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High Irisin levels in nondiabetic HIV-infected males are associated with insulin resistance, nonalcoholic fatty liver disease, and subclinical atherosclerosis

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Summary

Objective: HIV infection is associated with an increased risk of cardiovascular disease. Irisin is a miokyne secreted by skeletal muscle, which may influence insulin homeostasis, nonalcoholic fatty liver disease (NAFLD) and atherosclerosis. Our objective was to evaluate the relationships between serum irisin, insulin homeostasis, NAFLD and subclinical atherosclerosis in HIV-infected males.

Design: Cross-sectional study in a cohort of HIV-infected patients.

Patients: Inclusion criteria: men older than 18 years; antiretroviral therapy (ART) -naïve or on effective ART (<50 HIV-1 RNA copies/mL) without changes in the previous 6 months; no diabetes or hepatitis C.

Measurements: Irisin was measured by enzymatic immunoassay (Phoenix Pharmaceuticals), insulin sensitivity by homeostasis model assessment of insulin resistance (HOMA-IR), as well as the 2-hour continuous infusion of glucose with model assessment (CIGMA-HOMA). Hepatic steatosis was measured by 1-H magnetic resonance spectroscopy, subclinical atherosclerosis by evaluation of carotid intima-media thickness (C-IMT), measured by Ultrasonography.

Results: Eight nine men (age 42.0 ± 8.3 years, duration of HIV infection 7.9 ± 5.6 years, CD4 count 547 ± 279 cells/mL) were included. Circulating irisin was positively related to HOMA-IR and CIGMA-HOMA, hepatic triglyceride content, and to VAT/SAT ratio. Higher irisin concentrations were associated with higher C-IMT, although this association did not persist in multivariate analysis. Lipodystrophy and a higher baseline PAI-1 concentration were independently associated with C-IMT.

Conclusions: In male HIV patients without diabetes, higher irisin concentrations are positively associated with insulin resistance, NAFLD and subclinical atherosclerosis. However, waist-hip-ratio is the main determinant of insulin resistance, and PAI-1 and lipodystrophy were the strongest determinants of IMT in this population.

KEYWORDS

cardiovascular, carotid intima-media thickness, HIV, insulin homeostasis, irisin, nonalcoholic fatty liver disease, subclinical atherosclerosis

1 | INTRODUCTION

In spite of modern antiretroviral therapy (ART), HIV infection is associated with insulin resistance,[1] nonalcoholic fatty liver disease (NAFLD),[2] and an increased risk of cardiovascular disease (CVD), especially coronary heart disease, compared with people not infected with HIV.[3] In the HIV-infected population, the role of biomarkers of endothelial dysfunction, inflammation and coagulation as predictors of CVD remains controversial. HIV-infected individuals with viral suppression have higher levels of endothelial dysfunction and inflammation markers compared to uninfected individuals.[4]

Skeletal muscle has recently emerged as an endocrine organ through the secretion of myokines, hormones released into the circulation during or after physical activity. Irisin is a myokine which is secreted by skeletal muscle in mice and humans.[5] It has been proposed that irisin drives the brown-fat-like conversion of the white adipose tissue, increasing energy expenditure[6] and consequently is thought to be able to influence insulin resistance (IR), to regulate cardiometabolic parameters and potentially influence atherosclerosis.[5,7] In the liver, irisin may be associated with lipid and carbohydrate metabolism, and also with hepatic IR and hepatic steatosis.[7]

Most clinical studies performed in different populations have found a positive association among irisin concentrations and insulin resistance[8-10] after adjusting for potential confounders. By contrast, Moreno-Navarrete et al[11] found a positive association with insulin sensitivity. Few clinical cross-sectional studies evaluate irisin in human NAFLD and the results are controversial. In biopsy-proven NAFLD adults, there were no differences in serum irisin with matched controls; however, irisin was independently and positively associated with portal inflammation.[12] Zhang et al[13] demonstrated an independent and inverse association between irisin and higher intrahepatic triglyceride content, assessed by ^{1}H magnetic resonance spectroscopy. Choi et al observed higher irisin levels in NAFLD adults, assessed by abdominal ultrasonography, than in controls; in the NAFLD group, patients with mild steatosis had higher irisin levels than those with moderate-to-severe steatosis. The authors speculated that irisin increases in early steatosis, as a compensatory mechanism, but as the steatosis progresses this mechanism is exhausted and irisin decreases.[14]

Little is known about the relationship between subclinical atherosclerosis and irisin. In the available literature, only two studies have investigated the relationship between irisin and subclinical atherosclerosis measured by coronary artery calcium (CAC) or, as intima-media thickness (IMT), showing a positive correlation between these indices and irisin concentrations.[10,15]

To our knowledge, there is only one study addressing irisin in HIV patients[16] after lifestyle modification.

