Natural Course of Nonalcoholic Fatty Liver Disease and Type 2 Diabetes in Patients With Human Immunodeficiency Virus With and Without Combination Antiretroviral Therapy-associated Lipodystrophy : A 16-Year Follow-up Study

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Natural course of NAFLD and type 2 diabetes in HIV-positive subjects with and without combination antiretroviral therapy (cART)-associated lipodystrophy: a 16-year follow-up study

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Summary: We determined the natural course of NAFLD and type 2 diabetes in HIV-positive subjects during 16 years. Diabetes develops more frequently in HIV-positive than in control subjects. Progression of NAFLD is rare compared to development of diabetes in HIV-positive subjects.

ABSTRACT

Background and aims: Abnormal glucose metabolism and non-alcoholic fatty liver disease (NAFLD) are common in HIV-positive (HIV+) subjects but longitudinal data are lacking. We determined the natural course of NAFLD (liver fat, LFAT) and type 2 diabetes (T2DM) in HIV+ subjects with and without lipodystrophy (LD) during a 16-year longitudinal study.

Methods: LFAT [by proton magnetic resonance spectroscopy (^{1}H-MRS)], and clinical characteristics were measured in 41 HIV+ subjects at baseline and after 16 years. Liver fibrosis was estimated by measuring liver stiffness using transient elastography (TE) and magnetic resonance elastography (MRE) at 16 years. We also longitudinally studied 28 healthy control subjects.

Results: During follow-up, the HIV+ group gained more body fat (8.6±0.7%) than the control subjects (4.5±0.6%, $p<0.001$). Features of insulin resistance (fasting glucose, insulin and HOMA-IR) increased significantly in the HIV+ but not the control subjects. A significant proportion (20%, $p<0.01$ vs. 0% at baseline) of the HIV+ but none of the control subjects developed T2DM. LFAT was significantly higher at baseline in the LD+ (4.3, 1.9-11.8) than the LD- (1.0, 0.5-1.5; $p<0.001$) HIV+ subjects. LFAT remained stable during follow-up in all groups. At follow-up, liver stiffness measured with TE was similar between all HIV, LD+, and LD- and control subjects, and between the LD+ and LD- subjects measured with MRE. Advanced fibrosis by MRE was observed in 3 of LD+ and none of LD- subjects.

Conclusion: During 16-years of follow-up, progression of NAFLD is rare compared to development of T2DM in HIV+ subjects.

Keywords: human immunodeficiency virus, non-alcoholic fatty liver disease, glucose metabolism, magnetic resonance elastography, liver fibrosis

ABBREVIATIONS

ALT	Alanine aminotransferase
ART	Antiretroviral treatment
BMI	Body mass index
cART	Combination antiretroviral therapy
HbA_{1c}	Glycosylated hemoglobin A_{1c}
HDL	High-density lipoprotein
hs-CRP	High-sensitivity C-reactive protein
HIV+	Human immunodeficiency virus-positive
^{1}H-MRS	Proton magnetic resonance spectroscopy
HOMA-IR	Homeostasis model assessment of insulin resistance
LD	Lipodystrophy
LD+	Presence of lipodystrophy
LD-	Absence of lipodystrophy
LDL	Low-density lipoprotein
LFAT	Liver fat
MRE	Magnetic resonance elastography
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
OGTT	Oral glucose tolerance test
ROIs	Regions of interest
T2DM	Type 2 diabetes
TE	Transient elastography
tNRTI	Thymidine analogue nucleoside reverse transcriptase inhibitor

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as a common cause of significant liver disease in human immunodeficiency virus-positive (HIV+) subjects [1]. In cross-sectional studies, the prevalence of NAFLD diagnosed by imaging methods may be more common in HIV+ subjects (35%) than the general population (25%) [2, 3] but data are not consistent [4]. In the MACS study, HIV+ subjects had a higher prevalence of features of insulin resistance (high HOMA-IR, low HDL cholesterol, high triglycerides) but paradoxically lower liver fat content than HIV-uninfected equally obese controls [4].

HIV+ subjects with lifelong exposure to combination antiretroviral therapy (cART), especially thymidine analogue nucleoside reverse transcriptase inhibitors (tNRTIs) (stavudine and zidovudine), have an increased risk of lipodystrophy (LD) [5]. Newer antiretroviral agents with a more favorable profile on metabolic parameters and body composition [6] have replaced older more toxic drugs in industrialized countries [7]. LD induced by antiretroviral drugs is characterized by lipoatrophy, i.e. loss of subcutaneous adipose tissue with or without lipohypertrophy intra-abdominally and/or in dorsocervical region and all features of insulin resistance including increased liver fat (LFAT) [8]. We previously reported that HIV+ subjects with LD had higher LFAT measured by proton magnetic resonance spectroscopy (^{1}H-MRS) and higher serum insulin and C-peptide concentration indicating disturbed glucose metabolism than those without [8]. Such individuals might be at risk for both type 2 diabetes and liver disease but there are no long-term longitudinal studies addressing the natural course of NAFLD and diabetes in HIV+ subjects.

In the present study, we examined a total of 41 HIV+ subjects at baseline and after a 16-year period of follow-up. A total of 28 healthy control subjects were also studied

longitudinally. The aim was to determine the natural course of NAFLD (LFAT by ^{1}H-MRS) and diabetes in HIV+ subjects with and without LD as compared to controls. At follow-up, liver fibrosis was also estimated using elastographic techniques [9].

