

# Metformin Increases HDL3–Cholesterol and Decreases Subcutaneous Truncal Fat in Nondiabetic Patients with HIV-Associated Lipodystrophy

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## Abstract

The purpose of this study was to assess metformin effects on high-density lipoprotein (HDL) composition of patients with HIV-associated lipodystrophy (LDHIV). Twenty-four adult outpatients were enrolled to receive metformin (1700 mg/d) during 6 months, but 2 were lost to follow-up and 6 stopped the drug due to adverse events (gastrointestinal in 5, and excessive weight loss in 1). From the 16 subjects who completed the study, 69% were female. At baseline, 3 and 6 months, we assessed: weight, waist and hip circumferences, blood pressure, fasting glucose and insulin, homeostasis model assessment of insulin resistance (HOMA2-IR), lipids, and HDL subfractions by microultracentrifugation. At 0 and 6 months, body fat distribution was assessed by computed tomography (CT) scan (L4 and middle femur). Metformin use was associated with reduction of mean weight (-2.4Kg at 6 months;  $p < 0.001$ ), body mass index, waist, waist-to-hip ratio and a marked decrease in blood pressure ( $p < 0.001$ ). Subcutaneous ( $p = 0.01$ ) and total abdominal fat ( $p = 0.002$ ) were reduced, but no change was found in visceral or thigh fat. No difference was detected on plasma glucose, insulin, HOMA2-IR, cholesterol or triglycerides, except for an increase in HDL3–cholesterol (from 21 mg/dL to 24 mg/dL,  $p = 0.002$ ) and a reduction of nascent HDL (the fraction of plasma HDL-cholesterol not associated to subfractions HDL2 or HDL3) ( $p = 0.008$ ). Adverse effects were very common, but most were gastrointestinal and mild. Thus, metformin use in LDHIV increases HDL3–cholesterol (probably due to improved maturation of HDL) and decreases blood pressure, weight, waist, and subcutaneous truncal fat, making this an attractive option for preventing cardiovascular disease in this population.

## Introduction

**H**IV-ASSOCIATED LIPODYSTROPHY (LDHIV) is a complex syndrome characterized by body fat redistribution and metabolic abnormalities, including insulin resistance and dyslipidemia.<sup>1</sup> It can be found in 40%–65% of HIV-infected outpatients, and it seems to be strongly associated with highly active antiretroviral therapy (HAART), in special with protease inhibitors (PI).<sup>1,2</sup> LDHIV is of paramount clinical significance due to the elevated risk of cardiovascular events observed in these patients.<sup>2</sup>

HIV-infected patients usually present reduced values of plasma HDL-cholesterol, and after introduction of PI there is an additional worsening in lipid profile, by reducing the larger and more buoyant particles of HDL ( $\alpha$ -1 and  $\alpha$ -2, or

HDL2), classically known as the more cardioprotective.<sup>3</sup> This lipoprotein profile is very similar to the one of non-HIV-infected patients with coronary heart disease.<sup>3</sup> Thus, any intervention capable of improving HDL composition could be valuable for prevention of cardiovascular disease in these patients.

A possible way to reach that goal could be metformin, an insulin-sensitizing agent that is reported to be useful for improving HDL composition in non-HIV-infected patients with type 2 diabetes.<sup>4</sup> In HIV-infected patients, several studies showed that metformin reduces visceral fat accumulation and has beneficial effects on lipid profile, insulin sensitivity, and endothelial function, all important surrogate markers of cardiovascular risk.<sup>5</sup> To our knowledge, however, the effects of metformin on HDL composition of subjects infected by

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HIV have not been assessed until now. So, we conducted this study aiming to assess HDL subfractions in patients with LDHIV submitted to a 6-month course of metformin.

