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Circulating metabolomic profile can predict dyslipidemia in HIV patients undergoing antiretroviral therapy

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ABSTRACT

Background and aims: Dyslipidemia in HIV-infected patients is unique and pathophysiologically associated with host factors, HIV itself and the use of antiretroviral therapy (ART). The use of nuclear magnetic resonance spectroscopy (NMR) provides additional data to conventional lipid measurements concerning the number of lipoprotein subclasses and particle sizes.

Methods: To investigate the ability of lipoprotein profile, we used a circulating metabolomic approach in a cohort of 103 ART-naive HIV-infected patients, who were initiating non-nucleoside analogue transcriptase inhibitor (NNRTI)-based ART, and we subsequently followed up these patients for 36 months. Univariate and multivariate analyses were performed to evaluate the predictive power of NMR spectroscopy.

Results: VLDL-metabolism (including VLDL lipid concentrations, sizes, and particle numbers), total triglycerides and lactate levels resulted in good classifiers of dyslipidemia (AUC 0.903). Total particles/HDL-P ratio was significantly higher in ART-associated dyslipidemia compared to ART-normolipidemia ($p = 0.001$). Large VLDL-Ps were positively associated with both LDL-triglycerides (ρ 0.682, $p < 0.001$) and lactate concentrations (ρ 0.416, $p < 0.001$), the last one a marker of mitochondrial low oxidative capacity.

Conclusions: Our data suggest that circulating metabolites have better predictive values for HIV/ART-related dyslipidemia onset than do the biochemical markers associated with conventional lipid measurements. NMR identifies changes in VLDL-P, lactate and LDL-TG as potential clinical markers of baseline HIV-dyslipidemia predisposition. Differences in circulating metabolomics, especially differences in particle size, are indicators of important derangements of mitochondrial function that are linked to ART-related dyslipidemia.

1. Introduction

Hypertriglyceridemia, hypercholesterolemia and decreased high-density lipoprotein cholesterol (HDL-C) are the most pronounced lipid abnormalities that promote atherogenesis and

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contribute to an increase risk of cardiovascular disease (CVD) in HIV infection [1–4]. The prevalence of dyslipidemia (DL) in this population is unique and pathophysiologically associated with host factors, HIV itself, and the use of antiretroviral drugs [2,5,6]. The introduction of combination antiretroviral therapy (cART) to successfully control HIV viremia and improve the prognosis of HIV patients has been associated with lipid metabolism derangements and abnormalities in fat distribution [1,7,8]; in fact, even newer well-tolerated drugs that exerts less influence on the lipid profile are still associated with metabolic side effects [9,10]. Indeed, the incidence of cardiovascular events is higher in HIV-infected patients than matched-controls; consequently, premature atherosclerosis has become one of the leading causes of morbidity and mortality among these patients [11–13]. Therefore, a better understanding of HIV-dyslipidemia (HIV-DL) is needed to address the effective interventions for clinical care.

Nuclear magnetic resonance spectroscopy (NMR) offers additional value to conventional lipid measurements. The use of NMR allows for the fast and reproducible quantification of circulating lipoproteins and the most abundant metabolites. Spectral analysis of lipoprotein subclasses is used to directly quantify particle size and concentration and thus provides additional data regarding CVD risks in different populations [14,15]. Indeed, according to NMR, increased low-density lipoprotein (LDL) particle number, decreased LDL particle size and decreased high-density lipoprotein (HDL) particle have been found to be associated with high atherogenic profile and mitochondrial oxidative stress in HIV patients [15–18].

In this study, we used a circulating metabolomic approach in a cohort of ART-naïve HIV-infected patients, who were initiating non-nucleoside analogue transcriptase inhibitor (NNRTI)-based ART, and subsequently followed up these patients for 36 months. NMR differences were evaluated for possible metabolomic signatures of HIV-DL, to firmly establish predictive NNRTI-based ART-related dyslipidemia (ART-DL) biomarkers and to improve the clinical management of dyslipidemia during ART.

2. Patients and methods

2.1. Study design

The study included 103 HIV-infected patients aged ≥18 years, who were enrolled, and initiated their first ART regimens between 2009 and 2011 at the HIV-clinic of the participating hospitals, and were followed-up for 36 months under NNRTI-based ART. The inclusion criteria for participation were a documented HIV-positive status, being ART-naïve upon enrollment, good adherence to ART achieving effective plasma viral suppression at 36 months (1.28 (1.28–1.30) log copies/mL) and having baseline laboratory measurements of cholesterol and triglycerides. We excluded patients with previous ART, patients taking drugs with known metabolic effects such as hypolipemiants, patients with missing information regarding usual lipid measurements, the presence of active opportunistic infections, current inflammatory diseases or conditions, acute or chronic renal failure, pregnancy or a history of vaccination during the last year. For study purposes, patients were categorized into 2 groups according to their baseline (ART-naïve) lipid levels, i.e., normolipidemia (NL) and dyslipidemia (DL). NL was defined by a total cholesterol (TC) < 5.2 mmol/L and total triglycerides (TTG) < 1.65 mmol/L (n = 51). DL was defined by a LDL-cholesterol (LDL-C) > 3.4 mmol/L, TTG < 1.65 mmol or a TTG > 1.65 mmol/L, HDL-C < 1.00 mmol/L. For a sub-analysis, DL was subcategorized into 3 groups according to different common types of dyslipidemia (Supplemental Materials and methods). The study complies with the declaration of Helsinki and signed consent for participation in the study was obtained from each volunteer upon enrollment according to the guidelines of each recruiting ethical committee center, which reviewed and approved the present project. Data collection included basic clinical, background and demographic information, antiretroviral drugs used, as well as CD4 cell count, HIV-1 RNA viral load measurements and prior AIDS-defining events. Fasting venous blood samples were collected in EDTA tubes and centrifuged at 1,500 g for 15 min at 4 °C. Plasma samples were aliquoted and used for usual lipid analysis. Glucose and insulin analyses were performed as previously described [19]. Insulin resistance was calculated according to the homeostasis model assessment for insulin resistance (HOMA-IR) method, according to the following formula: insulin (μIU/mL) x glucose (mmol/L)/22.5. All samples were then stored at −80 °C until further analysis at the BioBanc IISPV following standard operation procedures and with appropriate approval of the Ethical and Scientific Committees.