SUBJECTS AND METHODS

Study subjects

We contacted 47 previously studied HIV+ and 32 healthy control subjects [8, 10] to participate in a follow-up study, which was similar to a metabolic and imaging (^{1}H-MRS) study performed 16 years earlier. In addition, liver fibrosis was assessed by measuring stiffness by transient elastography (TE) in all subjects and by magnetic resonance elastography (MRE) in the HIV+ subjects at follow-up. The HIV+ subjects had been originally recruited from the HIV-outpatient clinic of the Helsinki University Hospital in the years 2001-2004 using the inclusion and exclusion criteria described in detail [8, 10]. The subjects were sub-grouped based on the presence (LD+) or absence (LD-) of LD at baseline. LD was defined as self-reported symptoms of subcutaneous fat loss with/without increased girth or buffalo hump [8]. Control subjects were recruited from occupational health services in the years 1999-2001 and were healthy as judged by history, physical examination and standard laboratory tests, and did not use any medications [8, 10].

The study was conducted in accordance with the Declaration of Helsinki. Each participant provided written informed consent after being explained the nature and potential risks of the study. The ethics committee of the Helsinki University Hospital approved the study protocol.

Study design

At the first follow-up visit, all subjects underwent a history and physical examination including measurements of body composition and liver stiffness using TE. At this visit, fasting blood samples were obtained for biochemical measurements and a 2-hour oral glucose tolerance test (OGTT) was performed except in patients with known diabetes. Subjects were re-screened for hepatitis B (serum HBsAg) and C (serum HCVAb) at the

time of the follow-up visit. Alcohol use was quantified by structural interview and expressed as doses (12 grams of alcohol) per week. At the second visit, all subjects underwent measurement of LFAT using ^{1}H-MRS. At the third visit, MRE was performed in the HIV+ subjects. The duration of follow-up was slightly shorter in the control [12.3 (range: 9.9-13.4) years] than the HIV+ [15.7 (range: 12.3-16.4) years] subjects as they participated in a larger follow-up study reported recently [11].

Measures of body composition

Measurements of whole-body fat, waist-to-hip ratio, body mass index (BMI) and skinfolds are described in supplementary material. The methods and devices used were identical at baseline and follow-up.

Measurement of LFAT using proton magnetic resonance spectroscopy (^{1}H-MRS)

LFAT content was measured using two generations of 1.5 Tesla clinical scanners (Magnetom Vision and Avanto^{fit}, Siemens Healthcare Diagnostics, Erlangen, Germany). NAFLD was defined as LFAT exceeding 5.56% as in the Dallas Heart Study [12]. Detailed technical information is described in supplementary material.

Measurement of liver stiffness using transient elastography (TE) and magnetic resonance elastography (MRE)

Transient elastography. Liver stiffness was measured using TE (Fibroscan, Echosens, Paris, France) after an overnight fast. Two physicians (SLB and EI) performed measurements using the same protocol. Detailed technical information is described in

supplementary material. Liver stiffness data are expressed as the median of 10 successful measurements in kilopascal (kPa). TE measurement failed with both probes in 2 HIV+ and 5 control subjects. We used the cut-off value of 8.7 kPa for to estimate clinically significant stage 3-4 fibrosis [13].

Magnetic resonance elastography. MRE was performed on a 1.5 Tesla GE Signa HDxt magnetic resonance scanner (GE Healthcare, Milwaukee, WI) using commercially available software and hardware (Resoundant Inc., Rochester, MN). The results are expressed as the median of regions of interest at the four elastograms in kilopascal (kPa). We used the cut-off value of 3.62 kPa for to estimate clinically significant stage 3-4 fibrosis [9]. Detailed technical information is described in supplementary material.

Analytical procedures and oral glucose tolerance test (OGTT)

Details of the biochemical measurements performed at baseline and at follow-up are described in supplementary material. Homeostasis model assessment of insulin resistance (HOMA-IR) was used as a proxy for insulin resistance [14]. At the follow-up visit, a 75-g OGTT was performed, during which glucose and insulin concentrations at 0, 30, 60 and 120 minutes were measured. Diagnostic criteria for diabetes and prediabetes are described in supplementary material.

Statistical analysis

Data are shown as mean ± standard error of mean if normally distributed and as median followed by 25^{th} and 75^{th} percentiles if non-normally distributed. OGTT results between three groups were compared using Kruskal-Wallis test followed by Dunn's multiple comparison test. Changes over time in the HIV+ and control subjects were adjusted for

duration of follow-up using analysis of covariance (ANCOVA). To analyse which factors are independently associated with changes in features of insulin resistance during a follow-up period, ANCOVA with HIV-status as the fixed factor, and baseline value and change in body fat% or weight as covariates was used. For categorical variables, changes over time were compared using McNemar's test. Pearson's correlation coefficient and binary logistic regression analyses were used to identify univariate predictors of LFAT and fibrosis. Variables correlating with LFAT or fibrosis at a significance level of <0.1 in univariate linear analyses were entered in stepwise multiple linear regression analyses. If variables in univariate analysis reflected the same biological process, only one variable most closely associated with the outcome of interest was included. A *p*-value of <0.05 was considered statistically significant. Additional statistical information is described in supplementary material.

RESULTS

Physical and biochemical features

HIV+ vs. control subjects at baseline. Of the HIV+ and control subjects, 41 (87%) and 28 (88%) agreed to participate in the follow-up study (Suppl. Fig. 1). Baseline characteristics of the HIV+ and control subjects are shown in Table 1. At baseline, the HIV+ subjects had a significantly lower % whole-body fat and higher waist-to-hip ratio than the control subjects despite similar BMI, age and gender (Table 1). Concentrations of fasting insulin, triglycerides and liver enzymes were higher in the HIV+ than the control subjects (Table 1).