The main objective of the present study was to evaluate the association between irisin levels, insulin resistance, NAFLD and subclinical atherosclerosis in HIV-infected males. In addition, as secondary outcomes, we evaluate the relationships between irisin, HIV and cardiovascular-related factors.

2 | MATERIAL AND METHODS

2.1 | Study population

A cross-sectional observational study was carried out in the Infectious Diseases and Endocrinology Units of a tertiary hospital in Alicante (Spain). The local Ethics Committee approved the study. All HIV-infected men belonging to a cohort of 600 HIV-infected patients, with regular assessment of endocrine parameters and cardiovascular risk, were proposed to participate in this study if they were ≥18 years of age, ART-naïve or on effective ART (<50 copies RNA/mL), with no changes in the previous 6 months. Only patients receiving two nucleoside reverse-transcriptase inhibitors (NRTIs) and a boosted protease inhibitor (PI/r) or a non-NRTI (NNRTI) (efavirenz or nevirapine) that had never been treated with PIs were included. Those with chronic hepatitis C, diabetes mellitus, active AIDS disease, active illegal drug use, or psychiatric illness were excluded. All patients provided written informed consent. The study was conducted from March 2009 to October 2010.

Participants were required to fast for 12 hours prior to the blood sample, which was performed between 8:00 and 9:00 AM. The samples were centrifuged and serum and plasma were stored at −70°C until determination. For the present work, stored serum stored was used to measure circulating irisin.

2.2 | Outcome variables

2.2.1 | Insulin homeostasis

Insulin homesotasis was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR), as well as the 2-hour continuous infusion of glucose with model assessment (CIGMA). High HOMA and CIGMA-HOMA scores denote higher insulin resistance. The 2-hour CIGMA consists of a 180 mg/min/m^2 glucose infusion over 120 minutes. Blood samples were taken at 4, 8 and 10 minutes for the measurement of glucose and insulin levels, and again at 120, 125 and 130 minutes. The means of these three samples were used to estimate the insulin resistance score using the HOMA formula. The HOMA-IR score was calculated as fasting serum insulin (mU/mL) x fasting plasma glucose (mmol/L)/22.5. Three baseline samples, taken at 5-minutes intervals, were averaged to yield the mean levels of glucose and insulin.

For beta cell function, the incremental insulin and glucose areas under the curve (AUCins and AUGglu, respectively) were calculated using a trapezoidal model.

The gold standard for beta cell function is the insulin secretion/insulin resistance (disposition) index (DI/DG ΔIR, where DI is the change in insulin concentration, DG is the change in glucose concentration, and IR is insulin resistance).[17] We defined the disposition index as (AUCins/AUCglu) ÷ HOMA-CIGMA.

2.2.2 | Abdominal fat distribution and intrahepatic lipids

Abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were measured by 10-mm single-shot axial magnetic resonance imaging (MRI), between the L4 and L5. Hepatic triglyceride content (HTGC) was measured by 1-H magnetic resonance spectroscopy (1H-MRS) (1.5 T Gyroscan Intera; Philips Medical Systems, Best, the Netherlands).[18] Hepatic steatosis - NAFLD was defined as a HTGC>5%, after exclusion of causes for secondary hepatic fat accumulation, such as significant alcohol consumption, use of steatogenic medication, or hereditary disorders.

2.2.3 | Subclinical atherosclerosis

Subclinical atherosclerosis was measured by evaluation of carotid intima-media thickness (C-IMT), measured by Ultrasonography (Hitachi EUB-5500HV), with a 7.5 Mhz linear probe, using the Mannheim criteria.[19] Both common carotid arteries were evaluated, and IMT was measured automatically in the posterior wall at the end of diastole in a region free of plaque. The volume of interest has a length of 11 cm, adjustable to the visible IMT segment. The mean C-IMT was the mean value of the whole segment. The maximal C-IMT was the maximal value of the region of interest. The measurement was taken automatically off-line with the Hitachi team software. Left and right common C-IMT was evaluated separately and bilateral C-IMT was obtained by the arithmetic mean of C-IMT of both sides. Results are expressed in mm.

2.3 | Explanatory variables

2.3.1 | Clinical evaluation

At enrolment, subjects underwent a full assessment based on their medical history, a physical examination and routine haematological and biochemical tests. Lipodystrophy was determined using a standard questionnaire.[20] Adequate physical activity was defined as at least 150 minutes/wk of moderate-intensity aerobic physical exercise. Variables related to HIV infection are mode of transmission, nadir and current $CD4^+$ lymphocyte count (cells/mL and percentage), plasma viral load (copiesRNA/mL; lower detection limit 39 copies/mL), duration of HIV infection in years, ART and CDC clinical state.

2.3.2 | Biochemical measurements

Routine biochemical laboratory determinations were made in the reference laboratory, in the clinical evaluation period in 2010. The measurement of systemic inflammatory markers was made after completion of clinical data recorded in 2011. Stored serum from the previous study was used to measure circulating irisin in 2014.