2.2. NMR measurements

For NMR measurements, 430 μl of plasma was transferred to 5-mm NMR tubes. All ^{1}H NMR spectra were recorded at 310 K on a Bruker Avance III 600 spectrometer operating at a proton frequency of 600·20 MHz and using a 5 mm CPTCI triple resonance (^{1}H, ^{13}C, ^{31}P) gradient cryoprobe. Detailed information about technical details of the NMR spectra pulse acquisition programs and sample preparation can be found in Supplemental methods. Metabolomics profiles, including lipids, lipoproteins and low-molecular weight metabolites (LMWMs), were obtained for each sample. Each metabolite was identified by checking for all its resonances along the spectra, and then quantified using line-shape fitting methods on one of its signals. The quantification units corresponding to the area under the curve of each metabolite were normalized by the mean of each of them throughout all samples. Lipid concentrations, sizes (z), and particle numbers (Ps) for very low-density lipoprotein cholesterol (VLDL) (38·6–81·9 nm), LDL (18·9–26·5 nm), and HDL classes (7·8–11·5 nm) as well as the particle numbers of nine subclasses were measured in 2D spectra from diffusion-ordered NMR spectroscopy (DOSY) experiments using the liposcale test [20].

2.3. Statistical analysis

Normality of the distribution of variables was assessed with Kolmogorov-Smirnov test. Medians and interquartile ranges or means and standard deviations were used to summarize the continuous variables, and comparisons between groups were performed with nonparametric Mann-Whitney *U* test (MW). The categorical variables are summarized as frequencies and percentages, and their associations with dyslipidemia were determined using the χ^2 test. Spearman correlation coefficients and the corresponding *p* values were calculated to assess the associations between the usual lipid values and the estimated NMR determinations. Additionally, the fold change (FC) of each variable was calculated as "A/B", where 'A' and 'B' are median values for each group, and results are represented with heat maps. Random forest (RF) analyses were performed as multivariate tests to identify the variables that best partitioned the overall study population according to dyslipidemia status. The RF interpretations are represented using the mean decrease in accuracy (MDA) variable, which estimates how much excluding (or permuting) each variable reduces the accuracy of the model during the out of bag error calculation phase. The variables with large MDAs were selected, and logistic regression models that combined the statistically significant variables were generated in both univariate and multivariate tests. Receiver operating characteristic curves (ROC) were built

to quantify how accurately the NMR variables were able to discriminate between the groups. Statistical analyses were performed using SPSS (version 21.0, SPSS Inc., Chicago, IL) and the computing environment of the R software (https://www.r-project.org/). The graphical representations are based on both the graphic environment of R and the GraphPad Prism software (version 5.0, GraphPad Inc., San Diego, CA). The results were considered significant at $p < 0.05$.

3. Results

Fig. 1 outlines the study design. The majority of dyslipemic patients were male, significantly older, and more likely to be diagnosed with prior AIDS-related illness (Table 1). The precision of the NMR determinations was measured using lipid values from biochemical analysis (Supplementary Fig. 1).

3.1. ART-naïve (baseline) metabolomic profile

Based on ^{1}H-NMR spectroscopy, dyslipidemic patients (n = 52) exhibited increased LDL-TG ($p < 0.001$), HDL-TG ($p < 0.001$) and VLDL-particles ($p < 0.001$), including VLDL-cholesterol (VLDL-C) and VLDL-triglycerides (VLDL-TG) (Fig. 2A.1). All VLDL subclasses ($p < 0.001$) and small LDL-particles ($p = 0.024$) were also significantly higher in dyslipidemia. Alterations in VLDL metabolism were accompanied by decreased HDL-C ($p = 0.007$) and LDL–P sizes ($p = 0.030$) in dyslipidemia compared to normolipidemia. Univariate analysis (Mann-Whitney *U* test) also associated the glycoprotein peak ($p < 0.001$) and lactate ($p < 0.001$) and leucine ($p = 0.025$) concentrations to baseline dyslipidemia (HIV-DL).

Random forest (RF) analysis was used as a multivariate method to evaluate the classificatory value of NMR determinations in baseline dyslipidemia (Fig. 2B). RF algorithm confirmed that VLDL particles, TTG (mainly HDL-TG and VLDL-TG) and lactate levels were good classifiers of baseline dyslipidemia and that a large VLDL-P value was the best classifier (MDA>150). The prediction error in this model was 26%, and its accuracy was evaluated using ROC curves. Our 10 selected classifiers from RF algorithm (bold text) resulted in an area under the ROC curve (AUC) of 0.864, which was comparable to the values observed using all of the dyslipidemia classifiers as obtained with Mann-Whitney *U* test (AUC of 0.903). Because RF algorithm proved that baseline dyslipidemia development was mainly due to alterations in the VLDL particles, we also evaluate the ability of a VLDL panel (VLDL metabolism) to correctly classify dyslipidemia in this study population. VLDL metabolism, including VLDL lipid concentrations, sizes, and particle numbers, resulted in an AUC less accurate than that observed when using the10 selected classifiers from multivariate analysis (AUC of 0.854).

3.2. Metabolomic signature of NNRTI-based ART-dyslipidemia (36 months)

The majority of baseline dyslipidemic patients (n = 40, 77%) maintained alterations in lipid metabolism and, 47% (n = 24) of the normolipidemic patients developed dyslipidemia after 36 months on ART (Fig. 1). Univariate analysis revealed 29 parameters significantly altered in ART-related dyslipidemia (ART-DL$_{36}$, n = 64) compared to normolipidemic individuals (ART-NL$_{36}$, n = 39). Large HDL-P values ($p = 0.048$), alterations in LDL metabolism and increased isoleucine levels ($p = 0.044$) were among the baseline NMR differences that were detected in dyslipidemia after 36 months on ART (Fig. 2A.2). Curiously, dyslipidemic patients (ART-DL$_{36}$) also exhibited decreased medium HDL-P levels (mHDL-P), and consequently, the Total P/HDL-P and LDL-P/HDL-P ratios, two important indicators of cardiovascular risk with greater predictive value, were significantly increased during ART (Fig. 2C). On the other hand, dyslipidemic patients also showed increased glucose levels ($p = 0.040$) that were confirmed using traditional biochemical analysis (4.89 (4.42–5.26) mmol/L in ART-NL$_{36}$ compared to 5.28 (4.80–5.52) mmol/L in ART-DL$_{36}$, $p = 0.031$). As expected, insulin resistance was strongly associated to ART-related dyslipidemia according to the HOMA-IR model (3.22 (2.23–6.56) in ART-NL$_{36}$ compared to 5.89 (3.80–8.68) in ART-DL$_{36}$, $p = 0.003$) calculated from fasting glucose and insulin concentrations (13.90 (11.39–24.77) μIU/mL in ART-NL$_{36}$ compared to 22.84 (15.06–34.92) μIU/mL in ART-DL$_{36}$, $p = 0.010$).

