ritonavir, stavudine, abacavir, lamivudine/zidovudine, didanosine, efavirenz. Regarding PIs, we also investigated the effect of dual PI treatment. Patients currently or previously treated with stavudine (group a +b) had significantly lower levels of adiponectin compared with patients, who had never received stavudine (group c) ($P = 0{\cdot}004$)

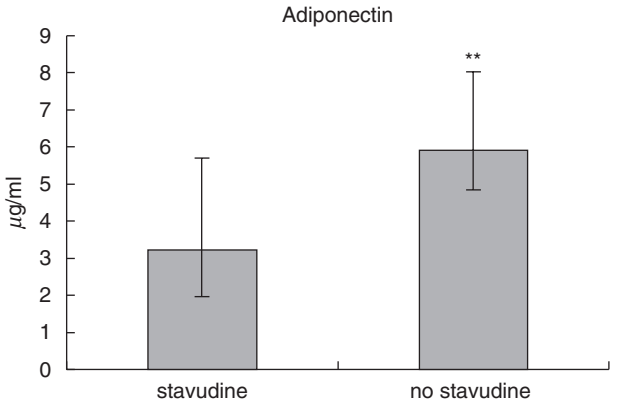

Fig. 2. Levels of circulating adiponectin in HIV-infected patients currently or previously treated with stavudine (stavudine) and HIV-infected patients never received stavudine (No stavudine) (**$P < 0{\cdot}01$).

(Fig. 2). When this result was adjusted for a possible type I error due to multiple comparisons (eight different drugs) by a Bonferoni correction the *P*-value was still significant ($P = 0{\cdot}03$). Furthermore, if we adjusted the *P*-value by a factor 16 due to that a *versus* c as well as a + b *versus* c were compared for the eight different drugs the *P*-value still tended to be significant ($P = 0{\cdot}06$). There was also a significant difference between patients currently or previously treated with abacavir (3·04 μg/ml (1·98–4·91) *versus* 5·59 μg/ml (3·42–7·85), $P = 0{\cdot}02$) (Data not shown). Since all patients in the abacavir group, received or had received stavudine, it was assumed that the findings of low adiponectin level in the abacavir group could be ascribed to an effect of stavudine. Stavudine was also strongly associated with the clinical diagnosis of lipodystrophy (Fisher's exact test). If the association between stavudine and adiponectin was adjusted for the effect of lipodystrophy in an ANOVA (including logadiponectin as the dependent variable) the effect of stavudine was no longer significant ($P = 0{\cdot}10$) whereas lipodystrophy still had a significant effect ($P = 0{\cdot}01$). The category of clinical lipodystrophy explained 26% (adjusted R^2) of the variability in adiponectin and the addition of stavudine intake added only 3% extra to this model (Data not shown). Accordingly, stavudine seemed to contribute importantly to lipodystrophy but it was not the only causative factor.

Metabolic parameters

The LD-FA group had increased level of insulin and HOMA-IR in comparison with the healthy group, whereas such a difference was not found for the LD-LA group. Plasma-triglycerides did not differ between the two LD groups, but was elevated compared to the healthy control group. Both LD groups had lower HDL compared to controls (Table 2).

Regarding LDL and glucose, there were no significant differences between any of the four groups.

Relationships between adiponectin and TNF-α, IL-6, lipid profile or insulin resistance

There was a negative correlation between adiponectin and triglycerides ($R_s = -0{\cdot}41$, $n = 26$, $P < 0{\cdot}05$) in the LD-group and in the total HIV positive group ($R_s = -0{\cdot}52$, $n = 39$, $P = 0{\cdot}002$), but not within the control group. A positive correlation was found between adiponectin and HDL in the total HIV positive group

($R_s = 0{\cdot}43$, $n = 39$, $P < 0{\cdot}02$) and in all subjects ($R_s = 0{\cdot}44$, $n = 48$, $P < 0{\cdot}02$).

There was no correlation between adiponectin on one hand and insulin, glucose and insulin resistance on the other, within neither the LD-group nor within the no-LD group. However, in the healthy HIV negative controls, a negative correlation existed between adiponectin and insulin ($R_s = -0{\cdot}79$, $n = 11$, $P < 0{\cdot}01$) and between adiponectin and HOMA-IR ($R_s = -0{\cdot}78$, $n = 11$, $P < 0{\cdot}01$).

Relationship between adiponectin, insulin resistance and body composition

In the total HIV positive group the adiponectin level correlated negatively with the percentage of truncal fat ($R_s = -0{\cdot}51$, $n = 31$, $P < 0{\cdot}01$) and with truncal to limb fat ratio ($R_s = -0{\cdot}48$, $n = 31$, $P < 0{\cdot}01$) and positively with the percentage of limb fat ($R_s = 0{\cdot}46$, $n = 31$, $P < 0{\cdot}01$).

HOMA-IR correlated positively with total trunk fat ($R_s = 0{\cdot}39$, $n = 41$, $P < 0{\cdot}05$) and with percentage trunk fat ($R_s = 0{\cdot}38$, $n = 41$, $P < 0{\cdot}05$).

DISCUSSION

The major finding in this study was that low level of adiponectin was found in patients with lipodystrophy, both with and without central fat accumulation. Low level of adiponectin was associated with low limb fat mass in accordance with previous findings [37]. In addition, we found an association between low adiponectin and high percentage of truncal fat. However, the causal relationship is most likely between adiponectin and limb fat, as truncal and visceral fat accumulation is probably a compensatory response to the loss of peripheral fat [8].