Glucose was measured using the hexokinase method (with the Modular autoanalyser; Roche Diagnostics, Indianapolis, IN, USA). Insulin levels were measured by solid phase sandwich chemiluminescent immunoassay (using the Immulite 2000 autoanalyser; Siemens, Flanders, NJ, USA). HbA1c levels were determined by high-performance liquid chromatography (HPLC) (using the Adams A1c HA-8160 autoanalyser; Menarini, Florence, Italy), adiponectin levels by an enzyme-linked immunosorbent assay (ELISA) (the Adiponectin ELISA; Mediagnost, Reutlingen, Germany).

Systemic inflammatory markers were measured: high-sensitivity C-reactive protein (hsCRP) (turbidimetry kinetics; IMMAGE, Beckmann Coulter, Inc.), plasminogen activator inhibitor-1 (PAI-1) (enzyme immunoassay, Quantikine, R & D Systems).

2.3.3 | Determination of circulating irisin

Serum irisin levels were measured by enzyme immunoassay (Phoenix Pharmaceuticals, Burlingame, California) following the user's manual. The sensitivity of the method was 1.57 ng/mL and the intra- and interassay variability were 10% and 15%, respectively. The assay range was 0.1-1000 ng/mL with a linear range of 1.57-47 ng/mL.

2.4 | Statistical analysis

Qualitative variables were expressed as relative and absolute frequencies. Parametric variables were expressed as means ± SD, nonparametric variables as medians and percentiles 25-75.

2.4.1 | Main objective

To determine whether an independent association existed between irisin (explanatory variable), insulin resistance (CIGMA-HOMA) and C-IMT (as main outcome variables), a multivariate unconditional linear regression analyses was performed, using all variables yielding statistical significance in the bivariate analysis; for hepatic steatosis (as main outcome variable), a logistic regression was performed.

In addition, the coefficient of determination for each of the variables in the multivariate linear regression models was calculated.

2.4.2 | Secondary objective

The associations between HIV and cardiovascular-related variables and irisin were calculated using the chi-squared test for qualitative variables and the Student's *t* test or Mann-Whitney *U*-test for quantitative variables. Logistic regression was used to identify factors associated with the presence of irisin ≥75th percentile. Correlations between quantitative variables were tested using Pearson and Spearman correlations where appropriate.

In all cases, a *P* value of <0.05 was considered statistically significant. The SPSS version 19.1 statistical package (SPSS Inc., Chicago, IL, USA) was used throughout.

3 | RESULTS

3.1 | Baseline characteristics

The participation in the study was offered to 109 men. Nineteen patients refused to give their consent, and a patient was enrolled but did not complete the study, so finally, 89 patients were included. The mean age was 42.0 ± 8.3 years, the mean duration of HIV infection was 7.9 ± 5.6 years, and the mean CD4 count was 547 ± 279 cells/mL; 59.6% and 19.1% of the patients were in the CDC class A and C categories, respectively. Seventy-five patients (84.2%) were receiving ART, with a mean of 67 ± 42 months, and 39 patients belonged to the PI group (ART based on protease inhibitor). Twenty-seven patients presented lipodystrophy. Mean body mass index (BMI) was 24.8 ± 3.4 kg/m^2, and only 5 patients had a BMI >30 kg/m^2. Mean irisin was 437.8 ± 108.1 ng/mL and was not influenced by the level of physical activity. Liver steatosis was present in 24 patients (available data for HTGC in 72 patients).

Regarding ART-naïve patients, they were younger (34.1 ± 5.2 years), with a shorter duration of HIV infection (2.7 ± 2.4 years), and the mean CD4 count was 611 ± 284 cells/mL; 92.9% and 7.1% of the patients were in the CDC class A and B categories, respectively. Mean BMI was 24.1 ± 2.6 kg/m^2, and mean irisin was 409.9 ± 103.5 ng/mL, without differences with patients on ART. Liver steatosis was present in 1 patient.

3.2 | Irisin and insulin homeostasis

Irisin was positively related to IR [HOMA-IR (Rho 0.359, $P < 0.001$), HOMA-CIGMA (Rho = 0.3, $P = 0.004$)] and to higher ratio AUC insulin/AUC glucose (Rho = 0.25, $P = 0.018$), and also, patients in the highest quartile of irisin concentrations had higher indices of insulin resistance ($P < 0.05$ for all) (Table 1). In multivariate analysis, we did not find an independent association between irisin concentrations (highest quartile vs lowest quartile) and insulin resistance (Table 2). The association between waist-to-hip-ratio and CIGMA-HOMA was the only that remained significant, explaining 74% of the insulin resistance change.