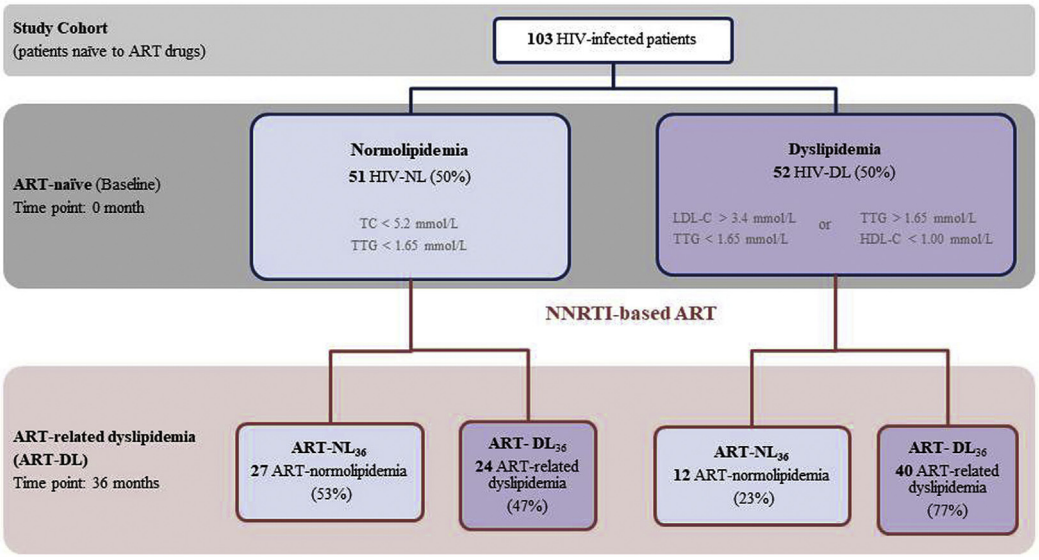

Fig. 1. Flow chart of subject cohort enrolment and analysis.
HIV-infected patients were included and categorized according to baseline lipid levels before starting antiretroviral therapy (ART) and follow-up to 36 months. ART-NL, ART-related normolipidemia; ART-DL, ART-related dyslipidemia; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TTG, total triglycerides.

Table 1
Baseline clinical characteristics (ART-naïve).

Characteristics	Normolipidemia	Dyslipidemia	*p* value*
No. of patients, n	51	52	
Age, years (IQR)	35 (29–40)	43 (36–55)	< 0.001
Male sex, n (%)	37 (72.5)	46 (88.5)	0.041
Prior AIDS-related illness, n (%)[a]	22 (45.8)	30 (66.7)	0.043
Risk transmission group, n (%)			0.518
Injecting drug use	2 (3.9)	4 (7.7)	
Homo/bisexual	24 (47.1)	21 (40.4)	
Heterosexual	22 (43.1)	18 (40.3)	
Other or unknown	3 (5.9)	9 (11.6)	
Hepatitis B and C coinfection, n (%)			0.763
Hepatitis-B surface antigen	19 (37.3)	20 (38.5)	
Hepatitis C antibodies	3 (5.9)	5 (9.6)	
Hepatitis B and C coinfection	2 (3.9)	1 (1.9)	
$CD4^+$ T-cell count, cells/μL (IQR)	234 (115–333)	175 (52–316)	0.105
Plasma viral load, log copies/mL (SD)	5.07 (0.66)	5.14 (0.76)	0.687
Glucose homeostasis			
Glucose, mmol/L (IQR)	4.90 (4.45–5.47)	4.78 (5.10–5.45)	0.486
Insulin, μIU/mL (IQR)	13.57 (10.29–18.80)	15.10 (9.78–25.88)	0.224
HOMA-IR (IQR)	3.21 (2.19–4.13)	3.61 (2.35–6.45)	0.194
Lipid panel			
Total cholesterol, mmol/L (SD)	3.87 (0.55)	4.37 (1.30)	0.008
LDL-cholesterol, mmol/L (SD)	2.30 (0.53)	2.71 (1.21)	0.090
HDL-cholesterol, mmol/L (IQR)	1.02 (0.90–1.19)	0.85 (0.68–1.03)	0.001
Total triglycerides, mmol/L (IQR)	1.04 (0.77–1.26)	1.96 (1.48–2.23)	0.001
Lipoprotein ratios			
TC:HDL-C (IQR)	3.83 (3.10–4.25)	5.24 (4.44–6.43)	< 0.001
LDL-C:HDL-C (IQR)	2.30 (1.65–2.76)	3.29 (2.44–4.02)	< 0.001

Values are medians when the interquartile range (IQR) is provided and means when the standard deviation (SD) is provided.
[a] AIDS was diagnosed according the CDC1993 criteria.AIDS, acquired immune deficiency syndrome; HDL-C, HDL-cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-C, LDL-cholesterol; TC, total cholesterol.

Moreover, the lactate and LDL-TG proved to be good classifiers of ART-related dyslipidemia (MDA>150) according to the RF regression model (Fig. 2D) at 36 months. In this situation, the two classifiers (bold text) resulted in an AUC of 0.956 with 80% sensitivity and 85% specificity that were less accurate than that observed when using all of the classifiers in the univariate test (AUC of 0.984). By contrast, NMR glucose did not result as much relevant as expected from the traditional parameters of glucose homeostasis, according to the RF regression model.

3.3. Correlation analyses for the key NMR parameters

Because our results revealed LDL-TG, lactate and large VLDL-Ps to be key factors in dyslipidemia as measured by MDA values > 150 in RF algorithm, we evaluated the possible associations between these NMR parameters. Interestingly, large VLDL-Ps were positively associated with both LDL-TG and lactate concentrations at baseline (ART-naïve) (Fig. 3A and B) and also after 36 months on NNRTI-based ART ($\rho = 0.576$, $p < 0.001$ and $\rho = 0.600$, $p < 0.001$, respectively).

NMR Total P/HDL-P and LDL-P/HDL-P ratios were significantly increased during NNRTI-based ART-DL_{36}. Thus, we also evaluated the association between these lipoprotein particle ratios and the baseline TC/HDL-C and LDL-C/HDL-C ratios based on traditional lipid measurements. The traditional CVD risk factors were positively associated with NMR lipoprotein ratios at baseline (Fig. 3C and D) and after NNRTI-based ART ($\rho = 0.460$, $p < 0.001$ and $\rho = 0.510$, $p < 0.001$, respectively).