Low level of adiponectin was found especially in patients with previous or current stavudine treatment. Multiple linear regression analysis revealed that the variation in adiponectin was explained primarily by the clinical lipodystrophy diagnosis, reflecting an altered fat distribution rather than stavudine treatment per se. However, the low number of subjects in the different groups limits the final conclusion that can be made. Stavudine has been associated with a higher risk of developing lipodystrophy [9–11,14,15] and drug-induced mitochondrial dysfunction in adipocytes has been suggested as a potentiel mechanism for the induction of lipodystrophy by NRTI [42,43].

In a study by Hadigan *et al*. [6] it was demonstated that the rates of lipolysis were significantly increased in HIV-subjects with LD and use of stavudine was predictive of increased free fatty acid flux. Moreover, use of stavudine and the rate of lipolysis were strong predictors of insulin resistance. This effect could be promoted by low adiponectin, which in our study is correlated with LD and stavudine use. Stavudine could mediate this effect by increasing fatty acid β-oxidation as demonstrated in mice [44] and/or by enhancing the expression of the malic enzyme [45].

The TNF-α levels were elevated in the total HIV groups compared with healthy controls consistent with other findings [46,47], but there were no differences between HIV subgroups. However, other studies of HIV-infected patients with LD have shown that sTNFR are elevated compared to patients without LD [46,47] and that TNF-α gene polymorphism is a determinant in the development of HIV-related lipodystrophy [48]. In addition, experimental research suggests that the regulation of adiponectin and TNF-α are related [27–29]. STNFR binds TNF-α with high affinity and may act either as inhibitors of TNF-α or carriers of this cytokine [49]. Plasma levels of sTNFR are strongly linked to the local as well as the systemic production of TNF-α but sTNFR are more stable in the circulation and accordingly they are often a more consistent marker in the TNF system than circulating levels of TNF-α [49]. This may explain why there was no difference between plasma TNF-α levels in HIV subgroups in the present study. Measurement of insulin resistance was obtained using the homeostasis model HOMA-IR, which exclusively reflects liver insulin resistance. With this limitation, only patients with LD-FA differed from the healthy controls with regard to insulin resistance. In addition, truncal fat mass (and not limb fat mass) was associated with HOMA-IR. In agreement, a recent study also found that lipoatrophy was not associated with insulin resistance [50].

Low levels of adiponectin have been associated with insulin resistance in subjects without HIV [32,36,36,51,52]. In the present

Table 2. Biochemical and metabolic determinations

	Patients with LD			
Characteristics	LD-FA ($n = 12$)	LD-LA ($n = 15$)	no LD ($n = 15$)	Healthy controls ($n = 12$)
Cholesterol(mmol/l)	6·3 (5·2–7·0)	6·0 (5·3–7·1)	5·9 (4·7–6·6)	5·8 (4·5–6·5)
HDL (mmol/l)	1·0 (0·9–1·1)	1·1 (0·9–1·3)	1·2 (1·0–1·5)	1·3 (1·15–1·65)††
LDL (mmol/l)	4·3 (3·3–4·9)	4·0 (3·1–5·0)	3·9 (2·9–4·9)	3·7 (3·0–4·6)
Triglycerides(mmol/l)	2·5 (1·9–3·5)	2·5 (2·3–3·5)	2·5 (1·3–2·5)*	1·1 (0·7–2·4)†††
Glucose (mmol/l)	5·0 (4·7–5·4)	4·9 (4·7–5·3)	5·0 (4·8–5·5)	4·8 (4·6–5·0)
Insulin (ρmol/l)	97·5 (64·5–110·5)	55·0 (38·0–90·0)	55·0 (43·5–74·0)	34·5 (20·0–94·0)‡‡
IR-HOMA	3·74 (2·15–4·58)	2·0 (1·55–3·6)	2·02 (1·67–2·84)	1·12 (0·71–3·36)‡‡

Results expressed are median and 25% and 75% quartiles; LD, lipodystrophy; LD-FA, HIV-infected patients with lipodystrophy with fat accumulation; LD-LA, HIV-infected patients with lipodystrophy without fat accumulation; no-LD, HIV-infected patients without lipodystrophy; Controls IR-HOMA, insulin resistance as measured by homeostasis mode of assessment [39]; LDL, low density lipoprotein; HDL, high density lipoprotein. (controls). LD-FA and LD-LA did not differ with regards to the lipid profile. * denotes significant difference (*$P < 0{\cdot}05$) between LD-FA/LD-LA and no-LD; † denotes significant difference († = $P < 0{\cdot}05$; †† = $P < 0{\cdot}01$, ††† = $P < 0{\cdot}001$) between LD-FA/LD-LA and controls; ‡ denotes significant difference (‡‡ = $P < 0{\cdot}01$) between LD-FA and controls.

study, we confirmed a negative relationship between adiponectin and insulin resistance measured by HOMA in the healthy controls, whereas we were not able to confirm such a relationship within the HIV patients. In patients with LD, the level of adiponectin is linked to fat atrophy rather than to insulin resistance. Although, TNF-α has been suggested to induce insulin resistance [53] and IL-6 to counteract the effects of TNF-α [54,55], altered levels of these cytokines did not explain the insulin resistance in the LD-FA group.

We further found a positive correlation between adiponectin and HDL in HIV-infected patients and a negative correlation between adiponectin and triglycerides in patients with lipodystrophy as has been shown before in patients with lipodystrophy [36], dyslipidaemia [35] and in healthy humans [52].

In conclusion, low levels of adiponectin are associated with lipodystrophy and linked to treatment with stavudine. The finding of no difference between LD-FA and LD-LA with regard to adiponectin indicates that it is the low limb, more than the truncal fat mass, that is the link to the low adiponectin level. Only patients with LD-FA had insulin resistance measured by HOMA. The latter was related to truncal fat mass, but not explained by adiponectin or other cytokines.

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