3.3 | Hepatic steatosis (NAFLD), abdominal fat distribution and irisin

Although patients with NAFLD (n = 24) had higher irisin concentrations (496.7 ± 140 vs 413.5 ± 86, $P = 0.01$) and irisin was positively related to the HTGC in the right lobe of liver (Rho 0.37, $P = 0.001$) and in the left lobe of liver (Rho 0.44, $P = 0.001$), in multivariate analysis, we did not find an independent association between irisin concentrations (highest quartile) and hepatic steatosis (Figure 1). In addition, none of the variables included in the multivariate logistic regression model showed an association with the presence of hepatic steatosis, with waist-hip-ratio approaching to statistical significance.

With regard to abdominal fat distribution, irisin was positively related to the percentage of VAT (r 0.36, P 0.02), and patients in the highest quartile of irisin had higher VAT.

3.4 | Subclinical atherosclerosis and irisin

Irisin was correlated with the maximal left C-IMT($r = 0.22$, $P = 0.041$). Patients in the lowest quartile of left C-IMT had lower irisin concentrations compared to those in the highest quartile: 385.2 ± 64.9 vs 458.5 ± 140.9, $P = 0.028$. Higher irisin concentrations were associated with several IMT-related measures as maximal common carotid IMT ($P = 0.05$), maximum left bifurcation IMT ($P = 0.01$) and mean left bifurcation IMT ($P = 0.04$) (Figure 2).

However, the multivariate linear regression model did not show an independent association between irisin concentrations and IMT-related measures. Lipodystrophy and a higher baseline PAI-1 concentration were independently associated with maximal left C-IMT and mean left C-IMT, these two variables explained 50% of maximal left C-IMT variation (41% lipodystrophy and 9% PAI-1 concentrations, $P < 0.03$), and 48% of mean left C-IMT variation (11% lipodystrophy and 37% PAI-1 concentrations, $P < 0.02$).

3.5 | Irisin and factors related to HIV infection

The association between factors related to HIV infection and irisin concentrations are shown in Table 3. There was a direct association between time of exposure to ART - and specifically NRTIs - and irisin concentrations. When we explored the associations between irisin and duration of NRTI exposure in adjusted models that included hepatic steatosis, waist-to-hip-ratio and VAT as possible confounding factors (all of them associated with higher irisin concentrations), this association persisted (OR 1.01 [1.002-1.02], $P = 0.01$).

3.6 | Metabolic parameters, systemic inflammatory markers, cardiovascular risk factors and irisin concentrations

Mean irisin concentration was 437.8 ± 108.1 ng/mL. Irisin levels were correlated positively with weight ($r = 0.22$, $P = 0.03$), waist-to-hip-ratio ($r = 0.25$, $P = 0.01$) and triglycerides ($r = 0.31$, $P = 0.003$). There was an inverse relationship between irisin and HDL cholesterol ($r = -0.25$, $P = 0.01$). Patients in the highest quartile of irisin concentrations had lower HDL, higher LDL and higher triglycerides, higher diastolic blood pressure and higher waist-to-hip-ratio ($P < 0.05$ for all) (Table 3).

There was a positive correlation between serum irisin and baseline PAI-1 ($r = 0.42$, $P < 0.001$). Higher irisin concentrations (above percentile 75) were associated with higher baseline PAI-1 (11.5 ± 4.7 vs 8.4 ± 3.1 ng/mL, $P = 0.007$) and with lower, adiponectin concentrations (5.2 (2.8-6.7) vs 7 (4.3-11.6) μg/mL, $P = 0.01$). There was no difference in hsCRP between irisin quartiles.

4 | DISCUSSION

In summary, the present study shows that, in HIV-infected males, higher irisin concentrations are associated with higher insulin resistance, higher HTGC and a greater carotid IMT. However,

Table 1. Cardiovascular-related factors, insulin sensibility parameters and irisin concentrations