3.4. NMR discriminates among the most common types of dyslipidemia

NMR variables proved to be an accurate classifier of both baseline HIV-dyslipidemia and ART-related dyslipidemia after 36 months on ART, thus we studied the NMR discriminatory values for the most common types of dyslipidemias (Supplementary Fig. 2). Univariate analysis associated decreased LDL particle size (FC -1.003, $p = 0.018$) and HDL cholesterol (FC 1.272, $p = 0.010$) to hypertriglyceridemia whereas the Total P/HDL-P (FC 1.635, $p < 0.001$) and LDL-P/HDL-P (FC 1.649, $p < 0.001$) ratios were associated to hypercholesterolemia. After 36 months of NNRTI-based ART, hypertriglyceridemia was related to important alterations in VLDL metabolism, whereas hypercholesterolemia was associated with alterations in LDL metabolism. As expected, alterations in VLDL and LDL metabolism were related to combined hyperlipidemia.

3.5. Potential predictive NMR variables of HIV-dyslipidemia

Because NMR metabolomic changes at baseline and after 36 months on NNRTI-based ART exhibited an excellent ability to discriminate dyslipidemia from normolipidemia, we established a predictive metabolomic panel of HIV-dyslipidemia. The potential predictive panel included all 29 parameters significantly associated to dyslipidemia after 36 months on NNRTI-based ART. So, we produced ROC curves using this potential metabolomic profile on baseline NMR values to search for predictive markers of HIV-DL. Thus, the new model was constructed with 29 parameters from the univariate analyses (Fig. 2A), 19 markers of baseline HIV-dyslipidemia, and 10 parameters of ART-related dyslipidemia. Surprisingly, the model improved the AUC of the ART-naïve univariate analysis from 0.903 with 80% sensitivity and 83% specificity to 0.935 with 80% sensitivity and 93% specificity (Fig. 4A). A heatmap of FCs for the predictive NMR variables is presented in Fig. 4B. Thus, the 29 classifiers could be potential predictive markers to develop dyslipidemia after stable ART and/or long-term

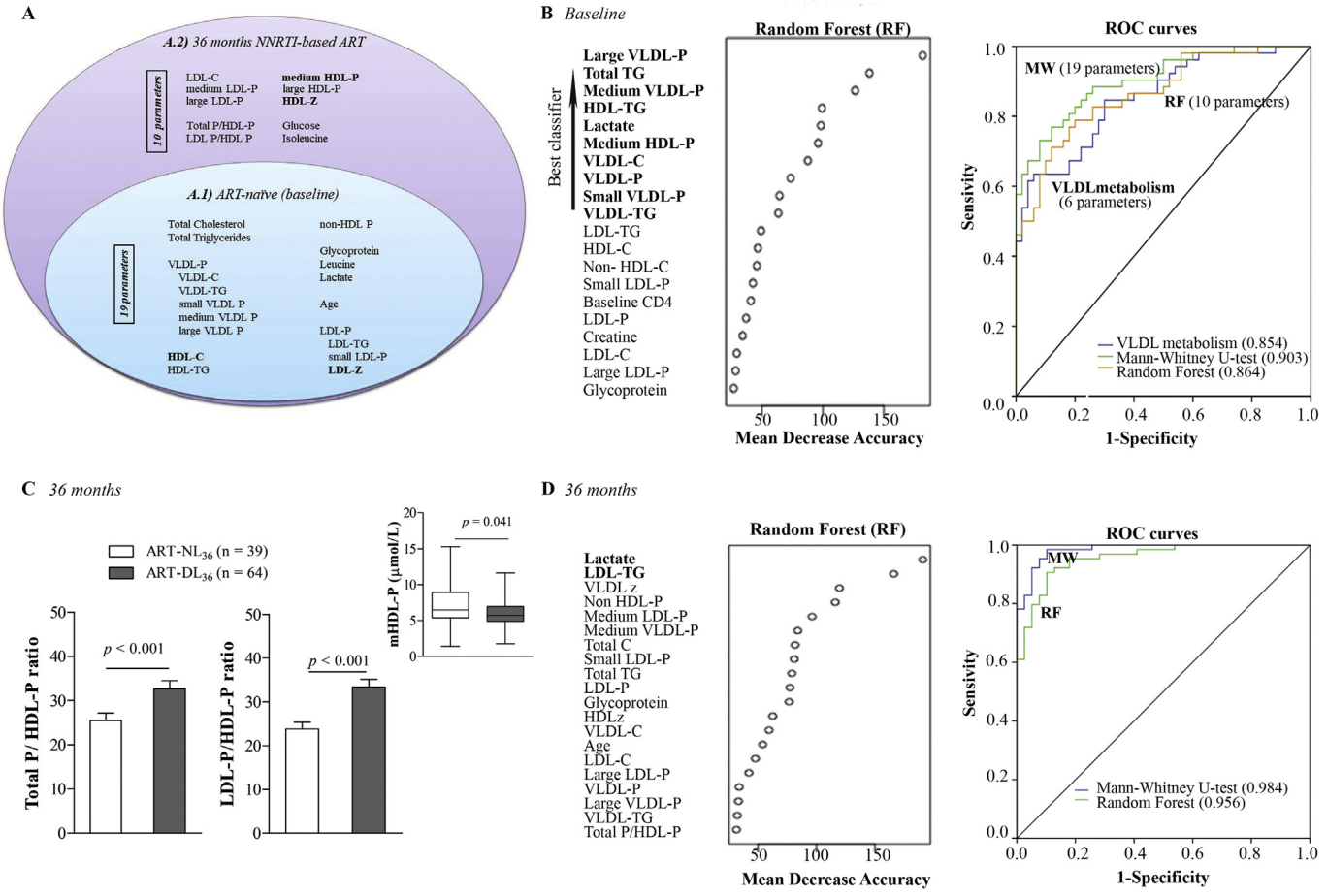

Fig. 2. NMR characteristics at baseline and after 36 months of NNRTI-based ART.
(A) Venn Diagram shows the overlap between variables statistically significant ($p < 0.05$) from Mann-Whitney *U* test at baseline (A.1) and at 36 months of ART (A.2), between normolipidemia (NL) and dyslipidemia (DL). All discriminatory baseline parameters remain significant after 36 months on ART. Bold indicates decreased values in DL compared to NL. (B) Random Forest (RF) variable importance plots and ROC curves to classify patients according to baseline dyslipidemia. VLDL metabolism includes VLDL lipid concentrations (VLDL-C and VLDL- TG), sizes (small VLDL-P, medium VLDL-P and large VLDL-P), and particle numbers (VLDL-P). (C) Graphic representation of increased cardiovascular risk ratios (Total P/HDL-P and LDL-P/HDL- P) due to decreased medium HDL-P (insert) in DL compared to NL after 36 months on ART. (D) RF variable importance plots and ROC curves to classify patients according to ART-related dyslipidemia after 36 months on ART. C, cholesterol; mHDL, medium HDL-P; MW, Mann-Whitney *U* test; P, particle; TG, triglycerides; z, particle size.

dyslipidemia status in HIV-infected patients.