## Materials and Methods

HIV-infected outpatients, aged 18 to 65 years, were selected from the population followed in HIV specialized clinic of State University of Londrina (HC/UEL). The inclusion criteria were: use of HAART for at least 18 months, without any changes in antiretroviral drugs in the previous 6 months or during study, and presence of LDHIV. LDHIV was clinically defined, requiring the detection of both the following: (1) self-perception of body changes compatible with fat redistribution (central lipohypertrophy with or without lipotrophy), first noticed after introduction of HAART and (2) confirmation of these body changes at clinical examination.<sup>1,6</sup> Patients with isolated lipotrophy were not included, since metformin can worsen peripheral fat loss.<sup>7</sup> All patients consecutively seen in HC/UEL from February to June 2006 that filled inclusion criteria were invited to participate. Exclusion criteria were as follows: viral load 10,000 or more copies per milliliter; fasting plasma glucose  $\geq 126$  mg/dL or previous diagnosis of diabetes; hypertriglyceridemia  $> 1.000$  mg/dL; total plasma cholesterol  $> 300$  mg/dL; creatinine  $> 1.4$  mg/dL (female) or  $> 1.5$  mg/dL (male); liver enzymes above 3 times normal upper limits; severe opportunistic infections or active malignancy; body mass index (BMI)  $< 18.5$  kg/m<sup>2</sup> or  $> 30$  kg/m<sup>2</sup>; congestive heart failure classes II–IV; symptomatic chronic obstructive pulmonary disease; poor adherence to HAART; alcohol or illicit drug abuse; current use of glucocorticosteroids, estrogen, progesterone, anabolizing agents or growth hormone; use of lipid-lowering drugs for less than 2 months; pregnancy, and conditions that difficult follow-up (e.g., inmates). The research was previously approved by Institutional Review Board, and all patients were voluntary and signed an Informed Consent Form.

All patients received metformin chlorhydrate (Glifage, Merck S. A., São Paulo) during 6 months, without any orientation of lifestyle changes. In the first 2 weeks, patients took half a 850-mg tablet orally after lunch and supper (850 mg/d), and then the dosage was increased to 1 full tablet twice daily (1.700 mg/d) until 6 months of treatment. One visit was scheduled at 30 days in order to check adhesion to protocol and possible adverse effects. Anthropometric measurements and venous blood samples after 12 hours fasting were collected at baseline, 3, and 6 months. An inelastic tape was used to determine the waist circumference (dorsal decubitus, at umbilical level) and hip circumference (orthostatic position, at the greater trochanteres). Resting systolic and diastolic blood pressures were determined by mercury sphygmomanometer, in seated position, considering the arithmetical mean of three measurements each time.

Blood samples were immediately processed in the Clinical Laboratory of HC/UEL. Complete blood cell count was performed in automated Cell Dyn 3700 (Abbott, Abbott Park, IL), and glucose, creatinine, transaminases and lactate were determined in automated Dimension RXL (Dade Behring, Deerfield, IL). Women of childbearing age also had measured  $\beta$ -HCG (AxSYM, Abbott) for pregnancy exclusion. Plasma was obtained from additional 15 mL venous blood drawn into 0.1% tubes containing ethylenediaminetetra-

acetic acid (EDTA), added the following conservatives per milliliter: 2 mmol/L benzamidine (5  $\mu$ L), 0.5% gentamycin and 0.25% chloramphenicol (20  $\mu$ L), 10 mg/mL aprotinin (5  $\mu$ L), and then stored at  $-70^{\circ}\text{C}$  until further analysis.

Plasma cholesterol (chol) and triglycerides (Tg) were measured with commercial kits (colorimetric-enzymatic assay, Roche, Mannheim, Germany), and LDL-chol was calculated by Friedewald's formula. Plasma apolipoprotein B-100 (apoB) and apolipoprotein A-I (apoA-I) were determined by immunoturbidimetric assay (Randox, Oceanside, CA), and insulin by radioimmunoassay (AxSYM, Abbott). Plasma HDL-chol and Tg were measured in supernatant after precipitation of apoB-containing lipoproteins with phosphotungstic acid/MgCl<sub>2</sub>. HDL2 and HDL3 subfractions were separated from this supernatant by microultracentrifugation in Airfuge (Beckman, Fullerton, CA), carried out in Laboratory of Clinical Pathology, State University of Campinas (UNICAMP).<sup>8</sup> Chol and Tg were measured in these subfractions by commercial kits (Roche) in automatized Cobas Mira (Roche). Plasma nascent HDL-chol was calculated by: [plasma total HDL-chol - (HDL2-chol + HDL3-chol)].