Factor	Irisin >P75th n = 22	Irisin <P25th n = 22	OR (95% CI)	*P* value
Age, years Mean ± SD	44.1 ± 7.7	40.6 ± 8.6		0.16
Hypertriglyceridemia, (n) yes	13	6	3.58 (1.09-13.7)	**0.03**
HDL <40 mg/dL, (n) yes	7	1	9.8 (1.1-88.2)	**0.02**
BMI (kg/m^2) Mean ± SD	26.3 ± 3.9	24.2 ± 2.7		0.16
WHR Mean ± SD	0.97 ± 0.04	0.92 ± 0.09		**0.03**
SBP (mm Hg) Mean ± SD	130.7 ± 16.5	120.1 ± 20.4		0.08
DBP (mm Hg) Mean ± SD	81.5 ± 11.5	74.2 ± 9.8		**0.04**
TC (mg/dL) Mean ± SD	197.5 ± 44.6	172.5 ± 34.3		0.55
HDL (mg/dL) Mean ± SD	44.9 ± 12.8	58.9 ± 17.7		**0.01**
LDL (mg/dL) Mean ± SD	135.5 ± 44.4	106.8 ± 37.3		**0.03**
TG (mg/dL) Median (P25-P75)	172.5 (77-613)	90.0 (40-296)		**0.01**
ALT Median (P25-P75)	26 (10-81)	19 (12-72)		**0.01**
GGT Median (P25-P75)	58 (41-413)	22 (12-113)		**0.004**
HOMA-IR Median (P25-P75)	2.52 (0.82-6.16)	1.39 (0.45-9.27)		**0.003**
CIGMA-HOMA Median (P25-P75)	16.59 (2.43-37-40)	5.33 (1.59-59.83)		**0.005**
AUC ratio (insulin/glucose) (mU/mg) Median (P25-P75)	23.6 (16.7-36.4)	15.2 (9.5-19)		**0.001**
Disposition index Mean ± SD	2.08 ± 1.4	2.2 ± 1.2		0.68
Glucose (mg/dL) Mean ± SD	97.2 ± 11.8	93.9 ± 10.1		0.3
HbA1c (%) Mean ± SD	4.4 ± 0.4	4.5 ± 0.7		0.7

Qualitative variables were expressed as relative and absolute frequencies. Parametric variables were expressed as means ± SD, nonparametric variables as medians and percentiles 25-75. The associations between the different variables and irisin were calculated using the chi-squared test for qualitative variables and the Student's *t* test or Mann-Whitney U-test for quantitative variables.

The *P* values are bold where they are less than the significance level cut-off of 0.05.

ALT: alanine aminotransferase; AUC: area under the curve; BMI; body mass index; CIGMA: continuous infusion of glucose with model assessment; DBP, dyastolic blood pressure; Disposition index: as (AUCins/AUCglu) ÷ HOMA-CIGMA; GGT: Gamma-Glutamyl Transpeptidase; HDL: high-density lipoprotein; HOMA-IR: Homeostatic model assessment-insulin resistance; LDL: low-density lipoprotein; OR: odds ratio (for qualitative variables); SBP, systolic blood pressure; SD: standard deviation; TC: total cholesterol; TG: triglycerides; WHR: waist-to-hip-ratio.

multivariate analysis showed that: first, waist-to-hip-ratio seems to explain most of the irisin influence on insulin resistance, second, none of the variables evaluated show an association with the presence of hepatic steatosis and finally, the presence of lipodystrophy and a higher baseline PAI-1 concentration were independently associated to a greater IMT carotid thickness, explaining 50% of its variation.

We evaluated the association among several factors related to HIV infection and irisin concentrations. However, only the time of exposure to NRTIs was related to higher irisin concentrations. To our knowledge, there is no data about the influence of HIV-related factors on irisin concentrations, because in the previous study by Srinivasa et al[16] this aspect was not addressed. Further studies are needed, to corroborate this data.

Table 2. Association between explanatory variables, insulin resistance and subclinical atherosclerosis

	Multiple stepwise regression								
	CIGMA-HOMA			Maximal Left CC IMT			Mean Left CC IMT		
	B	$r^2 = 0.88$	***P*** = 0.003	***B***	$r^2 = 0.78$	***P*** = 0.002	***B***	$r^2 = 0.72$	***P*** = 0.01
Age (10 y)	−5.5		0.28	−0.02		0.6	−0.02		0.52
Duration of HIV (y)				0.04		0.37	0.01		0.7
HIV-VL RNA/mL <50 (yes)				−0.05		0.36	−0.03		0.4
ART exposure (y)									
PI/r exposure (y)	−0.53		0.57	0.02		**0.03**	0.01		**0.03**
NRTI exposure (y)	1.92		0.23	0.001		0.95	0.005		0.5
BMI (kg/m^2)	0.27		0.8			0.3	−0.01		0.7
WHR	200		**0.003**	−0.66		0.06	−0.16		0.6
VAT	0.81		0.17						
VAT/SAT ratio	−6.4		0.14						
Right lobe HTGC (%)	−0.08		0.89						
Lipodystrophy (yes)	−2.7		0.68	0.14		**0.02**	0.04		**0.04**
Irisin >P75th (yes)	−7.2		0.22	0.04		0.9	0.02		0.55
FG (mg/dL)				0.004		0.22	0.002		0.5
HbA1c (%)				0.11		**0.04**	0.09		**0.03**
TG (mg/dL)				0.003		0.8			
hsRCP (mg/dL)							0.006		0.9
PAI-1 (ng/mL)				0.02		**0.01**	0.01		**0.03**
Vitamin D insufficiency (yes)	−0.87		0.07	−0.01		0.15	−0.08		0.08
Constant	−161		0.002	0.26		0.3	0.25		0.4

Dependent variables are in bold and underlined; explanatory variables with statistically significant results in multiple regression analysis, and their significance are in bold.