Then, to refine the predictive model, the FCs between the patients were calculated based only on ART-related dyslipidemia regardless of the presence of baseline HIV-dyslipidemia. Thus, we only selected HIV normolipidemic patients (NL), and we differentiated those who maintained NL (ART-NL_{36}) and those who developed DL (ART-DL_{36}) on ART (Fig. 4C). In addition to the traditional risk factors, such LDL-C and age (FC 1.196 and FC 1.178, $p < 0.020$), an increased number of small and medium LDL-P (FC 1.162 and FC 1.188, $p < 0.05$) and lower numbers of HDL-P (FC -1.006, $p < 0.020$) were related to the development of ART-DL_{36}. RF analysis again revealed medium HDL-P and LDL-TG to be the best classifiers of dyslipidemia predisposition (Fig. 4D.1) and again, glucose did not play any relevant role in the classificatory power of RF model (MDA<20). Therefore, we evaluated the predictive value of a combined model that used all of the variables obtained in univariate test and the selected parameters from RF analyses (9 parameters, bold text). The model improved the AUC from 0.921 with 80% sensitivity and 93% specificity to 0.946 with 80% sensitivity and 88% specificity. Therefore, increased lipid content in terms of LDL-P and decreases in both LDL and HDL size and the consequent increases in both Total P/HDL-P and LDL-P/HDL-P ratios were found to be related to dyslipidemia predisposition.

4. Discussion

^{1}H-NMR spectroscopy enabled us to strongly predict an atherogenic profile in terms of lipid and lipoprotein compositions and functions in treated HIV-infected patients. Our data suggest that circulating metabolites have better predictive values for HIV-dyslipidemia onset than do the biochemical markers that are associated with conventional lipid measurements. Using metabolite profiling, we identified good markers of dyslipidemia predisposition in a cohort of HIV-infected patients. Previous studies have reported the use of NMR spectroscopy in CVD [14, Supplementary Table 1] and also in atherogenic lipoprotein profiling among HIV patients on stable cART [15–18]. However, to the best of our knowledge, this is the first study to search for predictive metabolomic biomarkers of dyslipidemia over 36 months from baseline (ART-naïve) in HIV-infected patients.

In this study, we reported on the changes in VLDL metabolism, and TTG and lactate levels that were significantly affected as a function of HIV-dyslipidemia and independently associated with the use of NNRTI-based ART. Indeed, NMR profile associated with HIV-DL proved to be similar to the profiles that have previously been observed in non-infected subjects with metabolic disturbances associated with dyslipidemias (Supplementary Table 1). We

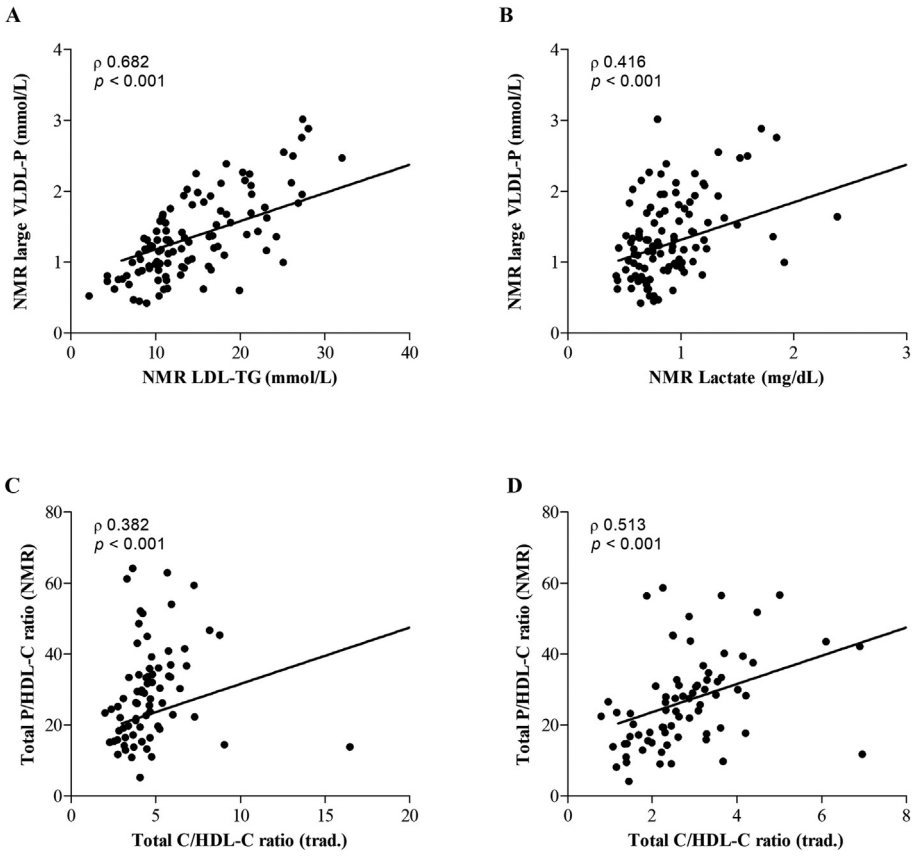

Fig. 3. Correlation analyses for the principal NMR parameters related to HIV-dyslipidemia.
(A and B) Baseline large VLDL-P concentrations were positively correlated with both baseline LDL-TG and lactate NMR parameters. Spearman's correlation test was used and the correlation coefficient is showed as a ρ value. (C and D) Baseline Total P/HDL-P and LDL-P/HDL-P ratios were significantly associated with baseline Total C/HDL-C and LDL-C/HDL-C ratios, respectively, based on cholesterol concentrations obtained from laboratory traditional measurements. Trad., laboratory traditional measurements.

observed increased VLDL-C, VLDL-TG and all VLDL-P subclasses that accounted for the increase in TTG. Both large VLDL-P and increased VLDL-C values have previously been reported to be independent indicators for CVD risk and the progression of coronary artery disease [21–23]. Specifically, our data suggest that large VLDL-Ps are the best classifiers of HIV-DL. It is known that VLDL-Ps promote the formation of small dense LDLs (sdLDL), via both cholesterol ester transfer protein (CETP) and hepatic lipase [24]. Thus, increased large VLDL values lead to triglyceride exchange from VLDL-Ps to LDL-Ps by CETP, the resulting triglyceride-rich LDLs become a preferred substrate for hepatic lipase, and the consequent triglyceride lipolysis and structural remodeling results in the formation of sdLDLs, which are associated with CVD risk [Supplementary Table 1]. The actions of CEPT and hepatic lipase also favor triglycerides exchange with HDL, which alters their compositions and the ensuing increased catabolism of HDL-Ps that leads the formation of sdHDL-Ps [24]. However, the lipid and lipoprotein changes examined herein were also related to an increase in circulating lactate level, which is a marker of low oxidative capacity. When oxidative capacity decreases, lactate levels increases as a consequence of increased flux through glycolytic pathways [27] and are independently associated with the development of cardiac dysfunction in the general population [28,29]. The results of this study indicate that HIV-DL is linked to increased large VLDL-P that are indicative of decreased HDL- C and increased triglyceride-rich lipoproteins that ultimately promotes the formation of sdLDL particles. Small LDLs are related to decreased mitochondrial oxidative function [17]; hence sdLDL particles might be associated with increased lactate levels in HIV-DL and independently linked to the use of NNRTI-based ART.