HOMA2-IR was calculated from fasting plasma glucose and insulin, using HOMA2 Calculator For Excel, downloaded from: [www.dtu.ox.ac.uk](http://www.dtu.ox.ac.uk).<sup>9</sup> The most recent results of CD4<sup>+</sup> lymphocytes count (flow cytometry, Becton Dickinson, Franklin Lakes, NJ) and HIV viral load (reverse transcriptase-polymerase chain reaction [RT-PCR], AMPLICOR Roche) were collected from medical charts. Body fat distribution was assessed by CT scan (Somatom AR Star, Siemens), in dorsal decubitus with arms abducted above head, at baseline, and 6 months. A 1-cm-thick slice was made on midpoint of the femur (for thigh subcutaneous fat area assessment), and another at L4 pedicle level (in order to assess abdominal fat area: total, visceral and subcutaneous compartments). Adipose tissue was defined by attenuation between  $-50$  and  $-250$  Hounsfield units.

Statistical analysis was performed using SPSS 15.0 software (SPSS Inc., Chicago, IL). The primary end point of the study was the HDL2-chol/plasma total HDL-chol ratio. Numeric continuous variables were compared using analysis of variance (ANOVA) for repeated measures (general linear model), with post-hoc analysis by least significant difference (LSD). Student's *t* test was used to compare fat areas by CT scan. Nonparametric continuous variables were compared by Kruskal-Wallis test, and proportions were compared by  $\chi^2$  (or Fisher's exact test, when appropriate). Correlations among CT scan fat areas and anthropometric/biochemical data were performed by bivariate Pearson correlation. Significance was defined as  $p < 0.05$ .

## Results

From 24 subjects initially assigned, 2 were lost to follow-up and 6 discontinued metformin due to adverse effects (Fig. 1). Sixteen subjects who completed the study were included in analysis. Two of these patients only tolerated 850mg/day, one due to nausea and the other due to dyspepsia and peripheral fat loss. Mean age was 40 years, and most were female. Mean time since HIV diagnosis was 9.5 years, and mean AIDS duration (defined in 75% by opportunistic infection) was 8.4 years. Almost 90% had had previous or cur-

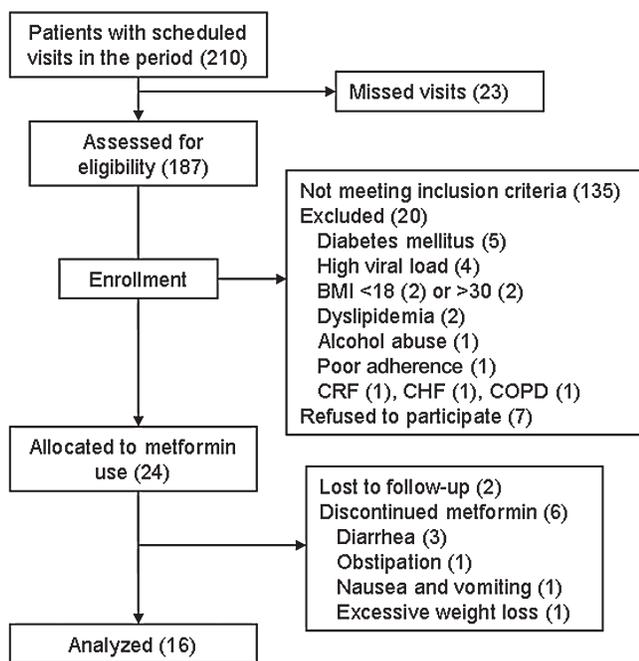


FIG. 1. Summary of selection process. BMI, body mass index; CRF, chronic renal failure; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease.

rent PI use. All (but one) patient had CD4<sup>+</sup> lymphocytes count above 200 cells/mm<sup>3</sup>, and 13 had undetectable (< 400 copies per milliliter) viral load at baseline. Clinical and virological data can be seen in Table 1.

TABLE 1. CLINICAL AND VIROLOGIC CHARACTERISTICS OF SUBJECTS AT BASELINE (MEAN ± STANDARD DEVIATION)

n	16
Gender (M:F)	5:11
Age (years)	40.4 ± 6.5
HIV infection known duration (months)	113.8 ± 31.1
AIDS duration (months)	101.4 ± 22.0 <sup>a</sup>
CD4 <sup>+</sup> nadir (median and range)	159.5 (30–436)
<50	1 (6%)
50–99	5 (31%)
100–199	3 (19%)
200 or more	7 (44%)
HAART duration (months)	97.1 ± 21.0
Antiretroviral classes used (previous or current)	
NRTI	16 (100%)
NNRTI	10 (62%)
PI	14 (88%)
Duration of use of antiretroviral classes (months)	
NRTI	96.6 ± 21.0
NNRTI	40.1 ± 20.0
PI	76.8 ± 32.3

<sup>a</sup>AIDS diagnosed in 25% by CD4<sup>+</sup> cell count only. HAART, highly-active antiretroviral therapy; NRTI, nucleoside-analogue reverse transcriptase inhibitors; NNRTI, non-nucleoside-analogue reverse transcriptase inhibitors; PI, protease inhibitors.