Changes in body composition and features of insulin resistance during follow-up. During follow-up, the HIV+ group gained significantly more body fat (8.6±0.7%) than the control subjects (4.5±0.6%, $p<0.001$) (Table 1). Body weight increased almost 2-fold more in the HIV+ than the control subjects, which was marginally significant ($p=0.06$). Insulin and HOMA-IR increased significantly in the HIV+ but not the control subjects (Table 1). These increases were explained by the change in body fat% ($p=0.015$ for insulin and $p=0.005$ for HOMA-IR), not HIV-status or baseline values. Regarding the increase in fasting glucose, both HIV-status ($p=0.001$), baseline glucose ($p=0.007$) and change in body fat% ($p=0.03$) were significant independent determinants.

LD+ (n=30) vs. LD- (n=11) groups. At baseline, the LD+ group was more insulin-resistant than the LD- group (Suppl. Table 1). At the follow-up visit, the duration of the HIV infection was comparable between the LD+ and LD- groups (23.5±0.7 vs. 22.4±1.6 years). Of the LD+ group, 86% had been exposed to stavudine during their lifetime as compared to 18% in the LD- group ($p<0.001$). Zidovudine exposure was similar (100% vs. 90%, respectively). At follow-up, 20 out of 30 LD+ subjects still reported symptoms of LD. Additional characteristics of the LD+ vs. LD- group are shown in Suppl. Table 1 and 2.

During the 16-year follow-up period, the thicknesses of all four skinfolds increased in the LD+ group and were not significantly different from those in the LD- group at the time of the follow-up visit. At follow-up, a greater proportion of the LD+ (60%) than the LD- (18%, p=0.03) or the control (31%, p=0.04) subjects were using statins (Fig. 1A).

Type 2 diabetes (T2DM) and prediabetes

HIV+ vs. control subjects. No subject had T2DM at baseline (Fig. 1B). The HIV+ subjects developed T2DM (20 %, p<0.01 for follow-up vs. baseline) significantly more often than the control subjects (0 %) during follow-up period (Fig. 1B). During the OGTT at follow-up, the HIV+ subjects had higher glucose and insulin concentrations than the control subjects at 0, 60 and 120 min (Fig. 2) and the HIV+ subjects had a significantly more prediabetes (60%) than the control subjects (17%, p=0.002).

LD+ vs. LD- groups. A significant increase in the proportion of T2DM was observed in the LD+ but not the LD- group at follow-up as compared to baseline (Fig. 1B). During the OGTT, the LD+ group had significantly higher insulin concentrations than the LD- group at 120 min (Fig. 2). The prevalence of prediabetes was similar in the LD+ (63%) and LD- (50%) groups.

Predictors of T2DM. Baseline measures of obesity and insulin resistance predicted T2DM 16 years later (Table 2). In multiple logistic regression analysis, baseline waist circumference remained an independent predictor of T2DM at 16 years (Table 3).

NAFLD

At baseline, LFAT was significantly higher in the LD+ [4.3 (1.9-11.8)%] than the LD- [1.0 (0.5-1.5)%, p<0.001] group (Suppl. Table 1) and the control subjects [1.9 (1.0-3.3), p=0.004]. Median LFAT remained stable during follow-up in both the HIV+ (Table 1 and Suppl. Table 1) and control subjects. Of the HIV+ subjects, 35 vs. 32% had NAFLD at baseline vs. follow-up and of the control subjects 18% vs. 17%, respectively. Median LFAT remained stable also in the LD+ and LD- groups (Suppl. Table 1).

The change in body weight during follow-up was significantly correlated with that in LFAT both in HIV+ (r=0.47, p<0.005) and control (r=0.47, p<0.05) subjects. The slopes and intercepts of the linear regression lines relating changes in body weight and LFAT were similar in all groups (Fig. 3A). Baseline LFAT correlated with LFAT at follow-up in both HIV+ and control subjects (Fig. 3B, Table 4).

In univariate analysis, baseline measures of obesity and insulin resistance and baseline LFAT correlated with LFAT at 16 years (Table 4). In multiple linear regression analysis, baseline alanine aminotransferase (ALT) and the waist-to-hip ratio remained independent correlatives of LFAT at 16 years (Table 5).

Liver fibrosis

Of the HIV-positive subjects who died during follow-up (Suppl. Fig. 1), one death was attributed to complications of NASH cirrhosis.

TE and MRE at follow-up. Liver stiffness measured with TE was similar between all HIV+ subjects [5.2 (4.1, 6.6) kPa], LD+ [5.6 (4.2, 7.2) kPa] and LD- [4.7 (3.9, 5.2) kPa] and control [6.3 (4.4, 10.0) kPa] subjects. Liver stiffness measured with MRE averaged 2.49

(2.27, 3.04) kPa in HIV+ subjects with no difference between LD+ [2.47 (2.26, 3.07) kPa] and LD- [(2.51 (2.35, 2.82) kPa] subjects. Advanced fibrosis estimated by MRE was observed in 3 of LD+ and none of LD- subjects (p=0.54). Measures of obesity and features of insulin resistance including HOMA-IR, high-sensitivity C-reactive protein (hs-CRP), and baseline LFAT were significant predictors of liver fibrosis estimated by MRE at 16 years (Table 4). The duration of HIV-infection correlated significantly with liver fibrosis (r=0.36, p=0.03) estimated by MRE. In multiple linear regression analysis, baseline BMI and LFAT remained the best predictors of liver fibrosis measured by MRE at 16 years (Table 5).