The proved inverse correlation between increased LDL triglyceride-rich lipoproteins and the catabolism of HDL-Ps emphasizes the predictive capacities of lipoprotein particle ratios in CVD risk prevention. Previously, some studies have proposed NMR methodology for the identification of coronary and CVD risks via the quantification of LDL-Ps [30,31], [Supplementary Table 1] because these are more accurate indicators than routine LDL-C and non-HDL-C measurements. However, studies of the predictive values of total P/HDL-P and LDL-P/HDL-P ratios on CVD risk are scarce [32], and additional studies are needed. Our data confirmed that both total P/HDL-P and LDL-P/HDL-P ratios are as useful predictive markers of HIV-DL during NNRTI-based ART. With the multivariate random forest model, we also found that the LDL-P/HDL-P ratio may have more predictive power for CVD risk when medium HDL-P (protective) and TTG (atherogenic) are accounted for. Regarding the CVD, we have to mention the contribution of insulin resistance (IR) that has been increasingly recognized in HIV patients undergoing antiretroviral treatment [33]. Dyslipidemia and IR are the two metabolic derangements which play a central role in the development and progression of CVD. Our data confirmed both the impact of antiretroviral therapy in the development of IR and also the strict relationship between both IR and dyslipidemia in the increased CVD. Additionally, HIV-DL may be associated with a poor immune recovery predisposition. Notably, decreased CD4+T cell values have previously been associated with higher concentrations of TG and lower levels of TC and LDL-C in HIV infection [34].