Current smoking was reported by 31%, and previous smoking by another 31%. Three (19%) reported hypertension and 10 (62%) have previously received the diagnosis of “dyslipidemia,” 1 of whom used pravastatin and 2 used bezafibrate (all for more than 2 months). Half presented metabolic syndrome, by NCEP-ATP III criteria.<sup>10</sup>

Lipodystrophy was first detected by the patient himself in most cases (88%), and the majority (75%) presented mixed lipodystrophy (central lipohypertrophy associated to lipoa-trophy in face or limbs).

Anthropometric data at baseline, 3, and 6 months are shown in Table 2. Mean weight loss was 1.4 kg (2.2% of initial body weight) at 3 months, and 2.4 kg (3.8% of initial weight) at 6 months, with concomitant reduction in BMI (*p* < 0.001). Reduction of waist (by 2.4 cm at 3, and 4.3 cm at 6 months) and hip circumferences (1.4 cm and 2.8 cm) were detected (*p* = 0.001), resulting in lower waist-to-hip ratio at 6 months. A remarkable decrease was also noticed in blood pressure at 3 months, which was sustained until 6 months; this was more pronounced for systolic (*p* < 0.001), but it was also significant for diastolic and mean pressures (*p* < 0.01). The changes in blood pressure did not correlate with weight or BMI (data not shown).

Baseline mean plasma glucose was 91 ± 9 mg/dL; insulin was 12.5 ± 7 mU/L and HOMA2-IR was 1.6 ± 0.9 (HOMA2-%B: 128% ± 58%, HOMA2-%R: 77% ± 33%), with no difference from baseline to 3 or 6 months. HOMA2-IR correlated with total abdominal (*r* = 0.35, *p* = 0.04) and subcutaneous abdominal fat areas (*r* = 0.37, *p* = 0.03), but not with visceral or thigh fat. Other correlations were found among HOMA2-IR and BMI (*r* = 0.47, *p* = 0.006) and waist (*r* = 0.39, *p* = 0.02), but not with body weight, hip circumference or waist-to-hip ratio.

No significant difference was detected in plasma total cholesterol and its fractions (LDL, HDL), Tg, apoA-I, or apoB. HDL2-cholesterol and the primary outcome, HDL2-cholesterol/total HDL-cholesterol, did not change as well. However, metformin use was associated to a significant increase in HDL3-cholesterol (*p* = 0.002) and a trend to increased HDL3-cholesterol/total HDL-cholesterol ratio (*p* = 0.05; Table 2). We also observed that nascent HDL-cholesterol (the fraction of plasma total HDL-cholesterol in excess from the sum of HDL2-cholesterol and HDL3-cholesterol) was reduced from 15% at baseline to 2% at 6 months (*p* = 0.008; Fig. 2). No difference in lipid profile or HDL subfractions was observed among males and females (data not shown).

CT scan showed remarkable reduction in total abdominal adipose tissue (-10.3%) from baseline to 6 months (*p* = 0.01). The subcutaneous compartment was the more affected: -15% (*p* < 0.01). In fact, 87% of fat loss in abdomen occurred in subcutaneous. No changes were seen in visceral abdominal or thigh subcutaneous fat (Table 3). Abdominal total fat area correlated well with waist circumference (*r* = 0.70, *p* < 0.001) and BMI (*r* = 0.75, *p* < 0.001).

Total HDL-cholesterol correlated with visceral fat area (*r* = 0.46; *p* = 0.007), visceral/subcutaneous abdominal fat ratio (*r* = 0.60, *p* < 0.001), and visceral/total abdominal fat ratio (*r* = 0.50, *p* = 0.003). On the other hand, HDL3-cholesterol correlated inversely with subcutaneous abdominal fat area (*r* = -0.36, *p* = 0.03), and directly with visceral/subcutaneous fat ratio (*r* = 0.49, *p* = 0.004), and with visceral/total abdominal fat ratio (*r* = 0.42, *p* = 0.01). Visceral/subcutaneous fat ratio also correlated with total cholesterol/HDL-cholesterol (*r* = -0.53, *p* = 0.002) and LDL-cholesterol/HDL-cholesterol ratio (*r* = -0.40, *p* = 0.02).