DISCUSSION

We determined the natural course of insulin resistance, diabetes and NAFLD in HIV+ subjects. Insulin resistance increased over time more in the HIV+ than the control subjects due to a greater increase in body fat over 16 years in the HIV+ than the control group. The HIV+ subjects developed more type 2 diabetes than the control subjects. HIV+ status, baseline glycemia and change in body fat all independently contributed to the increase in glycemia over 16 years. The increase in diabetes and also statin use (Fig. 1A) was especially observed in the LD+ group (Fig. 1B). Median LFAT was similar at baseline and follow-up. Of the initial cohort, one patient died of NASH cirrhosis but clinically significant fibrosis was not more frequent in the HIV+ than the control subjects or between LD+ vs. LD- subjects. These data show that LFAT remains stable in HIV+ and control subjects and that T2DM is a more frequent complication than advanced liver fibrosis in HIV-positive subjects.

At baseline, most of the participants were taking tNRTIs which were replaced during follow-up by less toxic antiretroviral agents. Skinfold thickened at all originally lipoatrophic anatomic sites (biceps, triceps, thigh), in line with earlier data [15] and most likely because of drug switches. The increase in body fat was higher during follow-up in the HIV+ than the control subjects. This seemed to explain the increase in insulin resistance, while both baseline glucose, HIV-status and the body fat gain seemed significant associates of changes in glycemia. The increase in diabetes was significant in the entire HIV+ group and the LD+ but not the LD- group consistent with cross-sectional studies [16] and confirm longitudinal data from the Data Collection on Adverse Events of anti-HIV drugs [17].

Aging and potentially accelerated aging may be involved in the pathogenesis of comorbidities caused by HIV and/or ART [18]. We observed significant differences in the

incidence of diabetes and the response to OGTT between the two HIV+ study groups (LD+ vs LD-) but not between LD- and HIV- groups, independent of age. If these differences were driven by accelerated aging in the LD+ group, it cannot be explained by HIV infection per se, but the more toxic ART agents and/or lipodystrophy in the LD+ group.

Median LFAT remained unchanged in HIV+ including LD+ and LD- and control subjects. LFAT was attributable to NAFLD as alcohol consumption was within healthy limits and comparable between all groups and the subjects did not have evidence of other causes of steatosis including viral hepatitis. Because of multiple changes in drug regimens during follow-up, effects of individual ARTs on the change in LFAT could not be evaluated. The change in body weight correlated significantly with that in LFAT (Fig. 3), and this relationship was similar in all groups. Thus, weight loss might be equally beneficial for HIV+ and control subjects and weight gain equally harmful in increasing LFAT, regardless of changes in antiretroviral agents in HIV+ subjects. It is interesting that LFAT remained unchanged in the HIV+ group during follow-up although insulin, glucose and HOMA-IR increased significantly. One could have predicted LFAT to increase concomitant with insulin [19]. Possibly, the increase in insulin reflected fat accumulation in the periphery rather than in the liver. Consistent with this, we have previously shown the intercept of the relationship between fasting insulin and liver fat to be higher in LD+ than LD- subjects [8].

We found liver stiffness to be similar in all groups at follow-up. This suggests that the risk of liver fibrosis unlike that of diabetes was not increased in the HIV+ as compared to the control subjects. Of note, methods to determine non-invasively liver stiffness were not available 16 years ago. Baseline associates of liver stiffness at follow-up included abdominal obesity, LFAT, insulin and hs-CRP. These data closely resemble those of non-HIV subjects [20][21]. In non-HIV-positive subjects, fibrosis has been estimated to progress

by one stage in 14 years starting from steatosis [22]. This implies that even 16 years of follow-up may be too short to observe appearance of advanced fibrosis.

We conclude that HIV+ subjects, especially those with LD, are at increased risk of developing T2DM compared to control subjects (Fig. 1B). Independent associates of this risk were HIV-status, baseline glycemia and change in body fat, which was greater in the HIV+ than the control group. For the same reason, *i.e.* change in body fat especially peripherally at former lipoatrophic sites, the HIV+ subjects also became more insulin resistant than control subjects during follow-up. LFAT remained unchanged in all groups during follow-up. The relationship between changes in body weight and LFAT was similar in the HIV+ and control subjects suggesting that lifestyle changes are likely to be equally effective in both groups (Fig. 3). Since obesity is rapidly increasing among HIV+ persons, the risk for future diabetes is highly significant. Although NAFLD is prevalent in both HIV+ and control subjects [2, 3] and can progress to fibrosis [22], the present measurements of liver stiffness at the time of follow-up suggest that advanced fibrosis is a very rare complication compared to type 2 diabetes in HIV+ subjects.

NOTES

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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Table 1. Characteristics of the study subjects at baseline and follow-up.