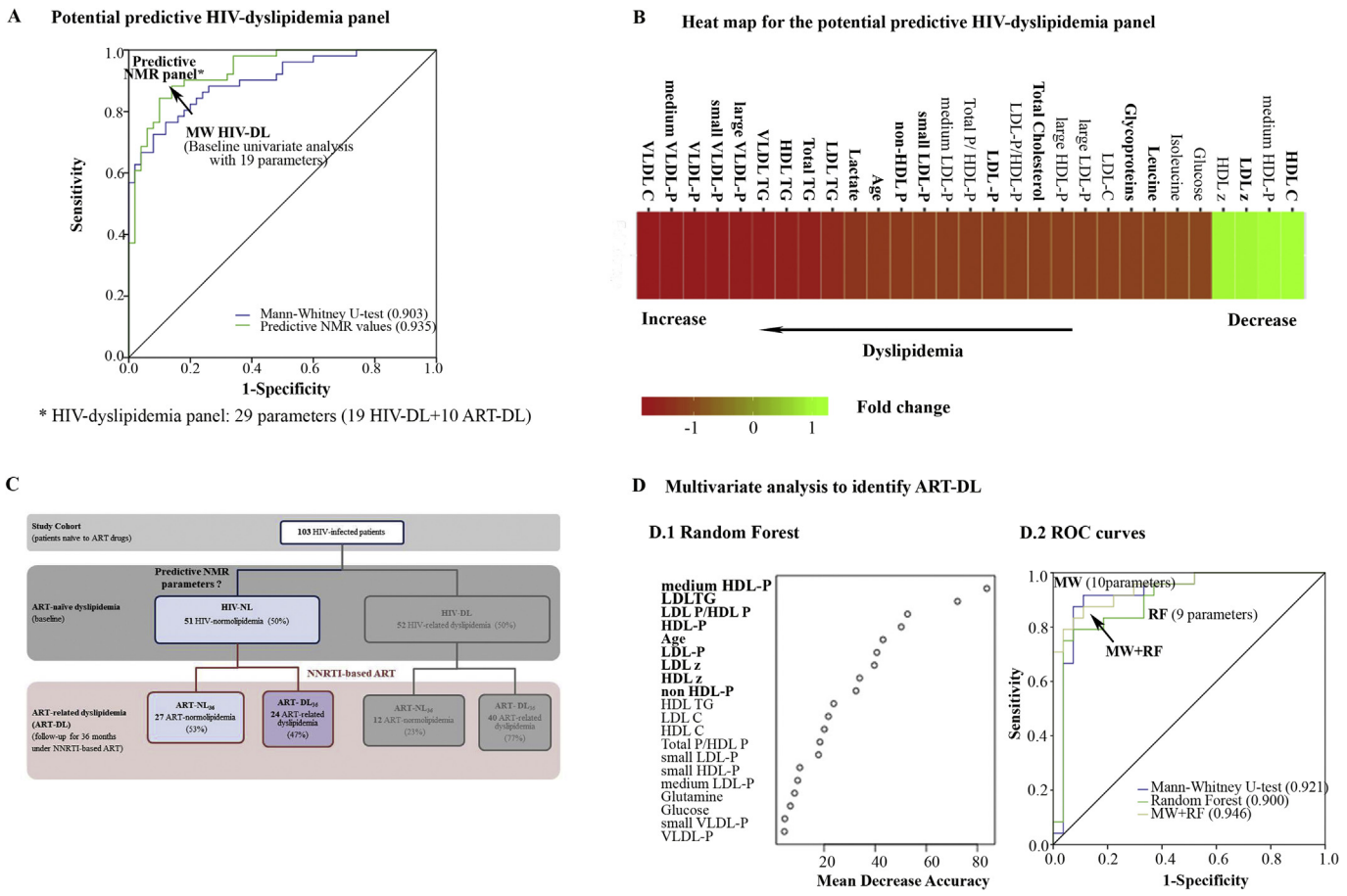

Fig. 4. Predictive NMR values of dyslipidemia in HIV-infected patients.
(A) ROC curve and AUC of the univariate analysis (Mann-Whitney *U* test) at baseline (19 parameters) is improved when the test is conducted using 29 baseline variables, which will be statistically significant after 36 months on NNRTI-based ART (19 + 10 parameters). (B) Heat map of the fold change values of NMR baseline parameters from the HIV-dyslipidemia panel (29 parameters) between HIV-NL and HIV-DL, with green bands indicating increased NMR parameters in NL and red bands indicating increased NMR values in HL. Bold text indicates statistically significant differences only at baseline. (C) Flow chart of subject cohort sub-analysis. HIV-NL at baseline (n = 51) were classified according to dyslipidemia development after 36 months and FCs were calculated on ART-naïve patients (baseline). (D) RF variable importance plots to classify normolipidemic patients at baseline according to dyslipidemia development. ROC curves and AUC of the univariate (Mann-Whitney *U* test), multivariate (RF, AUC 0.900 with 80% sensitivity and 84% specificity) and the combination of both univariate and multivariate model. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Our study has some limitations, including the relatively small sample size and the lack of demographic information that deserve mention. The number of patients per groups was relatively small, and consequently the power to detect significant association between biomarker expression and dyslipidemia markers was reduced. In the Supplementary material, we referred several studies measuring lipid and lipoprotein profiles by NMR spectroscopy in non-HIV-infected patients with metabolic derangements associated to dyslipidemia (Supplementary Table 1). However, case-control differences in dyslipidemia among our group of HIV-infected patients and own control group of healthy non-HIV infected volunteers would have been useful to confirm no metabolomics changes associated to HIV infection per se. Although patients taking drugs with known metabolic effects were already excluded for the present work, more data on lifestyle risk factors (diet, physical activity, and tobacco or alcohol use) would have been helpful to evaluate the implication of CVD risk factors on lipid and lipoprotein composition and function. Data on measurable clinical outcome would have been meaningful to improve the predictive benefit of the markers proposed. In resume, our findings should be interpreted with great caution and all limitations displayed should be taken in consideration for future studies.

In conclusion, this study reiterates that NMR lipoprotein subclass profiles can be powerful predictive tool of HIV-dyslipidemia and atherogenic risk indicators. HIV patients with ART are at increasing CVD risk and, thus, the possibility to classify patients for lipid lowering treatment, although they do not develop marked dyslipidemia, may be a useful tool for clinical management. Also, NMR not only classifies HIV-dyslipidemia from HIV-normolipidemia but also discriminates among the most common types of dyslipidemia. Our data indicate that changes in VLDL particles, lactate and LDL-TG are clinical markers of dyslipidemia in patients on stable NNRTI-based ART and are also predictive markers of HIV-dyslipidemia predisposition. An increase in the amount of large VLDL-P results in the formation of sLDL-P that might be related to low oxidative capacity measured by increased lactate levels through glycolytic pathways. Mechanistic studies addressing the implication of dyslipidemia associated to mitochondrial derangement are necessary to confirm and weigh up the relevance of the lipoproteins and metabolites observed in HIV-dyslipidemia.

Conflicts of interest

The authors declare they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

All authors have seen and approved the submitted version of the manuscript. ER-G and JG contributed to experimental design, data analysis and interpretation, and manuscript preparation. PD contributed to study design and provided intellectual guidance. SF-M contributed to recruitment of subjects and sample procurement. JP, CV, SV and ML-D contributed to experimental design, recruitment of subjects, sample procurement, and provided intellectual guidance. RB-D, VA, MV and AJC contributed to sample procurement and data collection. ML, YMP, ER-M and FG contributed to experimental design and manuscript preparation. FV and AR were responsible for the study design, data analysis, and article development. FV and AR reviewed and edited the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2018.04.008.

References

[1] F. Ballocca, S. Gili, F. D'Ascenzo, W.G. Marra, M. Cannillo, A. Calcagno, S. Bonora, A. Flammer, J. Coppola, C. Moretti, F. Gaita, HIV infection and primary prevention of cardiovascular disease: lights and shadows in the HAART Era, Prog. Cardiovasc. Dis. 58 (2016) 565–576.

[2] T. Kelesidis, J.S. Currier, Dyslipidemia and cardiovascular risk in human immunodeficiency virus infection, Endocrinol. Metabol. Clin 43 (2014) 665–684.

[3] J.V. Baker, D. Duprez, Biomarkers and HIV-associated cardiovascular disease, Curr. Opin. HIV AIDS 5 (2010) 511–516.

[4] C. Hadigan, J.B. Meigs, C. Corcoran, P. Rietschel, S. Piecuch, N. Basgoz, B. Davis, P. Sax, T. Stanley, P.W.F. Wilson, R.B.D. agostino, S. Grinspoon, Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy, Clin. Infect. Dis. 32 (2001) 130–139.

[5] W. El–Sadr, C. Mullin, A. Carr, C. Gibert, C. Rappoport, F. Visnegarwala, C. Grunfeld, S. Raghavan, W. El-Sadr, C. Mullin, A. Carr, C. Gibert, C. Rappoport, F. Visnegarwala, C. Grunfeld, S. Raghavan, Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naïve cohort, HIV Med. 6 (2005) 114–121.

[6] S.A. Riddler, E. Smit, S. Cole, R. Li, J. Chmiel, A. Dobs, F. Palella, B. Visscher, R. Evans, L.A. Kingsley, Impact of HIV Infection and HAART on serum lipids in men, J. Am. Med. Assoc. 289 (2003) 2978.

[7] B.F. Asztalosa, E.J. Schaefera, K.V. Horvatha, C.E. Cox, S. Skinner, J. Gerrior, S.L. Gorbach, C. Wanke, Protease inhibitor-based HAART, HDL, and CHD-risk in HIV-infected patients, Atherosclerosis 184 (2006) 72–77.

[8] M. Duro, R. Sarmento-Castro, C. Almeida, R. Medeiros, I. Rebelo, Lipid profile changes by high activity anti-retroviral therapy, Clin. Biochem. 46 (2013) 740–744.

[9] P. Domingo, J. Espinet, F. Vidal, ART regimes and fat: the healing hand wielding the sword, Lancet HIV 4 (2017) e2–e3.

[10] K. Lamarca, A. García Sarasola, F. Vidal, P. Domingo, Drug therapies for HIV-related metabolic disorders, Expert. Opin. Pharmacother. 17 (2016) 1327–1338.

[11] S.M. Ingle, M.T. May, M.J. Gill, M.J. Mugavero, C. Lewden, S. Abgrall, G. Fätkenheuer, P. Reiss, M.S. Saag, C. Manzardo, S. Grabar, M. Bruyand, D. Moore, A. Mocroft, T.R. Sterling, A. D'Arminio Monforte, V. Hernando, R. Teira, J. Guest, M. Cavassini, H.M. Crane, J.A.C. Sterne, Impact of risk factors for specific causes of death in the first and subsequent years of antiretroviral therapy among HIV-infected patients, Clin. Infect. Dis. 59 (2014) 287–297.

[12] S.E. Lipshultz, C.M. Mas, J.M. Henkel, V.I. Franco, S.D. Fisher, T.L. Miller, HAART to heart: highly active antiretroviral therapy and the risk of cardiovascular disease in HIV-infected or exposed children and adults, Expert Rev. Anti Infect. Ther. 10 (2012) 661–674.