TABLE 2. ANTHROPOMETRIC DATA AND LIPID PARAMETERS AT BASELINE, THREE MONTHS, AND SIX MONTHS (MEAN  $\pm$  STANDARD DEVIATION)

	Baseline	3 months	6 months	p <sup>a</sup>
<b>Anthropometric</b>				
Weight (kg)	62.3 $\pm$ 9.5	60.9 $\pm$ 8.9 <sup>b</sup>	59.9 $\pm$ 8.6 <sup>b,c</sup>	< 0.001
BMI (kg/m <sup>2</sup> )	24.50 $\pm$ 2.79	23.92 $\pm$ 2.53 <sup>b</sup>	23.58 $\pm$ 2.68	< 0.001
Waist (cm)	85.9 $\pm$ 6.8	83.5 $\pm$ 5.9 <sup>b</sup>	81.6 $\pm$ 5.8 <sup>b,c</sup>	0.001
Hip (cm)	93.4 $\pm$ 6.1	92.0 $\pm$ 5.8 <sup>a</sup>	90.6 $\pm$ 5.5 <sup>b,c</sup>	0.001
WHR	0.92 $\pm$ 0.05	0.91 $\pm$ 0.04	0.90 $\pm$ 0.04 <sup>b</sup>	0.04
SBP (mm Hg)	132.7 $\pm$ 17.1	115.9 $\pm$ 15.0 <sup>b</sup>	117.6 $\pm$ 12.7 <sup>b</sup>	< 0.001
DBP (mm Hg)	88.7 $\pm$ 14.9	78.1 $\pm$ 10.3 <sup>b</sup>	82.3 $\pm$ 10.1	0.007
MBP (mm Hg)	103.3 $\pm$ 15.4	90.7 $\pm$ 11.3 <sup>b</sup>	94.1 $\pm$ 10.6 <sup>b</sup>	0.001
<b>Lipids in total plasma (mg/dL)</b>				
Total chol	206 $\pm$ 42	211 $\pm$ 38	193 $\pm$ 39	NS
Tg	272 $\pm$ 167	293 $\pm$ 200	224 $\pm$ 82	NS
Total HDL-chol	30.4 $\pm$ 8.0	30.0 $\pm$ 10.2	31.6 $\pm$ 7.3	NS
HDL-Tg	24.87 $\pm$ 7.52	23.38 $\pm$ 5.83	23.25 $\pm$ 4.78	NS
LDL-chol	117.5 $\pm$ 42.3	114.9 $\pm$ 29.4	115.3 $\pm$ 30.3	NS
ApoA-I	110.2 $\pm$ 17.8	112.3 $\pm$ 20.5	113.5 $\pm$ 19.1	NS
ApoB-100	109.5 $\pm$ 19.4	108.7 $\pm$ 19.8	111.8 $\pm$ 22.4	NS
<b>Lipids in HDL2 subfraction (mg/dL)</b>				
Chol	5.13 $\pm$ 1.93	5.63 $\pm$ 1.67	6.50 $\pm$ 2.00	NS
Tg	3.88 $\pm$ 1.71	5.50 $\pm$ 3.31	5.00 $\pm$ 2.42	NS
<b>Lipids in HDL3 subfraction (mg/dL)</b>				
Chol	20.62 $\pm$ 6.40	21.94 $\pm$ 5.53	24.28 $\pm$ 6.03 <sup>b,c</sup>	0.002
Tg	16.53 $\pm$ 5.43	16.23 $\pm$ 6.36	16.23 $\pm$ 3.96	NS
Relations (%)				
HDL3-chol/ total. HDL-chol	67.5 $\pm$ 10.3	76.0 $\pm$ 12.6	76.7 $\pm$ 7.1	0.05

<sup>a</sup>ANOVA for repeated measures.

<sup>b</sup> $p < 0.05$  compared to baseline (ANOVA).

<sup>c</sup> $p < 0.05$  compared to 3 months (ANOVA).

BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; chol, cholesterol; Tg, triglycerides; apoA-I, apolipoprotein A-I; apoB-100, apolipoprotein B-100; NS, non-significant.

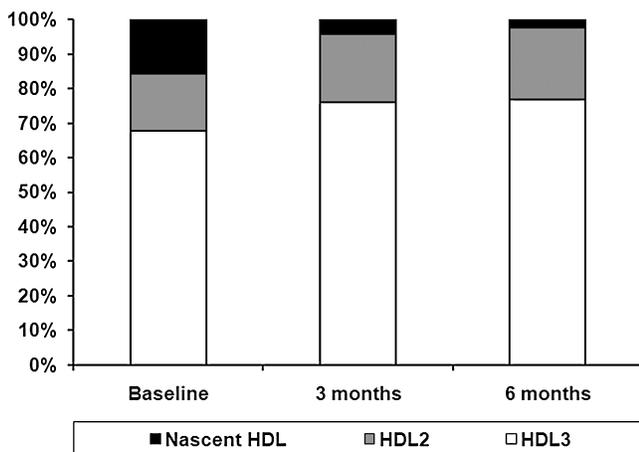


FIG. 2. Relative (%) mean contributions of HDL2-cholesterol, HDL3-cholesterol and nascent HDL-cholesterol for total plasma HDL-cholesterol, at baseline, 3 months, and 6 months. \* $p < 0.05$  compared to baseline (analysis of variance [ANOVA] for repeated measures). HDL, high-density lipoprotein.

Adverse effects of metformin were quite common. Evaluating all 24 subjects who initiated study, 93% of participants reported at least one symptom (79% in first 30 days, 53% at 3 months, and 81% at 6 months). Most symptoms (78%) were light and 21% were moderate. Most frequent complaints were diarrhea (50% of reported symptoms), abdominal pain (28%), nausea (24%) and dysgeusia (14%). Excessive weight or peripheral fat loss were reported by 5 patients. No significant change was detected in biochemical tests concerning safety: complete blood count, lactate, creatinine, or transaminases.

## Discussion

This is the first study on metformin effects on HDL composition in LDHIV patients. These are significant data because antiatherogenic activities of HDL seem to depend on its size and density: the larger and more buoyant the HDL particle, the greater its efficiency in reverse cholesterol transport. This difference may be at least partially due to the higher concentration of apoA-I in lipoprotein surface.<sup>11,12</sup> When HDL particles are fractionated by density gradient (as

TABLE 3. ADIPOSE TISSUE AREAS IN DIFFERENT BODY COMPARTMENTS, AS ASSESSED BY CT SCAN, AT BASELINE AND SIX MONTHS (MEANS  $\pm$  STANDARD DEVIATIONS)

Adipose tissue area (cm <sup>2</sup> )	Baseline	6 months	p <sup>a</sup>
Total abdominal	271.6 $\pm$ 94.6	243.5 $\pm$ 79.3	0.01
Visceral abdominal	107.4 $\pm$ 51.6	103.9 $\pm$ 58.0	NS
Subcutaneous abdominal	164.2 $\pm$ 82.6	139.6 $\pm$ 72.9	0.002
Subcutaneous thigh	62.0 $\pm$ 55.4	51.4 $\pm$ 52.5	NS
Visceral/total abdomen	0.414 $\pm$ 0.198	0.447 $\pm$ 0.221	0.02
Subcutaneous/total abdomen	0.585 $\pm$ 0.198	0.553 $\pm$ 0.221	0.02

<sup>a</sup>Paired student's *t* test.

CT, computed tomography; NS, not significant.

in ultracentrifugation), two subpopulations can be readily distinguished: the larger and less dense HDL2 (containing apoA-I), and the smaller and more dense HDL3 (containing apolipoprotein A-I and A-II).<sup>13</sup> In our patients, a 6-months course of metformin was associated with an increase in HDL3-cholesterol and no change in HDL2-cholesterol. These results are opposite to studies with non-HIV-infected subjects with type 2 diabetes mellitus, in whom metformin use increases the HDL2/HDL3 ratio, primarily by reduction of HDL3-cholesterol.<sup>4</sup>