	HIV+ (n=41)			Healthy controls (n=28)		
	Baseline	Follow-up	Change	Baseline	Follow-up	Change
Gender (*male/female*)	34/7	-	-	24/4	-	-
Age (*years*)	41.9±1.3	56.5±1.3	14.6±0.3***	42.9±1.6	55.2±1.5	12.3±0.2***
Weight (*kg*)	71.9±1.7	80.0±2.4	8.1±1.5***	76.2±1.6	80.8±1.9	4.3±1.0***
BMI (kg/m^2)	23.1±0.5	25.8±0.7	2.7±0.5***	23.7±0.4	25.1±0.5	1.3±0.3***
Waist-to-hip ratio	0.96±0.01	0.99±0.01	0.04±0.01***	0.91±0.01^	0.93±0.02^^	0.01±0.01
Liver fat content (%)	3.0 (1.0-9.0)	3.7 (1.0-9.9)	-0.1 (-5.5-4.1)	1.9 (1.0-3.3)	1.9 (0.7-3.9)	0.0 (-1.0-0.7)
Total body fat (%)	17.0±1.1	25.6±1.0	8.6±0.7***	20.5±0.9^	25.1±0.8	4.5±0.6***, aa
Biceps skinfold (*mm*)	3.3 (2.7-4.4)	5.6 (4.1-8.2)	1.8 (0.4-3.0)***	-	-	-
Triceps skinfold (*mm*)	4.7 (3.8-6.6)	7.3 (5.0-10.1)	2.3 (0.8-4.1)***	-	-	-
Scapula skinfold (*mm*)	13.1 (10.0-21.2)	16.9 (13.4-24.1)	2.4 (-1.7-7.6)*	-	-	-
Thigh skinfold (*mm*)	4.2 (3.7-6.2)	6.0 (4.9-7.8)	1.4 (-0.4-3.0)*	-	-	-
Systolic BP (*mmHg*)	123±2	143±2	20±2***	131±3^	142±4	11±3**
Diastolic BP (*mmHg*)	78±1	86±2	8±2***	85±2^^	91±2	6±2**
Alcohol consumption (*12g/week*)	3.5 (0.8-9.0)	2.0 (0.0-12.0)	0.0 (-3.0-2.0)	4.0 (2.0-6.0)	5.5 (1.0-12.0)	0.5 (-1.0-4.0)
fP-Glucose (*mmol/l*)	5.0 (4.8-5.4)	5.8 (5.5-6.7)	0.7 (0.2-1.4)***	5.4 (5.1-5.7)	5.2 (4.9-5.5)^^^	-0.3 (-0.4-0.0) aa
fS-Insulin (*mU/l*)	6.0 (4.3-12.7)	10.0 (6.6-16.4)	3.8 (-1.0-10.3)*	5.0 (3.5-7.0)^	4.4 (3.2-6.9)^^^	-0.4 (-1.8-1.3) aa
HOMA-IR	1.43 (1.02-3.02)	2.73 (1.60-4.62)	1.2 (-0.1-2.9)*	1.13 (0.80-1.77)	1.08 (0.70-1.70)^^	-0.1 (-0.4-0.3) aa
HbA_{1c} (*%*)	4.9±0.1	5.4±0.1	0.5 (0.1-0.8)***	5.5±0.1	5.4±0.1	-0.1 (-0.3-0.1)* , aa
S-ALT (*U/l*)	30 (23-50)	30 (22-62)	1.0 (-7.5-13.5)	23 (19-33)^	26 (21-36)	1.0 (-5.8-6.0) a
S-AST (*U/l*)	32 (28-44)	31 (24-43)	-3.5 (-8.0-9.0)	25 (21-34)^	28 (25-36)	2.0 (0.0-9.3)*
S-GT (*U/l*)	42 (28-69)	44 (28-131)	10.0 (-7.0-62.8)	19 (15-29)^^^	21 (17-41)^^	3.5 (-0.8-10.5)
fP-Triglycerides (*mmol/l*)	1.9 (1.6-3.3)	1.4 (1.0-2.0)	-0.5 (-1.2-0.0)***	1.0 (0.7-1.2)^^^	0.9 (0.7-1.3)^	0.0 (-0.2-0.4) aa
fP-HDL cholesterol (*mmol/l*)	1.3 (1.0-1.6)	1.3 (1.1-1.6)	0.1 (-0.2-0.3)	1.3 (1.2-1.6)	1.3 (1.2-1.6)	0.1 (-0.1-0.2)
fP-LDL cholesterol (*mmol/l*)	3.1±0.2	2.8±0.1	-0.2±0.2	3.2±0.1	3.2±0.1	0.0±0.2
S-hs-CRP (*mg/l*)	0.76 (0.40-1.75)	1.59 (0.70-3.1)	0.45 (-0.1-1.9)*	-	-	-
White blood cell count ($10^9/l$)	5.4±0.3	5.5±0.2	0.3±0.3	5.1±0.3	5.1±0.2	0.0±0.2

Data shown as median (25-75%) or mean±SEM. Comparison within group between baseline and follow-up: *p<0.05; **p<0.005; ***p<0.0005. Comparison of HIV+ to healthy controls at the corresponding time points: ^p<0.05; ^^p<0.005; ^^^p<0.0005. Comparison of change during a follow-up in HIV+ and healthy controls adjusted for follow-up time: [a]p<0.05, [aa]p<0.001 .

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; fP, fasting plasma; fS, fasting serum; GT, gamma-glutamyl transferase; HbA_{1C}, glycosylated hemoglobin A_{1C}; HDL, high-density lipoprotein; HIV+, human immunodeficiency virus-positive subjects; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high- sensitivity C-reactive protein; LDL, low-density lipoprotein; NS, non-significant; S, serum.

Table 2. Baseline predictors of type 2 diabetes in HIV+, LD+ and all subjects at follow-up (bivariate logistic regression).