[13] J.S. Currier, J.D. Lundgren, A. Carr, D. Klein, C.A. Sabin, P.E. Sax, J.T. Schouten, M. Smieja, Epidemiological evidence for cardiovascular disease in HIV-infected patients and relationship to highly active antiretroviral therapy, Circulation 118 (2008) e29–35.

[14] N.J. Rankin, D. Preiss, P. Welsh, K.E.V. Burgess, S.M. Nelson, D.A. Lawlor, N. Sattar, The emergence of proton nuclear magnetic resonance metabolomics in the cardiovascular arena as viewed from a clinical perspective, Atherosclerosis 237 (2014) 287–300.

[15] S.A. Riddler, X. Li, J. Otvos, W. Post, F. Palella, L. Kingsley, B. Visscher, L.P. Jacobson, A.R. Sharrett, Antiretroviral therapy is associated with an atherogenic lipoprotein phenotype among HIV-1-infected men in the multicenter AIDS cohort study, J. Acquir. Immune Defic. Syndr. 48 (2008) 281–288.

[16] A.M. Munger, D.C. Chow, M.P. Playford, N.I. Parikh, L.M.A. Gangcuangco, C.M. Shikuma, B.K. Nakamoto, K.J. Kallianpur, L.C. Ndhlovu, C.M. Shikuma, N.N. Mehta, Characterization of lipid composition and high-density lipoprotein function in HIV-Infected Individuals on stable antiretroviral regimens, AIDS Res. Hum. Retrovir. 31 (2015) 221–228.

[17] N.I. Parikh, M. Gerschenson, K. Bennett, L.M. Gangcuangco, M.S. Lopez, N.N. Mehta, M.P. Playford, B.K. Nakamoto, T.B. Seto, D.C. Chow, C.M. Shikuma, Lipoprotein concentration, particle number, size and cholesterol efflux capacity are associated with mitochondrial oxidative stress and function in an hiv positive cohort, Atherosclerosis 239 (2015) 50–54.

[18] F.C. Lampe, D.A. Duprez, L.H.H. Kuller, R. Tracy, J. Otvos, E. Stroes, D.A. Cooper, J. Hoy, N.I. Paton, N. Friis-Møller, J. Neuhaus, A.P.P. Liappis, A.N. Phillips, Changes in lipids and lipoprotein particle concentrations after interruption of antiretroviral therapy, J. Acquir. Immune Defic. Syndr. 54 (2010) 275–284.

[19] X. Escoté, M. Miranda, S. Veloso, P. Domingo, C. Alonso-Villaverde, J. Peraire, C. Viladés, V. Alba, M. Olona, A. Castro, M. López-Dupla, J.J. Sirvent, V. Vicente, J. Vendrell, C. Richart, F. Vidal, Lipodystrophy and insulin resistance in combination antiretroviral treated HIV-1-infected patients: implication of resistin, J. Acquir. Immune Defic. Syndr. 57 (2011) 16–23.

[20] R. Mallol, N. Amigó, M.A. Rodríguez, M. Heras, M. Vinaixa, N. Plana, E. Rock, J. Ribalta, O. Yanes, L. Masana, X. Correig, Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy, J. Lipid Res. 56 (2015) 737–746.

[21] S.B. Prenner, C.K. Mulvey, J.F. Ferguson, M.R. Rickels, A.B. Bhatt, M.P. Reilly, Very low density lipoprotein cholesterol associates with coronary artery calcification in type 2 diabetes beyond circulating levels of triglycerides, Atherosclerosis 236 (2014) 244–250.

[22] J. Ren, S.M. Grundy, J. Liu, W. Wang, M. Wang, J. Sun, J. Liu, Y. Li, Z. Wu, D. Zhao, Long-term coronary heart disease risk associated with very-low-density lipoprotein cholesterol in Chinese: the results of a 15-year Chinese multi-provincial cohort study (CMCS), Atherosclerosis 211 (2010) 327–332.

[23] J. Liu, C.T. Sempos, R.P. Donahue, J. Dorn, M. Trevisan, S.M. Grundy, Non-high-density lipoprotein and very-low-density lipoprotein cholesterol and their risk predictive values in coronary heart disease, Am. J. Cardiol. 98 (2006) 1363–1368.

[24] M. Adiels, S.O. Olofsson, M.R. Taskinen, J. Borén, Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 1225–1236.

[27] F. Zagari, M. Jordan, M. Stettler, H. Broly, F.M. Wurm, Lactate metabolism shift in CHO cell culture: the role of mitochondrial oxidative activity, Nat. Biotechnol. 30 (2013) 238–245.

[28] S.P. Juraschek, E. Selvin, E.R. Miller, F.L. Brancati, J.H. Young, Plasma lactate and diabetes risk in 8045 participants of the atherosclerosis risk in communities study, Ann. Epidemiol. 23 (2013) 791–796.

[29] K. Matsushita, E.K. Williams, M.L. Mongraw-Chaffin, J. Coresh, M.I. Schmidt, F.L. Brancati, R.C. Hoogeveen, C.M. Ballantyne, J.H. Young, The association of plasma lactate with incident cardiovascular outcomes: the ARIC study, Am. J. Epidemiol. 178 (2013) 401–409.
[30] W.C. Cromwell, J.D. Otvos, M.J. Keyes, M.J. Pencina, L. Sullivan, R.S. Vasan, P.W. Wilson, R.B. D'Agostino, LDL particle number and risk of future cardiovascular disease in the framingham offspring study - implications for LDL management, J. Clin. Lipidol. 1 (2007) 583–592.
[31] J.D. Otvos, S. Mora, I. Shalaurova, P. Greenland, R.H. Mackey, D.C.J. Goff, Clinical implications of discordance between LDL cholesterol and LDL particle number, J. Clin. Lipidol. 5 (2011) 105–113.
[32] B.T. Steffen, W. Guan, A.T. Remaley, P. Paramsothy, S.R. Heckbert, R.L. McClelland, P. Greenland, E.D. Michos, M.Y. Tsai, Use of lipoprotein particle measures for assessing coronary heart disease risk post-American Heart Association/American College of Cardiology Guidelines: the multi-ethnic study of atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 35 (2015) 448–454.
[33] L.R. Non, G.V. Escota, W.G. Powderly, HIV and its relationship to insulin resistance and lipid abnormalities, Transl. Res. 183 (2017) 41–56.
[34] S. Souza, L. Luzia, S. Santos, P. Rondó, Lipid profile of HIV-infected patients in relation to antiretroviral therapy: a review, Rev. Assoc. Med. Bras. 59 (2013) 186–198.