Despite some common features among LDHIV and type 2 diabetes (e.g., disturbed lipid metabolism, insulin resistance and high cardiovascular risk), biological mechanisms of dyslipidemia may be different between these two syndromes. In diabetes, low HDL, high TG and increased small-dense LDL result from lack of insulin action on key enzymes like lipoprotein-lipase.<sup>14-17</sup> In HIV-infected patients, otherwise, HDL is reduced even before antiretroviral therapy,<sup>3,18</sup> with further reduction in HDL particle size when protease inhibitors are introduced,<sup>3,19</sup> generating a lipid profile very similar to that reported in non-HIV-infected subjects with coronary heart disease.<sup>20</sup> Insulin resistance alone is an unlikely explanation for this lipid disturbance in our patients, once they presented fairly normal indices of insulin action (mean HOMA2-IR 1.6; normal is <2.7),<sup>21</sup> which did not change during the study. This contrasts with several studies that reported improvement in insulin sensitivity of HIV-infected subjects with metformin<sup>7,22-24</sup>; only one paper reported no change.<sup>25</sup> While most studies used HOMA-IR, we preferred a more accurate index of insulin sensitivity (HOMA2-IR, "computer model"), which incorporates non-linear solutions that the older HOMA-IR equation only approximates to.<sup>26,27</sup> Other difference from this study to the others is that we excluded more insulin-resistant subjects (obese and diabetic).

Other intriguing effect of metformin in our study was the reduction of HDL-cholesterol not contained in the HDL2 or HDL3 subfractions (Fig. 2). Indeed, we observed that the sum of [HDL2-cholesterol + HDL3-cholesterol] was always lower than the total HDL-cholesterol measured in plasma. This third nonmeasured HDL subfraction is very probably composed by immature particles of HDL particles (nascent HDL), which present higher density (>1.21 g/mL) and pre- $\beta$  motility in immunoelectrophoresis, rich in apolipoprotein-A1 and phospholipids.<sup>20,28</sup> The reduction observed in nascent HDL-cholesterol, with concomitant increase in HDL3-cholesterol, is compatible with an

improved maturation of smaller lipid-poor pre- $\beta$  HDL particles into larger lipid-enriched  $\alpha$ -2 or  $\alpha$ -3 (HDL3) particles.

Our HIV patients presented low plasma HDL-cholesterol (mean 30 mg/dL), from which only approximately 20% were composed by HDL2 (compared to approximately 30% in general population).<sup>29</sup> This lipid disturbance may be induced either by effects of virus itself, or by direct effects of antiretroviral drugs (protease inhibitors can inhibit hepatic lipase), or by acute-phase reactants and cytokines.<sup>1,3,30</sup> Similar HDL reduction has been reported in systemic inflammatory conditions, like sepsis, where it is due to severe impairment of lecithin:cholesterol acyltransferase (LCAT) activity.<sup>31,32</sup> Inhibition of LCAT is strongly related to plasma concentrations of proinflammatory cytokines, as interleukin-2, interleukin-6 and transforming growth factor (TGF)- $\beta$ .<sup>33,34</sup> Another model of systemic inflammation is polycystic ovary syndrome (PCOS), a condition in which inflammatory markers (C-reactive protein, endothelial adhesion molecules) are higher and HDL-cholesterol is lower and lipid-depleted, compared to non-PCOS women.<sup>35,36</sup> Interestingly, metformin use in women with PCOS was associated to reduction of these inflammatory markers.<sup>35</sup> Thus, the improvement of HDL maturation (reduction of nascent HDL-cholesterol and elevation of HDL3-cholesterol) observed in our patients, also likely affected by low-grade chronic systemic inflammation (by LDHIV and HIV infection itself), could be due to improved action of LCAT mediated by metformin, an effect already demonstrated in animal models.<sup>37</sup>

The clinical significance of HDL3 elevation is uncertain. Evidences of cross-sectional<sup>20</sup> and prospective studies<sup>38-42</sup> suggest that concentrations of the larger and less dense particles of HDL (HDL2) are more powerful predictor of cardiovascular risk, but considerable controversy still remains. One reason is the great heterogeneity of methods used to assess HDL composition (ultracentrifugation, electrophoresis, chromatography, spectroscopy by magnetic resonance), which prevents comparisons among the studies.<sup>20,43,44</sup> Asztalos et al.<sup>20</sup> concluded that the association between HDL subfractions and cardiovascular risk depends on the methodology adopted. To date, four prospective studies evaluated HDL2 and HDL3 levels by ultracentrifugation and their association with cardiovascular risk. All four showed an increased risk of ischemic events with both reduced HDL2 and HDL3.<sup>38-41</sup> In two of them, HDL2 was more strongly related to cardiovascular disease,<sup>39,41</sup> while in the other two, HDL3 was better correlated.<sup>38,40</sup> The largest of these studies was