B: standardized coefficient; BMI: body mass index; CC: common carotid; FG: fasting glucose; hsRCP: high-sensitivity-reactive C protein; IMT: intima-media thickness; NRTI: nucleoside reverse-transcriptase inhibitor; PAI-1: plasminogen activator inhibitor-1; PI/r: protease inhibitor; >P75th: >percentile 75 of irisin levels vs <percentile 25 of irisin levels; r^2: coefficient of determination; TG: triglycerides; WHR: waist-hip-ratio.

We report a consistent and inverse relationship between circulating irisin concentrations and insulin homeostasis in HIV patients, measured by CIGMA-HOMA score, a dynamic test to evaluate insulin resistance. Our data are in accordance with previous results observed in different populations as subjects with metabolic syndrome[9] and nondiabetic adult subjects[10] and suggest a plausible role of irisin in insulin resistance, and specifically in HIV patients. However, this association seems to be mediated mainly by changes in body fat distribution, without a causal relationship.

HIV males with liver steatosis had higher irisin concentrations, and irisin was positively related to HTGC, VAT, and ALP and GGT concentrations. This association between irisin concentrations and HTGC was in line with previous data showing higher irisin concentrations in NAFLD.[12,14] Moreover, the presence of higher irisin related to higher VAT might reinforce the hypothesis of the potential role of irisin as a compensatory mechanism for the abnormal adipose distribution in HIV patients.

Irisin concentrations have been shown to be associated with the development of major adverse cardiovascular events in patients with coronary artery disease.[21] In the available literature, we found only four studies investigating relationship between irisin and subclinical atherosclerosis as measured by CAC or IMT. According to our data, Sesti et al[10] in a cross-sectional evaluation, and Kwaśniewska et al[15] in a 25-year follow-up cohort study, found a significant positive relationship between irisin and carotid IMT. Conversely, irisin showed a negative association with carotid IMT in smaller studies conducted in selected populations as Behcet's disease[22] and dialysis patients.[23]

The explanation for the positive relationship between irisin and carotid IMT is speculative, but the increased release of irisin by adipose/muscle tissue could reflect a response to deterioration of insulin sensitivity or a compensatory increase to overcome an underlying irisin resistance. Also, the positive correlation between serum irisin and PAI-1, demostrated in the present study, supports the plausible influence of irisin in the atherotrombotic disease, as to our knowledge, PAI-1/irisin association has not been described previously.

The association between lipodystrophy and C-IMT in patients with HIV has been described recently, although age was the strongest

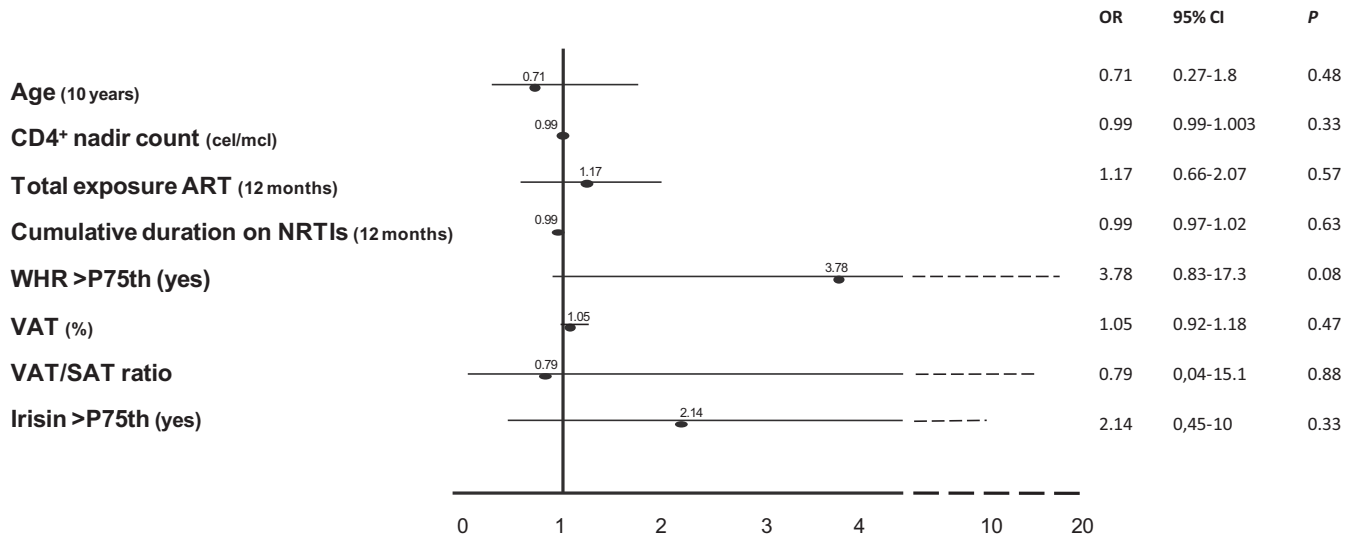

Figure 1. Risk factors associated with nonalcoholic fatty liver disease in multivariate logistic regression model. (.) OR, odds ratio; error bars are 95% confidence interval; dashed lines indicate a change in scale; ART: Antiretroviral Therapy; NRTIs: Nucleoside analog reverse-transcriptase inhibitors; WHR, waist-hip-ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; >P75th, >percentile 75 levels vs <percentile 75 levels