Baseline parameters	**HIV+**		**All** (HIV+/controls)	
	OR	95% CI	OR	95% CI
Gender (*female/male*)	1.56	0.16-15.12	1.37	0.15-12.42
Age (*years*)	1.05	0.96-1.15	1.04	0.95-1.14
Lipodystrophy (*no/yes*)	3.04	0.33-28.10	-	-
Weight (*kg*)	1.18	1.06-1.32**	1.16	1.04-1.29*
BMI (kg/m^2)	1.87	1.21-2.89**	1.93	1.23-3.02*
Waist (*cm*)	1.27	1.08-1.48**	1.24	1.08-1.43**
Waist-to-hip ratio (*per 0.01*)	1.29	1.07-1.57*	1.34	1.10-1.63**
Liver fat content (%)	1.03	0.95-1.11	1.04	0.96-1.12
Total body fat (%)	1.11	0.98-1.26	1.07	0.95-1.21
Biceps skinfold (*mm*)	1.35	0.96-1.89	-	-
Triceps skinfold (*mm*)	1.05	0.82-1.35	-	-
Scapula skinfold (*mm*)	1.08	0.99-1.17	-	-
Thigh skinfold (*mm*)	0.97	0.83-1.14	-	-
Systolic BP (*mmHg*)	1.00	0.93-1.08	0.98	0.92-1.04
Diastolic BP (*mmHg*)	1.05	0.93-1.18	0.98	0.91-1.06
Alcohol consumption (*12g/week*)	1.01	0.94-1.09	1.02	0.94-1.10
fP-Glucose (*mmol/l*)	1.83	0.35-9.45	1.08	0.20-5.74
fS-Insulin (*mU/l*)	1.17	1.04-1.33*	1.22	1.08-1.38**
HOMA-IR	1.92	1.17-3.16*	2.26	1.37-3.72**
HbA_{1c} (%)	1.23	0.25-6.02	0.44	0.12-1.62
S-ALT (*U/l*)	1.03	1.00-1.06	1.04	1.01-1.07*
S-AST (*U/l*)	1.00	0.95-1.05	1.03	0.98-1.08
S-GT (*U/l*)	1.00	0.99-1.02	1.01	1.00-1.02
fP-Triglycerides (*mmol/l*)	1.46	1.00-2.12*	1.70	1.17-2.46*
fP-HDL cholesterol (*mmol/l*)	0.01	0.00-0.46*	0.01	0.00-0.17**
fP-LDL cholesterol (*mmol/l*)	0.72	0.26-2.01	0.64	0.21-1.94
S-hS-CRP (*mg/l*)	1.31	0.68-2.53	-	-
White blood cell count ($10^9/l$)	1.58	0.90-2.78	1.54	0.98-2.41
Duration of HIV infection (*years*)	1.01	0.84-1.21	-	-
Duration of tNRTI medication (*years*)	1.07	0.87-1.31	-	-
Duration of d4T medication (*years*)	1.00	0.75-1.34	-	-
Duration of AZT mediation (*years*)	1.00	1.00-1.00	-	-

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AZT, zidovudine; BMI, body mass index; BP, blood pressure; d4T, stavudine; CI, confidence interval; fP, fasting plasma; fS, fasting serum; GT, gamma-glutamyl transferase; HbA_{1C}, glycosylated hemoglobin A_{1C}; HDL, high-density lipoprotein; HIV+, human immunodeficiency virus-positive subjects; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high- sensitivity C-reactive protein; LDL, low-density lipoprotein; LD+, HIV-positive subjects with lipodystrophy; OR, odds ratio; S, serum; tNRTI, thymidine analogue nucleoside reverse transcriptase inhibitor.

Table 3. Multiple logistic regression model to predict type 2 diabetes at 16 years in HIV+ subjects.						
	R^2	OR	IQR	P-value	B	SE
Predictors of type 2 diabetes	0.53, p<0.001					
Baseline waist circumference		1.27	1.08-1.48	0.004	0.235	0.081
Constant				0.003	-22.940	7.670

Logistic regression analysis: Backward LR. Model included baseline BMI, waist circumference, HOMA-IR and fasting plasma triglyceride and HDL cholesterol concentrations.

Abbreviations: B, coefficient; IQR, interquartile range; OR, odds ratio; R^2 , Nagelkerke *R* square; SE, standard error.

Table 4. Baseline predictors of liver fat content, liver fibrosis estimated by MRE and advanced fibrosis estimated by TE in HIV+ subjects at follow-up (Pearson's correlation coefficient and binary logistic regression).

	Liver fat (%)		Liver stiffness (kPa, MRE)		Advanced fibrosis (TE)	
Baseline parameters	*r*	P-value	*r*	P-value	OR	95% CI
Gender (male)	0.26	0.11	0.21	0.20	0.56	0.05-6.5
Age (*years*)	0.06	0.72	0.24	0.15	0.95	0.81-1.11
Weight (*kg*)	0.42	0.007	0.29	0.08	1.02	0.93-1.12
BMI (kg/m^2)	0.42	0.007	0.40	0.01	1.09	0.79-1.52
Waist (*cm*)	0.54	<0.001	0.36	0.03	1.04	0.94-1.16
Waist-to-hip ratio	0.58	<0.001	0.32	0.047	1.04 [a]	0.92-1.19
Liver fat content (%)*	0.39	0.01	0.38	0.02	1.03	0.93-1.13
Total body fat (%)	0.09	0.58	-0.06	0.73	1.02	0.87-1.19
Biceps skinfold (*mm*)*	0.10	0.55	0.16	0.34	1.06	0.68-1.65
Triceps skinfold (*mm*)*	-0.10	0.53	0.08	0.63	1.08	0.80-1.46
Scapula skinfold (*mm*)*	0.26	0.11	0.20	0.23	1.06	0.95-1.18
Thigh skinfold (*mm*)*	-0.16	0.32	-0.06	0.72	0.96	0.77-1.21
Systolic BP (*mmHg*)	0.13	0.45	0.31	0.07	0.99	0.90-1.10
Diastolic BP (*mmHg*)	0.09	0.59	0.36	0.04	0.96	0.83-1.12
Alcohol consumption (*12g/week*)*	0.25	0.15	-0.11	0.56	0.88	0.68-1.15
fP-Glucose (*mmol/l*)*	0.22	0.19	0.30	0.07	1.60	0.19-13.44
fS-Insulin (*mU/l*)*	0.25	0.13	0.35	0.03	0.93	0.74-1.15
HOMA-IR*	0.40	0.001	0.37	0.02	0.77	0.33-1.81
HbA_{1c} (*%*)	0.01	0.97	0.09	0.58	1.32	0.16-10.93
S-ALT (*U/l*)*	0.53	<0.001	0.22	0.19	0.98	0.92-1.04
S-AST (*U/l*)*	0.41	0.04	0.25	0.23	0.96	0.86-1.07
S-GT (*U/l*)	0.58	0.001	0.38	0.06	0.97	0.91-1.03
fP-Triglycerides (*mmol/l*)*	0.56	<0.001	0.14	0.41	0.84	0.43-1.62
fP-HDL cholesterol (*mmol/l*)*	-0.37	0.02	-0.25	0.13	1.10	0.08-15.89
fP-LDL cholesterol (*mmol/l*)	-0.15	0.42	-0.10	0.56	0.44	0.11-1.89
S-hS-CRP (*mg/l*)*	0.23	0.19	0.33	0.05	1.24	0.56-2.71
White blood cell count ($10^9/l$)	0.17	0.35	0.21	0.24	1.08	0.63-1.86