Quebec Cardiovascular Study, in which subjects with higher levels of HDL2 (first quartile) had 79% lower risk of cardiovascular events in follow-up, compared to the lower quartile. In the same study, however, subjects in the higher quartile of HDL3 also showed a 63% reduction in cardiovascular risk, compared to subjects in lower quartile.<sup>41</sup> Other actions of HDL besides reverse cholesterol transport may also be important for cardioprotection. A recent study compared HDL2 and HDL3 particles with respect to their antioxidant activity and concluded that this activity was more evident in HDL3.<sup>43</sup>

Beyond lipids, other noteworthy effects of metformin were detected in our study, as a marked decrease in blood pressure (also shown in previous studies),<sup>22</sup> along with reductions in weight, waist, hip circumference, waist-to-hip ratio, and subcutaneous abdominal fat. Other studies on metformin in HIV reported different patterns of fat loss on CT scan. Most reported decrease in visceral fat, either alone<sup>7,22</sup> or combined with subcutaneous abdominal fat,<sup>5</sup> or no change at all.<sup>23,25</sup> However, all studies were limited by small sample sizes (from 18 to 26), and subjects were different: many included only hyperinsulinemic subjects,<sup>7,22,23</sup> and one study included only men.<sup>5</sup> In our group, subcutaneous abdominal fat correlated better than visceral with insulin sensitivity parameters (HOMA2-IR, total chol/HDL-chol and LDL-chol/HDL-chol ratio). The importance of subcutaneous abdominal adipose tissue in glucose metabolism has been previously demonstrated by studies using euglycemic clamp.<sup>45-47</sup>

All these effects on surrogate markers like blood pressure, BMI and waist could render metformin an attractive option for prevention of cardiovascular disease in LDHIV patients. However, clinical consequences of HDL3–chol increase, as well as the impact of the drug on hard outcomes (cardiovascular death and/or myocardial infarction) still need be clarified by long-term prospective studies.

Despite its potential benefits, metformin presented common adverse effects, mainly gastrointestinal (diarrhea, nausea and abdominal pain), predominantly self-limited and mild. However, one quarter of patients who started protocol discontinued metformin because of side effects. Loss of peripheral fat was another common complaint, reinforcing recommendations for caution in subjects with significantly evident lipodystrophy.<sup>1,7,25</sup> No serious adverse effect, like lactic acidosis (reported with concomitant use of metformin and nucleoside reverse transcriptase inhibitors),<sup>48</sup> anemia, or hepatotoxicity was observed.

Considering these common adverse events, perhaps metformin should be reserved to patients with HIV with higher cardiovascular risk. Several clinical tools are available for risk stratifying, as the Framingham score and the PROCAM and SCORE equations, but it is still unknown which of these better applies to HIV-infected subjects. However, the Framingham identified more male patients with moderate and less with low cardiovascular risk in comparison to the other two scores, in a recent report.<sup>49</sup>

Some limitations of our study should be considered, as the small sample size and the lack of controls. However, we believe this should not compromise our results, since most patients were using HAART for more than 8 years, with no recent changes in antiretroviral drugs, with adequate viral suppression; in this situation, treatment-related metabolic or

morphologic disturbances should have already reached a new equilibrium. Also, we did not assess other potential markers of cardiovascular risk, on which metformin demonstrated improvement on previous reports: tPA, PAI-1,<sup>7,50</sup> MCP-1, paraoxonase-1,<sup>24</sup> and flow-mediated vasodilatation (FMD) of brachial artery.<sup>5</sup>

In conclusion, metformin use in LDHIV patients was associated with significant increase in HDL3–cholesterol and reduction of immature forms of HDL, reflecting an improved maturation of HDL particles, possibly due to facilitated action of LCAT. Along with other effects (decreases of blood pressure, body weight, waist, and subcutaneous abdominal fat), this could suggest a preventive role of metformin against cardiovascular disease in LDHIV patients, which needs to be confirmed by further research. In the meanwhile, physicians who treat HIV-infected subjects should be aware of the common adverse effects of metformin, specially gastrointestinal symptoms and worsening of lipodystrophy.

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