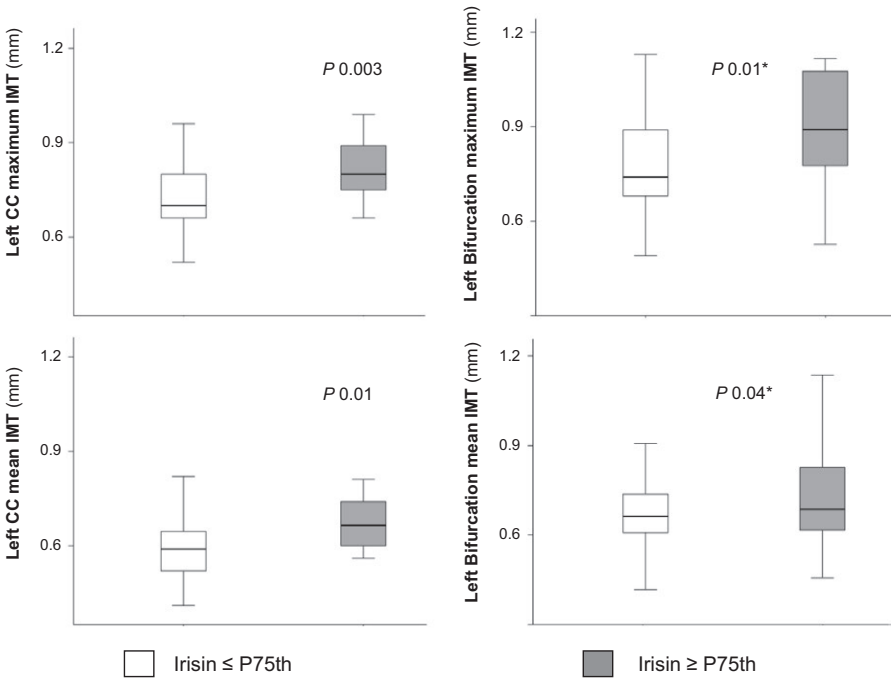

Figure 2. Carotid intima-media thickness by irisin concentration. CC, common carotid; IMT, intima-media thickness. Student's *t* test* or Mann-Whitney *U*-test, as appropriate; *P*, significance. Left CC maximal IMT 0.8(0.75-0.89) vs 0.7(0.66-0.8)mm; Left CC mean IMT0.66 (0.6-0.74) vs 0.59 (0.52-0.65)mm; Left bifurcation maximal IMT 0.95 ± 0.18 vs 0.83 ± 0.14 mm; Left bifurcation mean IMT 0.74 ± 0.11 vs 0.65 ± 0.14 mm

associated factor,[24] and in the 12-month follow-up, no association between the progression of C-IMT and the presence of lipodystrophy was found.[25] While to our knowledge, there is no published data about PAI-1 concentration and C-IMT in people living with HIV.

There are few data in the literature about the impact of the antifibrinolytic protein PAI-1 concentrations on CV morbidity in patients with HIV infection. Only a case-control study[26] described an association between levels of PAI-1, and subsequent development of a first-time MI among HIV-infected patients receiving ART. Thus, our observed relationship between PAI-1 and carotid IMT thickness reinforces its role in CV risk in HIV patients.

Finally, irisin concentrations were positively related to weight and waist-to-hip-ratio. Human data regarding BMI and irisin are conflicting, some authors reported a positive correlation[27-29] while others showed a negative correlation.[11,14] Differences between populations may influence these discrepancies, and also the relationship between irisin and BMI may be affected by the presence of diabetes, or it may differ across BMI categories. Irisin was associated with an adverse lipid profile, with higher irisin concentrations related to higher LDL and TGs, and lower HDL. Currently, the relation between irisin and circulating lipids has been analysed in different populations with mixed results.[13,27,30-33] Irisin induces adipocyte browning, with