*Logarithmic transformation for Pearson correlation coefficient analysis. [a], per +0.01. Liver stiffness was measured using TE and MRE to non-invasively estimate liver fibrosis. We used the cut-off value of 8.7 kPa for to estimate clinically significant advanced fibrosis by TE.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; CI, confidence interval; fP, fasting plasma; fS, fasting serum; GT, gamma-glutamyl transferase; HbA_{1c}, glycosylated hemoglobin A_{1c}; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MRE, magnetic resonance elastography; OR, odds ratio; *r*, Pearson's correlation coefficient; S, serum; TE, transient elastography.

Table 5. Multiple linear regression models to predict liver fat content and liver fibrosis at 16 years in HIV+ subjects.					
	Adjusted R^2	B	SE	*P*-value	Beta
*Predictors of liver fat content**	0.42, p<0.001				
Baseline waist-to-hip ratio		3.037	0.921	0.002	0.443
Baseline plasma ALT (*U/l*)		0.820	0.305	0.01	0.360
Constant		-3.633	0.821	<0.001	
Predictors of liver fibrosis estimated by MRE#	0.20, p=0.013				
Baseline BMI (kg/m^2)		0.014	0.007	0.049	0.329
Baseline liver fat content (%)		0.06	0.033	0.07	0.300
Constant		0.08	0.156	0.61	

*Model included baseline BMI, waist-to-hip ratio, plasma ALT (log), fasting plasma triglyceride (log), HDL cholesterol (log), HOMA-IR (log) and liver fat content (log).

#Model included baseline BMI, waist circumference, high-sensitivity C-reactive protein (CRP, log), HOMA-IR (log) and liver fat content (log).

Abbreviations: ALT, alanine aminotransferase; B, unstandardized coefficient; Beta, standardized coefficient; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; log, logarithmic transformed; MRE, magnetic resonance elastography; R^2, coefficient of determination; SE, standard error for unstandardized coefficient.

FIGURE LEGENDS

Fig. 1. Prevalence of statin use (**A**) and type 2 diabetes (**B**) in HIV+ subjects with (LD+) and without (LD-) lipodystrophy and control (HIV-) subjects at baseline and follow-up.

* p<0.05; **p <0.005; ***p<0.0005; ns, non-significant.

A: At baseline, 20% of the LD+ subjects used statins for dyslipidemia compared to 0% of the subjects without lipodystrophy (LD-, p=0.05) and 3% of the control subjects (p<0.05). At follow-up, 60% of the LD+ subjects used statins (p<0.001 for comparison between time points), which was significantly more than 18% of the LD- (p=0.03) or 31% of the control subjects (p=0.04). **B:** At baseline, none of the HIV+ or control subjects had type 2 diabetes. At follow-up, 23% of the LD+ group and 9% of the LD- group (27% of all HIV+ subjects) had type 2 diabetes, whereas none of the control subjects had type 2 diabetes (HIV+ vs. control subjects, p=0.01).

Fig. 2. Comparisons (Kruskal-Wallis test with Dunn's multiple comparison test) of plasma glucose (**A**) and serum insulin (**B**) concentrations between HIV+ subjects with (LD+) and without (LD-) lipodystrophy and control (HIV-) subjects during a 2-hour oral glucose tolerance test. Data are shown as mean ± SEM. ** p<0.05 and *** p<0.001 for comparison between LD+ and control subjects. ° p<0.05 for comparison between LD- and control subjects. # p<0.05 for comparison between LD+ and LD- subjects.

A: The LD+ subjects had significantly higher glucose concentrations compared to the control subjects at 0, 60 and 120 min. The LD- subjects had significantly higher glucose concentrations compared to the control subjects at 0 min. Concentrations of glucose did not differ significantly between the LD+ and LD- subjects. **B**: The LD+ subjects had significantly higher insulin concentrations compared to the control subjects at 0, 60 and 120 min, and compared to the LD- subjects at 120 min. Concentrations of insulin did not differ significantly between LD- and control subjects.