Table 3. HIV-related factors associated with irisin concentrations

Factor	Irisin >P75th n = 22	Irisin <P25th n = 22	OR (95% CI)	*P* value
CDC stage, n				
A	13	15		
B-C	9	7	1.43 (0.27-7.7)	0.67
Duration HIV, years Mean ± SD	9.2 ± 5.2	8.2 ± 6.2	1.03 (0.93-41.1)	0.56
Nadir CD4+, cells/mm^3 Median (P25-P75)	195.5 (5-781)	227.5 (25-985)		0.23
Current CD4+, cells/mm^3 Median (P25-P75)	520(216-1798)	450(149-1600)		0.91
Viral load <50 copies/mL, (n) yes[a]	16	16		0.97
Group, n				
Naive	2	5		
ART	20	17		0.34
Exposure, months Mean ± SD				
Total ART	90.6 ± 38.0	63.5 ± 34.4		**0.02**
non-NRTI	43.2 ± 32.4	34.3 ± 22.8		0.08
PI	77.2 ± 45.3	48.9 ± 37.6		0.38
Exposure to NRTIs, months Mean ± SD	164.9 ± 90.7	91.4 ± 85.7		**0.008**
Lipodystrophy, n yes	7	7	0.95 (0.51-1.82)	0.92
Total testosterone (ng/mL) Mean ± SD	4.6 ± 1.0	5.8 ± 1.7		0.53

Qualitative variables were expressed as number of patients. Parametric variables were expressed as means ± SD, nonparametric variables as medians and percentiles 25-75. The associations between the different variables and irisin were calculated using the chi-squared test for qualitative variables and the Student's *t* test or Mann-Whitney *U*-test for quantitative variables.
The *P* values are bold where they are less than the significance level cut-off of 0.05.
ART: Antiretroviral Therapy; CDC: Centers for Disease Control and Prevention; CI: confidence interval; HIV: human immunodeficiency virus; NRTIs: Nucleoside analog reverse-transcriptase inhibitors; OR: odds ratio (for qualitative variables); PI: protease inhibitors; SD: standard deviation.
[a]All ART-patients had an undetectable viral load in the recruitment phase, but at the time of the analysis there were 12 patients who presented detectable viral load.

increased lipid oxidation and thermogenesis, and may influence lipid profile.[5] However, our data showing increased irisin concentrations related to different metabolic disturbances, including an atherogenic lipid profile, give meaning to the rise in irisin as a compensatory mechanism.

4.1 | Clinical implications

On the basis of our results, irisin could play a role in the complex pathogenic mechanisms involved in the development of cardiovascular disease, insulin resistance and hepatic steatosis in HIV-infected males, and could serve as a starting point for new therapeutic targets. Nevertheless, the lack of consistency in the literature, together with the nonpersistence of the association between irisin and C-IMT in multivariate analysis, make that nowadays the role of irisin in atherosclerosis remains elusive and poorly defined, and preventive interventions should focus on traditional cardiovascular risk factors in nondiabetic males living with HIV.[34] According to our results, PAI-1 concentrations and the presence of lipodystrophy may be useful for risk stratification of HIV-infected individuals on effective ART, with regard to its relationship with subclinical atherosclerosis.

4.2 | Study limitations

Some limitations should be acknowledged in the interpretation of our results. The cross-sectional nature of the study precludes us to draw any conclusion on the role of irisin in the development of metabolic disorders, and no conclusion regarding cause-effect relationships can be made. Additionally, circulating irisin were measured at resting condition, and the possibility of obtaining different results after exercise cannot be excluded. The absence of significance after multivariate analysis indicates that irisin could have a role, but there must be other factors implicated in these processes. The inclusion of patients with ART with undetectable viral load in the initial evaluation in blood samples of the usual clinical follow-up, which was found to be detectable in the study's blood samples, is a bias; nevertheless, we decided not to exclude them, to not significantly affect the number of patients evaluated.

The present study has several strengths including the relatively large sample compared with other studies in this field and the exclusion of confounding conditions such as diabetes and hepatitis. The results are also strengthened by the use of a CIGMA-HOMA score, a dynamic test to evaluate insulin resistance, and by the use of the imaging gold standard ^{1}H-MRS to measure HTGC.

5 | CONCLUSIONS

In HIV males without diabetes, higher irisin concentrations are associated with higher insulin resistance, nonalcoholic fatty liver disease (NAFLD) (higher Hepatic triglyceride content (HTGC)) and subclinical atherosclerosis (greater carotid intima-media thickness (IMT)). However, waist-to-hip-ratio was the strongest determinant of insulin homeostasis, and PAI-1 levels and lipodystrophy were the main determinants of subclinical atherosclerosis. Irisin is positively associated with other cardiovascular risk factors and could behave as a surrogate marker of subclinical atherosclerosis. Among HIV infection-factors, only the time of exposure to nucleoside reverse-transcriptase inhibitors (NRTIs) was related to higher irisin concentrations.

At the present time, primary and secondary prevention of cardiovascular disease must be centred in the management of traditional cardiovascular risk factors. Only large prospective studies with clinical end-points will give a definitive answer, to clarify the role of irisin in cardiovascular and metabolic risk in the HIV-infected population.

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