Fig. 3. A: The relationship (Pearson's correlation coefficient) between change in body weight and change in liver fat measured by ^{1}H-MRS (logarithmic transformed fold-change) in HIV+ (r=0.47, p=0.002) and control (HIV-, r=0.47, p=0.01) subjects. The dotted lines denote no change in body weight (0, X-axis) or liver fat (1, Y-axis) during follow-up. **B:** The relationship (Pearson's correlation coefficient) between liver fat content at baseline and at follow-up in HIV+ (r=0.39, p=0.01) and control (HIV-, r=0.61, p<0.001) subjects. The dotted lines denote 5.56% of liver fat which indicates non-alcoholic fatty liver disease (NAFLD).

Figure 1

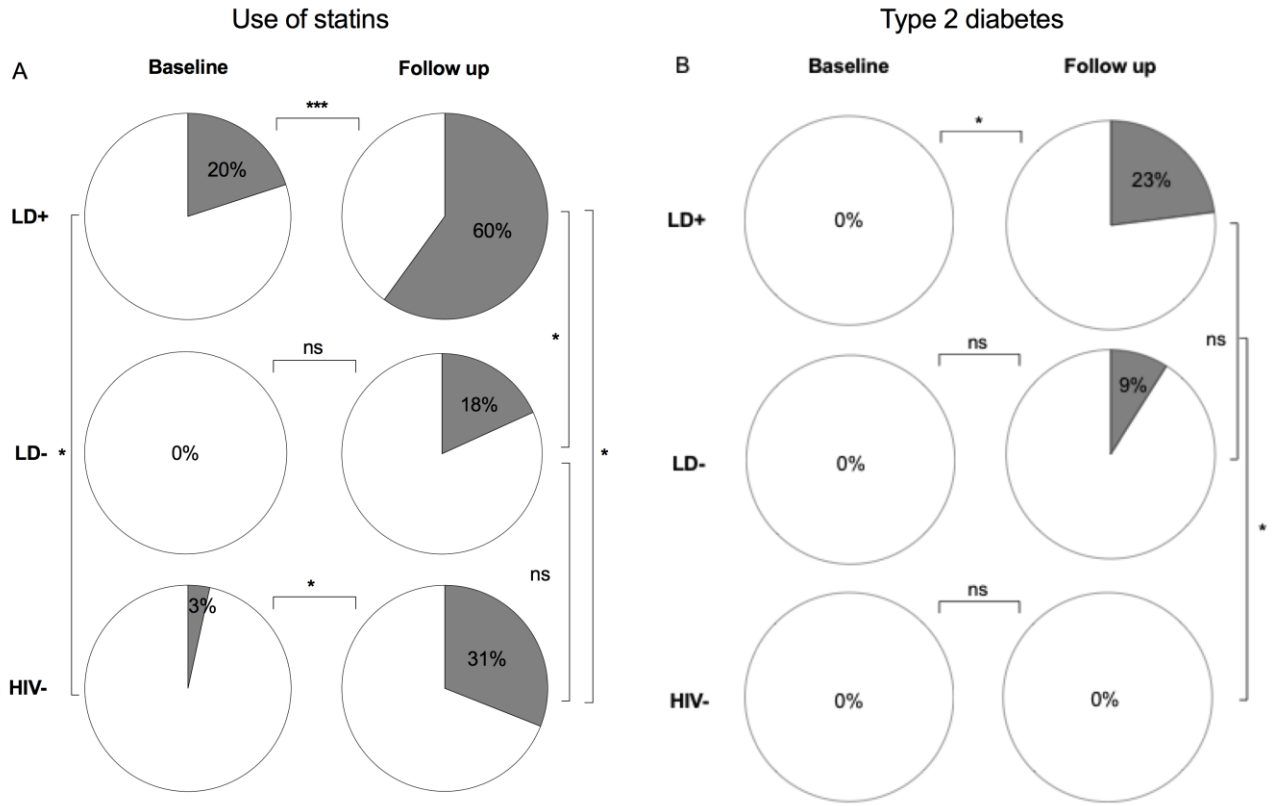

Figure 2

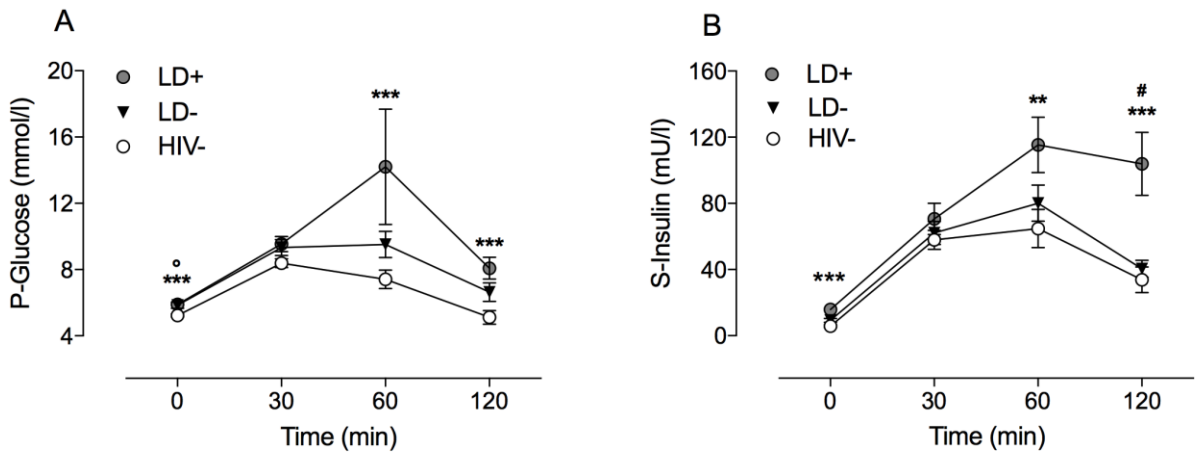

Figure 3